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Preparation of Polypropylene/Silver Nanoparticles Nanocomposite Film and Evaluation of its Mechanical and Antimicrobial Properties w.r.t it's Use in Packaging Applications

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Abstract: Polypropylene/silver nanoparticles nanocomposite films were prepared by melt compounding method by using polypropylene pallets and silver nanoparticles powder. The physical properties of the virgin polypropylene film and nanocomposite films were evaluated by mechanical testing. The effect of various silver nanoparticles content in the polymer nanocomposites with respect to its antimicrobial efficacy against the Gram positive bacteria Escherichia coli and Gram positive bacteria Staphylococcus aureus were studied. Nanocomposite film containing higher percentage of silver nanoparticles loading showed 99.9 % efficacy against the bacteria as compared to virgin polypropylene film.

Keywords: Polypropylene, Silver Nanoparticles, Escherichia coli, Staphylococcus aureus, antimicrobial activity, packaging

I. INTRODUCTION

Polymer nanocomposite due to their inherent properties is comprehensively and ingeniously used in many fields, particularly in medical devices, health care products and food packaging applications. Infections are produced when people touch, eat or drink something that is contaminated with germs. Every day we use many materials including food packaging items for our daily need. These packaging items as infected by many harmful bacteria; antimicrobial packaging is thought to be a subset of active packaging. It is the promising technology which effectively impregnates the antimicrobial agent into the food packaging materials which subsequently delivers over the stipulated period of time to kill the pathogenic microorganisms affecting food products thereby increasing the shelf life of food materials. Therefore incorporation of antimicrobial agents into food packaging materials has received considerable attention [1]. An antimicrobial nanocomposite film is particularly attractive due to its suitable structural integrity. Materials in the range of nano scale have higher surface to volume ratio as compared to their micro scale counterparts. This allows nanoparticles to attach more in numbers to the microorganisms, which confers greater efficiency [2]. Nanoparticles are cluster of atoms in the atomic size in between 1nm to 100 nm. Nano is Greek word meaning extremely small of dwarf. The use of nanoparticles in composites is of gaining importance due to improvement in mechanical, optical and many chemical properties. The metallic nanoparticles like copper, zinc, gold, titanium, magnesium, alginate and silver have been used in polymer composites for various purposes but the use of silver nanoparticles have proven to be most effective as it has good antimicrobial efficacy against viruses, bacteria and eukaryotic micro-organisms and the antimicrobial film used for food packaging applications are mainly based on silver (Ag), which is well known for its strong toxicity to a wide range of pathogenic bacteria, fungi and microorganisms. Thanks to these broad-spectrum antibacterial properties, silver has comprehensively used for biomedical applications and environmental disinfection process for long years. It is still used in a variety of commercially available products such as composites with endure slow silver release and which act as preservatives, or products containing silver thisulphate complexes which are introduced in packaging plastics for long-lasting antimicrobial protection of packed products. Till now silver ions (Ag⁺) have confirmed to be helpful and proficient for antibacterial purposes. But for the last few years silver nanoparticles due to its unique properties represent a reasonable alternative for boosting the development of new bactericides. Recently, silver nanoparticles (Ag-NPs) based antimicrobial polymeric materials have attracted considerable research interest ([3]-[5]). The antibacterial effect of silver has used in a variety of commercial available products and medical devices for many years, either in salt from or in metallic form. Several research papers have reported that the antibacterial effect of Ag-NPs is largely related to its particle size (usually <100 nm), shape and particle dispersion within the polymer ([6]-[8]).



