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Mechanical and Antibacterial Properties of ZnO Nanoparticles Incorporated HPMC Polymer Nanocomposite Films.

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ABSTRACT

The nanocomposite films were prepared by incorporating Zinc Oxide nanoparticles (ZnO NPs) into Hydroxypropyl methylcellulose (HPMC) polymer matrix. The films were prepared with different concentration of ZnO NPs and tested its mechanical and antibacterial properties, as a function of nanoparticles concentrations. The mechanical properties of nanocomposites were examined using universal testing machine (UTM). The antibacterial performance was evaluated as a function of ZnO NP concentration, by zone diffusion method, against gram-positive and gram-negative bacteria species. A decrease in tensile strength, Young's modulus and the elongation at break of the films were observed, as the ZnO NP concentration increased. The incorporation of ZnO NP with HPMC has a concentration dependent antibacterial activity on both Gram-positive and Gram-negative species.

Keywords: HPMC, ZnO nanoparticles, nanocomposites, tensile strength, antibacterial activity

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INTRODUCTION

Recently, polymer – based nanocomposites, with inorganic nanoparticles dispersed in polymer matrix are interesting because of their improved properties, simple processing steps and relatively low cost. With the addition of nano sized inorganic particles into the polymeric matrices, the new composite material will exhibit changed physical, mechanical, thermal, optic, effective bactericidal function, intensive UV and infrared absorption, which greatly differ from those of conventional materials [1]. During the past few years, several investigators have studied the incorporation of nanoparticles such as metal and metal oxide nanoparticles, nanofibres and nanotubes into polymers to provide additional barriers or functional properties for food packaging purposes [2]. Silica nanoparticles were inserted into polypropylene and starch polymer matrices to improve mechanical or barrier properties of composites and biodegradable films [3]. Starch nanocrystals have been found to improve the mechanical properties of polymer films and inhibit their elongation [4]. Biopolymers, such as cellulose and its derivatives, may offer attractive alternatives as long as their properties can be tailored to specific end-use applications [5]. Hydroxypropyl methylcellulose (HPMC) is one of the cellulose ethers which are used in the food industry as an emulsifier, protective colloid, stabilizer, suspending agent, thickener, or film former. The films obtained from HPMC are resistant to oils and fats, flexible, transparent, odourless, and tasteless but tend to have a moderate strength [6]. HPMC is also being adopted as a film coating or a sustained-release tablet material in the pharmaceutical field [7].

The addition of nano sized inorganic particles into polymer matrix results new composites material which exhibit enhanced properties. ZnO as an environment-friendly material and has relatively little toxicity makes it desirable for bio-applications [8]. Researchers have shown that ZnO NPs have biodegradability and biocompatibility properties [9]. The additional advantages of using biocompatible nanoparticles like ZnO in food-packing materials is that they can act as antimicrobial agents are the greater effectiveness on resistant strains of pathogens. Further, with special reference to ZnO NP, it can provide a mineral element essential to human cells since Zn has important physiological roles and nutritional significance; thus, ZnO nanoparticles is considered as one of the multifunctional inorganic nanoparticles [10].

We have investigated the effect of addition of ZnO nanoparticles on the mechanical properties and antibacterial performance of HPMC polymer film with the hypothesis that it will enhance its utility in food and biomedical fields with improved qualities. Hence in this paper we have reported tensile strength (*TS*), elongation (*E* in %) and Young's modulus (*Y*) and antibacterial activity of HPMC polymer films with and without incorporated with ZnO NP at different concentrations.

MATERIALS AND METHODS

Preparation of nanocomposite films

The commercial grade HPMC and ZnO nanoparticles used in this work and were obtained from Sigma-Aldrich. HPMC was in the form of white powder, having approximate molecular weight of 120,000 Dalton and size of ZnO nanoparticles were less than 100 nm. The HPMC-ZnO nanocomposite films were prepared by the solution casting method, with varying the concentration of ZnO NPs [11,12]. The thickness of the obtained films was 60 µm.

The mechanical properties of polymer samples were measured with universal testing machine (Lloyd Instruments, UK, 5kN load cell). Each sample was tested at the specifications of 50mm of gauge length, with the crosshead speed of 20mm/min at room temperature. A model LRX Plus was used to determine the maximum tensile strength (TS), maximum percentage elongation at break (E in %), and elastic (Young's) modulus (Y).

