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Original Article

Preparation and Characterization of Bio-nanocomposite Films, Incorporated with Silver Nanoparticles

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ABSTRACT

Background: The present work was conceded to know the improved and comparable properties with bio-nanocomposite films. The films were prepared by using blends of Carboxy Methyl Cellulose (CMC), a cellulose derivative; pectin by wet casting was used to create the films with incorporation of silver nanoparticles that had been produced. **Materials and Methods:** The silver Nanoparticles (AgNP's) were synthesized by eco-friendly method by using *Psidium guajava* leaf extract. Incorporation of AgNP's into CMC and pectin films (bio-nanocomposite films) was characterized by SEM. Also its mechanical properties, WVTR activity and anti- microbial studies were carried out in the present work. **Results:** SEM results have shown that formation of spherical shaped nanoparticles which is Ag nanoparticles coated bio-nanocomposite films. According to the WVTR results, adding more AgNP's to films decreased their humidity and ability of water absorption. The mechanical properties of the films were improved. The films showed remarkable antibacterial activity against *E. coli, Bacillus, S. aureus*, and *Klebsiella pneumoniae*. **Conclusion:** These characteristics imply that AgNP's-based bio-nano composite films find use in the food industry as an active packaging material.

Keywords: Antimicrobial properties, Biodegradable polymers, Carboxy methyl cellulose, Nanocomposites, Silver nanoparticles.

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INTRODUCTION

Based on unique properties including size, shape, and dispersion, nanoparticles typically range in size from 1 to 100 nm. The environmentally friendly method used in chemistry is called the "green synthesis method," and it was developed in response to the widespread issue of environmental concerns.[1-5] The newest developments in the realm of nanotechnology are related to nanomaterials.[6] Activated carbon materials that have shown antibacterial efficacy against very dangerous organisms are among the several silver nanomaterials.[7]

Silver nanoparticles production is anticipated to rise during the coming years as a result of its significant contributions to catalysis, bio-molecular detection, medicine, and biosensors. It is known to have potent bactericidal and inhibitive actions. The advancement of polymer properties has improved due to the incorporation of nanoparticles to biocomposite films to prepare bio nanocomposites films.[8] Bio-Nanocomposite can be considered as green technology shows various applications packaging, agriculture, and pharmaceutical technology.[9-11] Silver nanoparticles have attractive property to improve the antimicrobial property, which is essential to use for food packaging applications.

The green process is an alternative to traditional methods for the preparation of silver nanoparticles. In this case, silver ions were bio-converted to nanoparticles using *Psidium guajava* (also known as guava) leaf extract. The leaves of this plant are widely used in the field of medicine, and they are easily accessible in India. As there are many natural biopolymers, important cellulose ether, CMC, is created by carboxylating cellulose. The uses for Carboxy Methyl Cellulose (CMC) are incredibly varied.[12] The polysaccharide category with the most brilliant research and importance is Carboxy Methylcellulose (CMC). The water-soluble ionic derivative of cellulose introduced in 1918 is Carboxy Methylcellulose (CMC). It is widely utilized in the food, paper, textile, detergent, cosmetic, and pharmaceutical industries.

CMC is a cellulose derivative. The chemical reaction that occurs between cellulose and mono chloro acetic acid in the presence of NaOH makes CMC water soluble, despite the fact that cellulose is not water soluble. CMC is a linear organization polymer that

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can be found as a colorless, nontoxic, fragrance-free powder or a highly transparent gel. Additionally, it has high dispersion and binding forces in a mild alkaline solution. Pectin is also used as a reducing agent and a stabilizing agent to synthesize Ag nanoparticles. Pectin is a key cell wall polysaccharide of higher plants. Dicotyledonous plants have pectin mostly in the middle lamella and the primary cell walls, where it is essential for the cell,[13] mechanical strength,[14] and defensive mechanisms.[15] Pectin is mostly generated from citrus peel in commercial extraction operations. From food to medicinal items, it is employed as a gelling, thickening, and emulsifying ingredient in a variety of applications.

In this study, biodegradable CMC and pectin blends were employed to create a film. In addition to this, cross-linkers, plasticizers, and carbohydrates have frequently been thought of as a way to enhance the textural features of films. Glycerol can be employed to provide significant plasticizing characteristics at a moderately low concentration. Plasticizers lower the intermolecular Vander Waals force between polymers, making plastics more flexible and less rigid. Mixtures of homopolymers or copolymers with different molecular structures make up the polymer blend. Combining a polymer with another that has a higher-valued property can effectively mitigate the drawbacks of a polymer that lacks that trait.