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Because of their high surface area to volume ration and their high active surface with highly active facets metal nanoparticles (MNPs) exhibit remarkable and outstanding properties. Further, it helps in increased catalytic activity. As Ag-NPs having very large surface area could be more reactive and become more anti-microbiologically active than the bulk counterpart. The mechanism of antimicrobial activity is due to interference of cell membrane with reactive oxygen and hydrogen ions in presence of silver nanoparticles leads to bacterial death. Silver nanoparticles are curbed within the cells and then release silver ions. The ions so released interrupt in the DNA replications and adenosine triphosphate (ATP) preparation. The mechanism so followed is called Trojan horse mechanism. Applications like respirators, household water filters, cutting boards, cell phones, laptop keyboards , contra-conceptive, cosmetics, detergents, children's toys are typical products currently in the global market that exploit the antimicrobial properties of silver nanoparticles. But, even if the use of Ag-NPs seems to unwrap a new window of promises in the advancement of new age antibacterial agent, major concern of health issues and environmental safety risks may also considered. Accordingly the use of Ag-NPs in various applications has come under scrutiny by both private and public enterprises and government institutions. Metallic Ag is considered to be a non-reactive material but in this physical state it can chemically combine with moisture and getting ionized to form highly reactive silver ions (Ag⁺) ([5], [9]). The Ag⁺ ions can attach to negatively charged components in proteins and nucleic acids, causing structural variations in the cell membranes. Many antimicrobial polymer nanocomposite films have been prepared by melt mixing method ([10]-[12]). Literature review reveals the preparation of nanocomposite films incorporated with Ag-NPs with various polymers like polypropylene (PP), polyurethane, polyester, polyamide and polyacrylate by melt compounding technique ([12]-[17]). This melt compounding method is the most efficient method for the preparation of nanocomposite film for antimicrobial applications, compared to other methods like conventional deposition of metallic particles directly on the surface of the substrate, solution blending and vapour coating.

Polypropylene-silver nanoparticles (PP/Ag-NPs) nanocomposite film with low release potential of Ag⁺ ion shows outstanding long term antimicrobial activity. It has been confirmed that the prolonged and steady release of Ag⁺ ions in the aqueous atmosphere is the reason behind its antimicrobial activity. Ag-NPs can progress the water penetration characteristics of the nanocomposite film either by generating some additional voids within the nanocomposite film or dropping the crystallinity of the same to permit the access of more water molecules. In the present investigation, PP/Ag-NPs nanocomposite films were prepared at the different weight percentage of Ag-NPs as filler and polypropylene grafted maleic anhydride (PP-g-MAH) as compatibilizer by the melt blending method. The synergetic effect on mechanical, thermal, rheological and morphological properties has been comprehensively studied. The potential use of the prepared nanocomposite film for food packaging applications were evaluated by investigating the gas barrier and antimicrobial properties.

II. MATERIALS AND METHODS

The PP homopolymer pellets (Repol H100EY) of density 0.96 and Melt Flow Index 11g/10min (230°C/2.16 kg, ASTM D 1238) supplied by Reliance Polymers was used in this work which meets FDA requirements for all food contact and cooking application in the Code of Federal Regulations in 21 CFR 177.1520. Maleic anhydride (MAH) grafted into PP was used as a compatibilizer between nanofillers and matrices. The commercial grade Ag-NPs used in this experiment is of 99.9% purity and it has average particle size ranging from 50-80 nm with specific surface area 5.37m²/gm. The Ag-NPs were fine powder with bulk density of 0.312 gm/cm³ and true density of 10.5 g/cm³. It has spherical morphology with cubic crystallographic structure. It was purchased from Nanoshel, Intelligent Materials Pvt. Ltd., Panchkula, Haryana, India.

A. Preparation of PP/PP-g-MAH/Ag-NPs nanocomposite film

Prior to extrusion, the Ag-NPs powder was dried in a vacuum chamber oven at 80°C for 12 hours to remove the absorbed moisture. Three PP master batches were prepared containing PP and Ag-NPs along with virgin PP master batch. The composition of each master batch containing Ag-NPs was in the range of 0.5%, 1%, and 2% by weight without using compatibilizer. Table-1 represents the master batch composition of different samples. Nanocomposite films were prepared by melt mixing of the two components using Torque rheometer (Haake Rheomix OS, Germany) with counter rotating roller rotors having a chamber size of 66 cm³. The screw speed was 100 rpm and the mixing time was 15 minutes for all the compositions. The barrel temperature profile was optimized from 175°C to 190°C from feed to die zone. After compounding, the mixed material was extruded with the help of blown film extrusion set up to produce cylindrical films. The mechanical test was performed to select the optimum filler loading. The optimized composition of the master batch was further mixed with different wt. % of PP-g-MAH as compatibilizer. The mixture was extruded after passing through twin screw extruder to homogenize the mixture thoroughly to get the pellets. The prepared pellets were further processed to obtain the desired film samples with the help of blown film extrusion set up for property evaluation.