Mechanical properties test

Antibacterial activity test

In vitro antibacterial activity test was performed by disc diffusion method *[*13*]*. 0.1ml (10-5 CFU /ml) of 24 hrs different bacterial cultures were placed on Nutrient agar medium and spread throughout the plate by spread plate technique. The sterile discs were soaked with suitable HPMC solution was placed on the surface

of the medium and incubated at 37°C for 24hrs. Antibacterial activity was recorded by measuring the diameter of inhibition zone. Streptomycin was used as the positive reference standard. The entire test was performed in triplicate.

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) with Tukey's multiple comparison tests at 95% confidence level using Graph Pad instant 3.

RESULTS AND DISCUSSIONS

Mechanical properties test

Mechanical properties of the films are important for packing materials. They measure stretch ability prior to breakage and film strength. For this purpose, the mechanical performance of developed HPMC films were studied by determining the tensile strength at break (MPa), Young's modulus (MPa) and elongation at break (%).

The effects of ZnO NP on the mechanical properties of the HPMC nanocomposite films were evaluated up to their failure. Table 1 shows the tensile strength, the Young's modulus and elongation at break as determined from the typical stress-strain curves of these materials. The tensile strength and Young's modulus of the pure HPMC film were 59.12±4.18MPa and 1585.28±77MPa respectively. The elongation at break of same film was 14.46±1.59%. One-way ANOVA indicated that the addition of ZnO NP to HPMC films resulted in a significant difference ($p<0.05$) in film's mechanical properties. As the ZnO concentration increases, the tensile strength, Young's modulus and elongation decreases. The extent of reduction in tensile strength is varying and we observed 30.46% reduction in 0.04% concentration. Elastic modulus goes on decreasing as the concentration changes and 37% reduction in 0.04% ZnO concentration which is given in Table 1. Also from the Table 1, we observed that elongation at break (%) decreases with increase in ZnO concentration.

Table 1: Mechanical properties of virgin and ZnO incorporated polymer films

Sample	Tensile strength (MPa)	Young's modulus (MPa)	Elongation (%)
Pure	59.12 ± 4.18^{a}	1585.28 ± 077.02^{a}	14.46 ± 1.59^{NS}
0.01%	55.43 ± 0.62^{b}	1293.04 ± 005.04^{b}	14.29±2.28
0.02%	$49.64\pm0.57^{b,c}$	$1171.55\pm115.94^{b,c}$	11.25±1.22
0.03%	$44.77\pm3.89^{c,d}$	$1145.26\pm106.62^{b,c}$	11.00±5.08
0.04%	41.11 ± 1.43^{d}	997.00 ± 036.18^{c}	13.02±2.41

The different superscripts in the same column indicate the significant differences ($p<0.05$). The data with same superscripts does not differ significantly.

There may be entanglement of ZnO at very low concentration. This entanglement was the main cause of decrease of elongation [14]. The addition of ZnO to the HPMC films did not result in any improvement in mechanical properties and rather we observed detriment effect. This could be due to strain fields occures at polymer matrix due to presence of ZnO nanoparticles. This is also an indication of fewer interactions at the interface of both components due to the lower surface area [15].

Antibacterial activity test

The results obtained from zone diffusion method clearly indicated that incorporation of ZnO nanoparticle in HPMC (ZnO NP-HPMC) has dose-dependent antibacterial activity against both Gram-positive and Gram-negative bacterial species (Table 2). With the increase in the concentration of ZnO nanoparticle embedded in HPMC, the growth inhibition was found to be increased showing the highest activity at 0.04% (Fig. 1). Among the bacterial species tested, ZnO NP-HPMC was more effective against *S. Aureus* (Gram-positive), *A. hydrophila and K. pneumonia* (Gram-negative). Statistically, a significant difference ($P<0.05$ and

P<0.01) between the results obtained for virgin HPMC and HPMC embedded with 0.04% ZnO as evident from both the parameters proves that incorporation of a small quantity of ZnO nanoparticle (i.e., 400 ppm) has a potential impact on a biological property of HPMC in terms of antibacterial activity. Furthermore, the susceptibility of both Gram-positive and Gram-negative bacteria as shown in the present study suggests HPMC polymer film can be made to possess broad-spectrum antibacterial activity by incorporation of ZnO NP. ZnO NP disrupts the cell membrane integrity of bacterial cells and upon entering into cytosol induces oxidative stress leading to inhibition of cell growth and eventually cell death [16,17]. Also decomposition of cell wall and subsequent decomposition of the cell membrane of bacteria exposed to ZnO NP leads to the leakage of cytosolic contents, causing cell death [10].