The current study aimed to develop model composite films based on commercial CMC, pectin and green synthesized silver nanoparticles. Typically, nanoparticles are dispersed inside a polymeric network matrix to create polymeric bio nano composites. The features of nanocomposite materials then depend on the morphology and interfacial properties of each component, in addition to the attributes of those components as a whole. Here, the polymeric bio-nanocomposite films have two steps i.e., eco-friendly synthesis of nanoparticles and preparation of films by a casting method. In the current paper, CMC, pectin, and silver nano composites were made using AgNP's as a filler.

MATERIALS AND METHODS

Materials

Silver nitrate, Pectin, carboxymethyl cellulose were purchased from Sigma Aldrich in Bangalore, India. Additionally, glycerol (a plasticizer) and calcium chloride (a cross-linking agent) were bought from SRL India Limited. We used double-distilled water for the entire project.

Silver nanoparticles are made using a Green Synthesis method[16]

In order to make its solution, silver nitrate GR was used as such (obtained from SRL Chemicals, India). In an Erlenmeyer flask, a 100 mL, 1mM solution of silver nitrate was made. Following that, 1, 2, 3, 4, and 5 mL of plant extract were added in various amounts to 10 mL of silver nitrate solution while maintaining a 1 mM concentration. According to this theory, silver nanoparticles might also be produced using different concentrations of silver nitrate. To decrease the amount of photo-activation of silver nitrate at ambient temperature, this setup was incubated in a dark environment. The transformation of the solution's hue from colorless to brown during this process confirmed the reduction of Ag+ to Ag. UV-visible spectroscopy also provided further evidence of its genesis.

Preparation of CMC, Pectin-silver Nanocomposite Films

The CMC, pectin, and AgNP's nano composite films were created utilizing the solution casting process. For this, 2.5 g of Carboxy methyl cellulose and 1.5 g of pectin were dissolved at 30°C in 250 mL of distilled water with 0.5 mL of glycerol. To ensure thorough dissolution, the solution was mechanically agitated for 1 hr at 800 rpm. After dissolution, silver nitrate of different concentration was added. Subsequently, the solution temperature increased to 70°C and 0.3 g calcium chloride dehydrate ($CaCl_2.2H_2O$) was added. To avoid the production of microbubbles in the films, these filmogenic solutions were placed in a vacuum sucker (BUCHI Vac-350) for a period of time. The solutions were then put onto a glass plate (24x30 cm), which was then leveled and exposed to IR lamps of 250 volts for 24 hr to dry. The dried films were taken out and prepared for further analysis in sealed plastic bags kept at room temperature. Finally, neat CMC, pectin-silver films were prepared by following the procedure mentioned above.

Characterization of Bio-Nanocomposite films by incorporation of Ag Nanoparticles

SEM or scanning electron microscopy

Using a SEM (LEO 435, VP LEO Electronic microscopy Ltd., UK.) at 15kV and magnifications at the range of 1000x, 5000x, and 10,000xs, the surface morphology, structure, and shape of the film samples were examined.

Percentage of Elongation

The elongation percentage (E%) at the break was measured using a Universal Testing LLOYDS (LLOYDA-50K, London, UK) instrument. Tests were conducted in accordance with ASTM D-882 standard test (ASTM 1995a) with an initial grasp spacing

$$\text{Percentage of Elongation} = \frac{\textit{elongation at rupture}}{\textit{initial gauge length}} \times \mathbf{100}$$

of 50 mm and a crosshead speed of 10 mm/min. The formula in the equation can be used to calculate the percentage of elongation.

The ratio of extension to sample length determines the percentage elongation. The ratio of stress to strain at the linear location of the curve is known as Machine Elongation (ME). When the resultant means were all compared to one another.

Water Vapor Transmission Rate (WVTR)

WVTR was measured according to ASTM E 96 (14) using aluminum cups. A 50 cm^2 diameter of the sample was sealed on the aluminum cups containing fused $CaCl_2$ which is highly hygroscopic. After that, the specimen was put on the cup, and 50 cm^2 of its surface were sealed with hot wax. The sample were first rested at room temperature and weighed.