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Table-1 Composition of developed PP/Ag-NPs nanocomposite films

	Weight %		
Sample	PP	PP-g-MAH	Ag-NPs
Virgin PP	100	0	0
PP/Ag-NPs	99.5	0	0.5
PP/Ag-NPs	99	0	1
PP/Ag-NPs	98	0	2
PP/PP-g-MAH/Ag-NPs	96	3	1
PP/ PP-g-MAH /Ag-NPs	94	5	1
PP/ PP-g-MAH /Ag-NPs	92	7	1

III. TESTING METHODS

A. Mechanical Properties

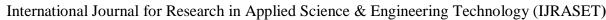
Mechanical properties of the nanocomposite were measured according to ASTM D-638 standard using a Universal Testing Machine (3882 Instron, UK). Test specimens were moulded in a size of 3.18 mm (width), 63.66 mm (length) and 3.00 mm (thickness) with a gauge length of 12.5 mm. Tensile strength and tensile modulus have been recorded at a crosshead speed of 50 mm/min for the virgin PP as well as for the PP/PP-g-MAH/Ag-NPs nanocomposite films. For each treatment process, five replicated specimens were tested and the average values were reported for analysis.

- B. Measurement of Antibacterial Properties
- 1) Shake flask method: Shake flask method was for quantitative assessment of bacterial reduction, which follows the test standard of ASTM E-2149. Film samples of 5 x 5 cm² in size were used in this method. Nutrient broth (NB) was used as a growing medium for Gram negative bacteria Escherichia coli and Gram positive bacteria Staphylococcus aureus. Peptone solution (prepared by 1 g/L peptone, pH 6.8-7.2) was chosen as a testing medium. In this work, the bacteria were cultivated in 5 mL of NB at 37°C for 24 h. The film samples and initial suspended bacteria of 10⁸ CFU/ mL were placed into a 250 mL flask with 50 mL of peptone solution. The flask was shaken on a reciprocal shaker at a speed of 100 rpm at 37°C 6±0.5°C at various contact times (30, 90, and 150 min). Where R is the reduction of bacteria (%), A is average number of bacterial colonies from thermoplastics without silver nanoparticles colloid (CFU/ml); B is average number of bacterial colonies from thermoplastics incorporated with nano-silver colloid (CFU/ml).
- Viable cell count method: Two typical food pathogens including one Gram-positive bacteria, Staphylococcus aureus ATCC-14458, and one Gram-negative bacteria, Escherichia coli ATCC-11775 were used to test the antimicrobial activity of PP/PP-g-MAH/Ag-NPs nanocomposite films using viable cell count method. Film samples were cut into square pieces (10 x10 cm) and placed in individual sterile flasks. The Gram-positive and Gram-negative bacteria were separately incubated in BHI (Difco Lab) broth and in TS (Difco Lab) broth at 30°C respectively under aerobic conditions for 16 h. Each 100 mL of the prepared inoculums with the 1/10th diluted broth was aseptically added to the flasks containing the test films to obtain inoculate of approximately 1.1–2.6x10⁻⁷ colony forming units (CFU)/ml. The flasks were incubated using an orbital shaker and rotated at 50 rpm and 30°C. Aliquots of 0.1 ml cell suspension were periodically taken from the flasks, diluted serially and plated on BHI agar for the Gram-positive bacteria cells, or on TS agar for the Gram-negative bacteria cells. The plates were incubated aerobically for 2 days at 37°C for BHI agar, and at 30°C for TS agar medium. Each experiment was performed in triplicates and the results were reported as the mean values in CFU/ml.