Table 2: Results of Agar diffusion test for antibacterial activity of HPMC polymer films with and without ZnO NP.

Test Samples	Diameter of inhibition zone (mm±SD)					
	Bacterial strain					
	Gram-positive		Gram-negative			
	B. subtilis	*S. aureus*	*E. coli*	*A.hydrophila*	*P.aeruginosa*	*K.pneumoniae*
Pure	--	--	--	--	--	--
0.01%	09.4±0.46^{a}	--	--	08.7±0.36^{a}	--	08.0±0.26^{a}
0.02%	12.6±0.36^{b}	08.9±0.20^{a}	10.1±0.56^{a}	11.3±0.46^{b}	08.3±0.66^{a}	10.7±0.46^{b}
0.03%	14.4±0.36^{c}	11.8±0.44^{b}	10.5±0.36^{a}	13.4±0.30^{c}	09.5±0.53^{b}	12.4±0.46^{c}
0.04%	16.8±0.36^{d}	13.9±0.27^{c}	12.7±0.46^{b}	16.9±0.30^{d}	12.7±0.21^{c}	14.3±0.46^{d}
Streptomycin	28.2±0.96^{e}	24.6±0.27^{d}	19.2±0.36^{c}	24.7±0.36^{e}	18.1±0.30^{d}	28.2±0.40^{e}

The data with different superscripts (a, b, c, d and e) differ significantly at probability level p<0.05. The data with same superscripts does not differ significantly.

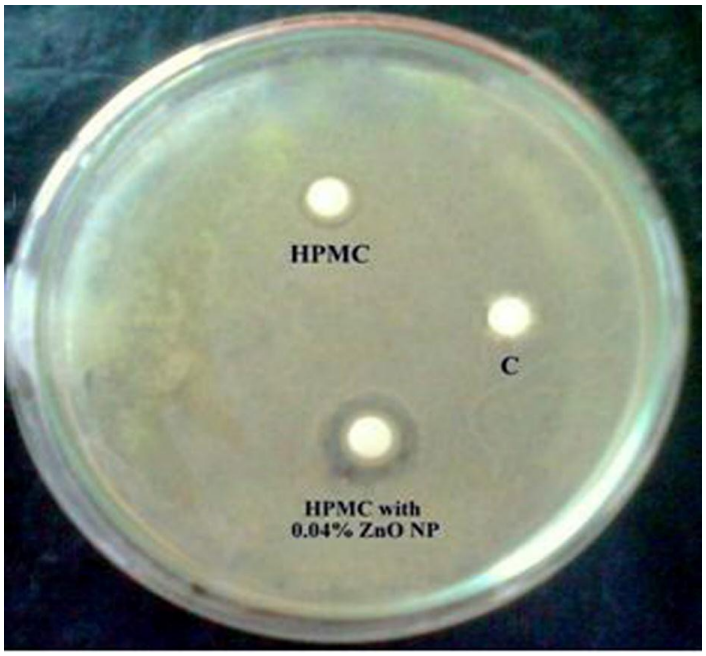

Figure 1: A representative photo of antibacterial activity by zone diffusion method. C - Control; pure HPMC and HPMC with 0.04% Zn NP, against *S. aureus*.

CONCLUSION

We observed that there are changes, both in mechanical and antibacterial properties, in HPMC films when ZnO nanoparticles were incorporated. Tensile strength, Young's modulus (Y) and percentage elongation decreases as the ZnO concentration increases. The mechanical properties decrease because of weakening of intermolecular van der Waal's forces and also due to the creation of strain fields due to indiscriminate dispersion of nanoparticles in the matrix of HPMC. In general, it is observed that the incorporation of the ZnO

nanoparticles negatively affected the mechanical properties but enhanced the antibacterial properties of the films. However, incorporation of ZnO NP into HPMC polymer film can be considered as a promising strategy to improve its usefulness in clinical applications in view of its potential antibacterial activity.

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