The prepared cups kept in a chamber at 38°C to 39°C and 90% RH. Weighing at regular intervals to monitor weight gain caused by $CaCl_2$, which in filtrated the film. Weight gain was plotted against time, and WVTR was determined using the linear least-squares approach.

$$\text{WVTR} = \text{Slope X area of sample gm/m}^2\text{/hr}$$

Determination of Antimicrobial Effects of Films

Using the agar diffusion method, the antibacterial effectiveness of films was evaluated against pathogens that can be found in food, such as *E. coli, Streptococcus* sp., *Bacillus*, and *Klebsiella* sp. On agar plates seeded with 0.1 mL of an overnight broth culture of indicator strains, the films of 5 mm diameter discs were laid out. The plates underwent an additional 24 hr of 37°C incubation. The antibiotic zone scale (Himedia, India) was used to calculate the diameter of the zone of inhibition. Each plate contained a positive control with sterile paper discs containing the standard antibiotic Tetracycline (30 g/discs).[17] There were three duplicates of each experiment.

RESULTS

Bio-Composite Films with AgNP's

Scanning electron microscopy Analysis

SEM of films is shown in Figures 1-5. The pure, or neat, CMC and pectin films have demonstrated a smooth surface. The SEM image revealed the uniformly dispersed particles upon the addition of $AgNO_3$ at various concentrations, indicating the creation of AgNPs. Scanning electron microscopy images were seen in various magnification ranges as shown in the figures. The shape of bio-nanocomposite films was shown in Figures 1-5. The structure of the produced nanoparticles was examined using the SEM. Only bio composite films were depicted in Figures 2-5. SEM image, and it was discovered that these films had Ag nanoparticles deposited on them. These SEM findings are consistent with data that have recently been published and demonstrate the production of spherical nanoparticles.[18]

The morphology of silver bio-nanocomposite films which was determined using Scanning Electron Microscopy analysis and the results were presented in Figures 1-5. The surface of the composite film was smooth, but that of the Ag bio-nanocomposite films have possess crystals which indicate the formation of silver nanoparticles, with increase in the concentration of silver NP'S the crystals on the surface had increased (10 mL, 20 mL, and 30 mL).

Break Elongation (E%)

The elongation at break (E%) is the indication of the food packaging film flexibility. It is an important property of packaging film. Break elongation was determined using universal testing machine instrument (Lloyd Instruments Ltd., England) and the results from the break elongation test for a biodegradable multifunctional film of CMC and Pectin with the incorporation of AgNP's as shown in Figure 6.

From the Figure 6, it was seen that the X-axis scale indicates the elongation at break (E%). The Y-axis scale indicates the concentration percentage of biodegradable multifunctional film

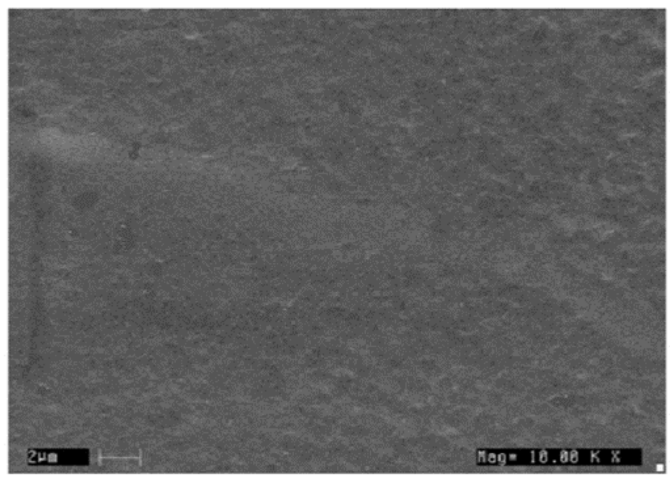

Figure 1: SEM image of CMC-Pectin film.

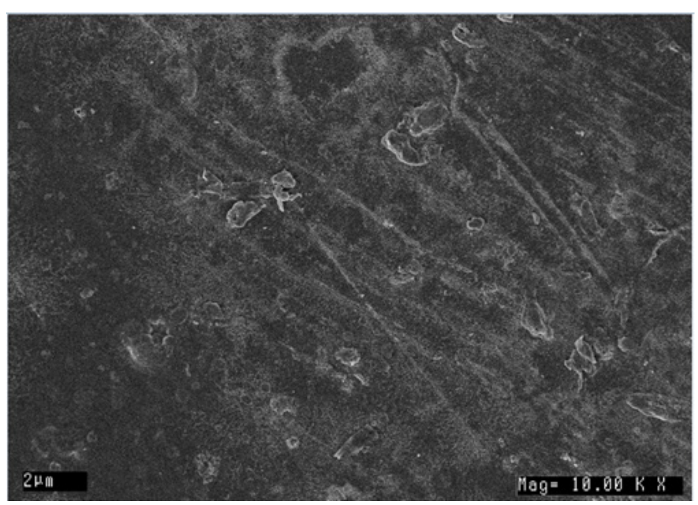

Figure 2: SEM image of CMC-Pectin with only leaf extract film.