IV. RESULTS AND DISCUSSIONS

A. Mechanical Properties

Mechanical properties of virgin PP and its nanocomposite film samples are shown in table-2. Incorporation of Ag-NPs within the PP matrix increases the tensile properties of the nanocomposite film dramatically. With 1 wt. % loading of Ag-NPs tensile strength of the nanocomposite film increases by 51.43 % and modulus by 65.85 % as compared to virgin PP film. This can be attributed to the homogeneous dispersion of Ag-NPs at the interface of PP matrix which results in better stress transfer from nanoparticles to matrix.





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It was also observed that at 2 wt. % Ag-NPs loading, there was decrease in strength and modulus by 8.83 % and 7.42 % in comparison to PP containing 1 wt. % Ag-NPs due to the higher percentage of Ag-NPs loading which creates cavities in the polymer matrix because of the de-bonding of the polymer from the surface of the nanoparticles [18]. As tensile modulus expresses the stiffness of the material, the insertion of the polymer chain into the Ag-NPs layer leads to increase in the surface area of interaction between Ag-NPs and polymer matrix, thus causing enhancement of strength and modulus. The strength and modulus of virgin PP has been increased with the incorporation of 5 wt. % of the compatibilizer in PP containing 1.0 wt % Ag-NPs. The crystal orientations were mainly depending on the size and aspect ratio of Ag-NPs in the presence of PP-g-MAH. It is seen that the presence of the PP-g-MAH improves the interface adhesion between PP matrix and the Ag-NPs. Therefore, the improvement in strength and modulus is related to better dispersion of Ag-NPs in PP matrix in the presence of compatibilizer. Similar results have also been reported earlier ([19], [20]). Further it is ascertained from the table-1 that there is a decrease in tensile strength for the nanocomposite film containing 7.0 wt. % loading of compatibilizer and 1.0 wt. % concentration of Ag-NPs. This is because of slippage of polymer chains at the inter face of the polymer matrix site due to the presence of the higher percentage of compatibilizer content as reported by many workers ([21],[22]).

Table- 2
Mechanical properties of virgin PP and PP/Ag-NPs nanocomposite films at different wt. % of Ag-NPs and compatibilizer content

1	<u> </u>
Tensile strength (MPa)	Tensile modulus (MPa)
19.13 ±0.95	495.04 ±24.7
22.33 ±1.1	510.12 ±25.5
28.97 ±1.4	820.99 ±41
26.41 ±1.3	760.17 ±38
27.21 ±1.3	768.31 ±38.4
31.21 ±1.5	910.23 ±45.5
26.32 ±1.3	680.12 34
	(MPa) 19.13 ±0.95 22.33 ±1.1 28.97 ±1.4 26.41 ±1.3 27.21 ±1.3 31.21 ±1.5

B. Antimicrobial Properties

Quantitative assessment has been made to measure the effects of Ag-NPs introduction into the PP matrix by the viable cell count technique using Gram-negative bacteria *Escherichia coli* ATCC 6538 and Gram-positive bacteria *Staphylococcus aureus* ATCC 8379. Table 3 shows the results of antimicrobial evaluation of the samples against the bacteria Escherichia *coli*. It shows that virgin PP and PP with higher concentration of silver nanoparticles did not have much antimicrobial effects. Similarly Table 4 shows the data relating to antimicrobial evaluation of the samples against the bacteria Staphylococcus *aureus*.

Table-3

Antimicrobial evaluation of the samples against Escherichia coli by Viable Cell Count method

Sample	Viable Cell Counts Log(CFU/ml)			
	Contact time (30 mins.)	Contact time (90 mins.)	Contact time (150 mins.)	Result interpretation
Virgin PP	5.77	6.26	6.34	Increasing trend
PP/0.5% Ag-Nps	4.95	5.65	5.79	
PP/1% Ag-Nps	5.0	5.69	5.83	
PP/2% Ag-Nps	5.17	5.71	5.84	

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Table-4
Antimicrobial evaluation of the samples against Staphylococcus aureus by Viable Cell Count method