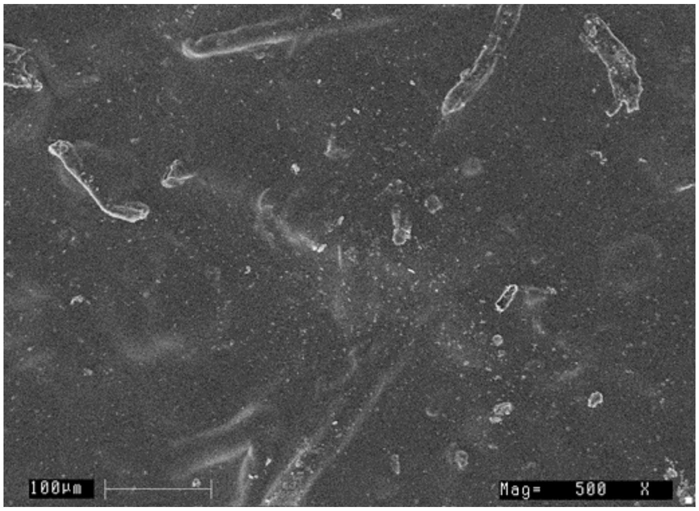

Figure 3: SEM image of CMC-Pectin with 10 mL AgNP's.

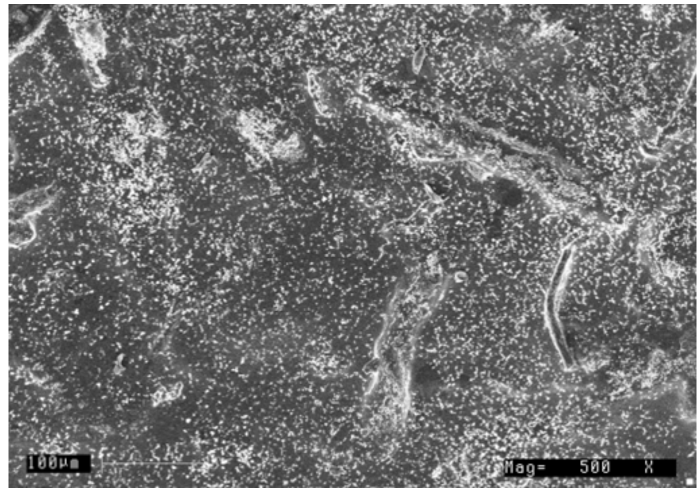

Figure 5: SEM image of CMC-Pectin with 30 mL AgNP's.

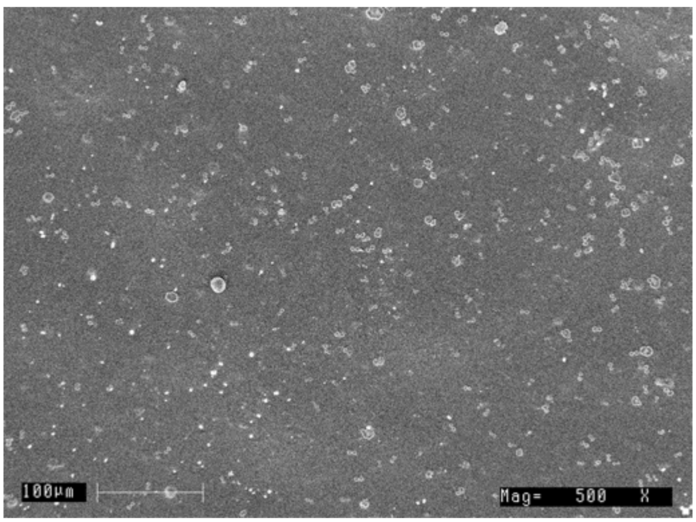

Figure 4: SEM image of CMC-Pectin with 20 mL AgNP's.

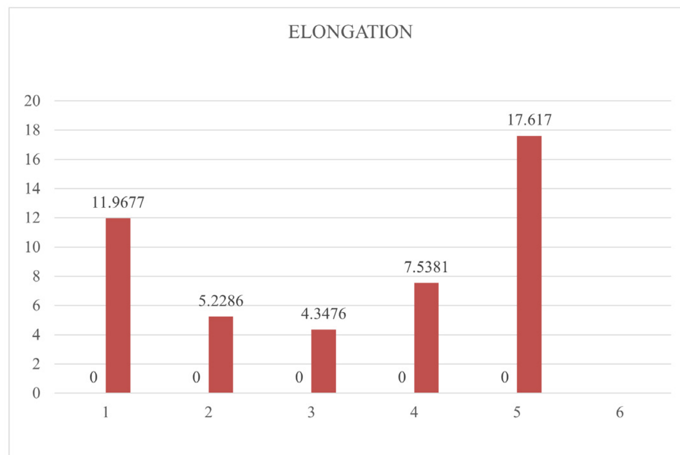

Figure 6: Elongation at break of the biodegradable multifunctional films.

Table 1: Antimicrobial activity of control, leaf extract and three different concentrations of synthesized Silver nanoparticles incorporated CMC-Pectin against some bacterial strains.

Antibacterial activity				
Minimal inhibition concentration				
	***E. coli* sp.**	***Klebsiella* sp.**	***Bacillus* sp.**	***Staphylococcus Aureus* sp.**
Control	0.88±0.18	0.86±0.07	0.84±0.18	1.2±0.28
Leaf extract	0.74±0.18	0.82±0.11	0.79±0.07	0.87±0.33
AgNP's 10 mL	0.81±0.11	1.93±1.74	0.95±0.21	0.83±0.09
AgNP's 20 mL	1.01±0.11	1.22±0.31	1.35±0.35	1.04±0.12
AgNP's 30 mL	1.15±0.11	1.15±0.02	0.95±0.07	0.96±0.23

of carboxy methyl cellulose and Pectin with the incorporation of AgNPs. The results of three different concentrations of composite films are as shown below. The first group from left shows for CMC and Pectin film (S-1). The next one is CMC and Pectin, leaf extract without AgNPs film (S-2), third one represents CMC and Pectin, leaf extract with 10 mL Ag NPs film (S-3), fourth represents is CMC, Pectin, leaf extract with 20 mL AgNPs film (S-4) and fifth represents is CMC, Pectin and leaf extract with 30 mL AgNPs film (S-5) groups.

The break elongation gradually decreased from control film to 10 mL of nanoparticles loading, with a maximum decrease at 10 mL

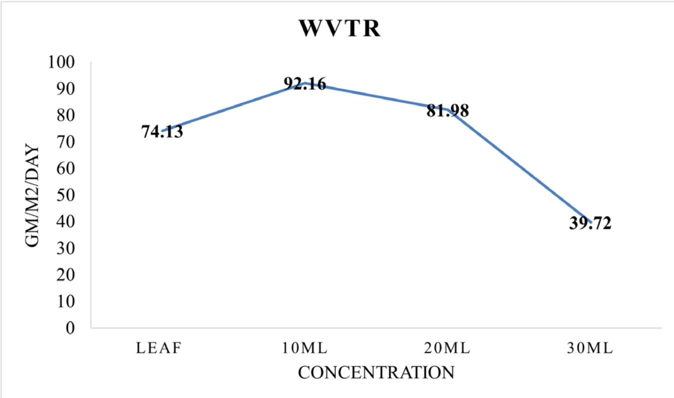

Figure 7: Water vapour transmission rate of CMC-Pectin at various concentrations of AgNP's blend films.

v/wt. %. The break elongation decreased from 5.22 to 4.34 MPa. For nanocomposite film containing 20 mL of Ag nanoparticles solution is increased (i.e., 7.53 MPa) than film having 10 mL of Ag nanoparticles solution. Elongation at break is greatly increased in a film containing 30 mL of Ag nanoparticles solution.

Water Vapour Transmission Rate (WVTR)

By using the water vapour transition rate machine it is possible to calculate the rate at which the water vapour crosses the film. Water vapour transition rate of Carboxy methyl cellulose-Pectin, leaf extract free from AgNP's. CMC-Pectin with 10 mL AgNP's, CMC-Pectin with 20 mL AgNP's and CMC-Pectin with 30 mL AgNP's films were determined at 28°C and 92% permanent RH chamber. The graph is drawn between different concentration of nanocomposite films and % of vapour transition rate of films and which as shown in the Figure 7. From the graph, it was seen that CMC-Pectin with 10 mL AgNP's nanocomposite film has transmission rate (92.16%) than others.

From above graph, it can clearly state that the film has no barrier property to water vapour. Among these films, the CMC, Pectin with the 30 mL film has reduced barrier property compared to others. The CMC, Pectin without silver nanoparticles film has shown poor barrier property than others. With increase of silver content in the composite film, there is lower WVTR rate observed.

Anti-microbial Activity of Bio-composite Films with Ag Nanoparticles

The agar disc diffusion method was utilized to carry out the experiment. Ampicillin disc was employed as a control. The anti-bacterial action of Ag NPs was seen against pathogens such as *E. coli*, *Streptococcus* sp., *Bacillus*, and *Klebsiella* sp.

The table below shows that the disc diffusion method was used to test the antibacterial activity of silver and leaf extract at three different concentrations (10, 20, and 30 mL) against four different bacterial strains (*E. coli, Bacillus, Klebsiella*, and *Staphylococcus aureus*). Table 1 contains a summary of the test samples antibacterial activity evaluation. The outcomes showed that the three Ag and leaf extract concentrations had varying degrees of potential efficacy in preventing the growth of poisoning bacteria. *E. coli*, a gram-negative bacterium caused the highest zone of inhibition, whereas *Bacillus* sp., gram-positive bacteria caused least zone of inhibition.

The tests were run in triplicate throughout. In *in vitro* antibacterial activity, the silver nanoparticles prevent microbial growth.[19,20] The most successful sample at inhibiting microbial growth was AgNPs 20 mL. Pathogenic bacteria including *E. coli, Klebsiella, Bacillus*, and *Staphylococcus aureus* were examined in a 20 mL Ag solution with an inhibition range of 1.15-0.11, 1.22-0.31, 1.35-0.35, and 1.04-0.12. The evaluated materials' leaf extract and Ag 10 mL concentration displayed decreased antibacterial efficacy against the same four bacterial strains that cause poisoning.

DISCUSSION AND CONCLUSION

In the current work, bio-nanocomposite films were created using a green production of AgNps using guava leaf extract, which was discovered to be environmentally beneficial was used. Synthesized AgNP's were combined with mixtures of CMC and Pectin bio-polymers to create multifunctional bio-nanocomposite films. SEM, mechanical properties, WVTR activity, and antibacterial activity were used to characterize the produced nanocomposite films.

The SEM findings are compatible with data that have recently been published and illustrate the creation of spherical nanoparticles, and the bio composite films were shown in Figures 2-5 SEM pictures, and it was found that these films had Ag nanoparticles deposited on them. The increase (i.e., 7.53 MPa) for nanocomposite film containing 20 mL of Ag nanoparticles solution compared to film having 10 mL of Ag nanoparticles solution. In a film containing 30 mL of Ag nanoparticles solution, elongation at break is significantly increased. The barrier properties of the CMC, Pectin without silver nanoparticles film have been inferior to others. Lower WVTR rates are seen as the composite film's silver content increases. In comparison to the same four bacterial strains that cause poisoning, the leaf extract and Ag 10 mL concentration of the tested components showed diminished antibacterial effectiveness.

AgNP's have been proven to increases the mechanical, water vapour, and barrier characteristics of biopolymer films. Water Vapour Permeability (WVP) significantly decreased when Ag NP concentration increased. The UV-screened AgNP's nanocomposite films demonstrated effective antibacterial properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CMC: Carboxy methyl cellulose; **Ag:** Silver; **AgNP's:** Silver nanoparticles; **SEM:** Scanning electron microscope; **WVTR:** Water vapour transmission rate; **nm:** Nano meter; **NaOH:** Sodium hydroxide; **ME:** Machine elongation; **$AgNO_3$:** Silver nitrate.

SUMMARY

In the present research, the CMC and pectin nanocomposite films have been prepared with incorporation of silver nanoparticles. The films with increase in the concentration of silver NP'S, the crystals on the surface had increased (10 mL, 20 mL, and 30 mL). The nano composite films with Ag silver nanoparticles have shown improved mechanical and barrier properties significantly. To maintain food safety and increase the shelf-life of packaged foods, CMC and pectin with Ag NPs nanocomposite films are anticipated to have great promise.

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