		1 0 17	-		
Sample	Viable Cell Counts Log(CFU/ml)				
	Contact time	Contact time	Contact time	Result interpretation	
	(30 mins.)	(90 mins.)	(150 mins.)		
Virgin PP	5.65	6.2	6.46		
PP/0.5% Ag-Nps	4.77	5.38	5.68	Increasing trend	
PP/1% Ag-Nps	4.84	5.30	5.66		
PP/2% Ag-Nps	4.96	5.25	5.62		

Figure-1 and 2 shows the viable colony count of *Escherichia coli* and *Staphylococcus aureus* for virgin PP film under a wide range of contact times (30 min, 90 min and 150 min). It was seen that the virgin film did not inhibit the growth of pathogenic bacteria; whereas nanocomposite films with varied concentration (0.5-2 wt. %) exhibited potent antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Further, at different contact time. Virgin PP and nanocomposite film showed the varying bactericidal efficacy against the tested bacteria ([23]-[25]). It was seen that the growth of *Escherichia coli* and *Staphylococcus aureus* increase with increasing contact time. As a result viable cell count increases. This has suggested that the virgin PP film did not show bactericidal behaviour. During the initial period of contact time the number of colony forming units (CFU/ml) was less for *Staphylococcus aureus* as compared to *Escherichia coli* for the virgin PP film ([26]-[29]). However, it was also experimented that there has been increasing magnitude of *Staphylococcus aureus* bacterial growth with increasing contact time in comparison to *Escherichia coli*. The mechanism behind such behaviour is that positively charged silver ions can interact with negatively charged bio-macromolecular component present inside the bacteria causing cell malfunction [30].

Further, the same figure-1 and 2 also show the viable cell colony count for Escherichia coli and Staphylococcus aureus for the Ag-NPs loaded nanocomposite films at various contact times. The results were compared with the virgin sample. It was found that the viable cell counts against the tested bacteria significantly decreased with incorporation of Ag-NPs within the PP matrix. It reveals that Ag-NPs act as antimicrobial agents inhibiting the growth of bacteria Escherichia coli and Staphylococcus aureus. It is well known that Ag⁺ hinders DNA replication and inhibits the working nature of ribosomal proteins and enzymes for ATP hydrolysis.²⁸ Must likely Ag-NPs play the same mechanism as Ag⁺ion, which create redox imbalance causing extensive bacterial death. Further, the large surface area of the nanoparticles makes this action more effective. It is shown that release of silver ion is controlled by an oxidation mechanism at the surface of the nanoparticles [29]. One unpublished study tracked tagged silver ions, indicating an absence of silver within the bacterial cells and pointed out that, only the surface -S-H groups were affected [30]. This could explain why only bacteria and viruses are affected by silver ions, as opposed to mammalian cells that do not have exterior sulphydryl groups. Silver ions may not be capable of permeating through cell membranes to react with the interior -S-H groups, rendering silver relatively nontoxic to humans and animals. The viable cell count increases with the increase in contact time for all the Ag-NPs loaded nanocomposite film. This effect was noticed for both Staphylococcus aureus and Escherichia coli. But the effect is more pronounced in case of Staphylococcus aureus ([22]-[26]). But with increase in Ag-NPs content, the number of surviving bacterial colonies decreases, which is related to the increasing number of Ag-NPs present per unit surface area within the polymer matrix. The result also shows that the nanocomposite film prepared with low concentration (0.5-1 wt. %) of Ag-NPs exhibits significant antimicrobial behaviour. As reported in literature, PP nanocomposite film with (10-25 wt. %) Ag-NPs and zeolites (acting as channels), require longer contact time (48 days) to reach efficient antimicrobial activity.³¹ Moreover, the PP nanocomposite film filled with copper nanoparticles prepared by Delgado et al reported that with concentration higher than 5 v/v % (equivalent to 30 w/w %) showed stronger antimicrobial properties than samples with lower filler concentration [32].

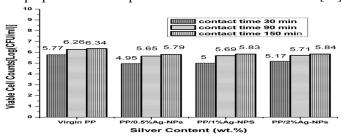


Fig. 1 Viable cell count for virgin PP and PP /Ag-NPs nanocomposite film with different Ag-NPs content at various contact times against *Escherichia coli*

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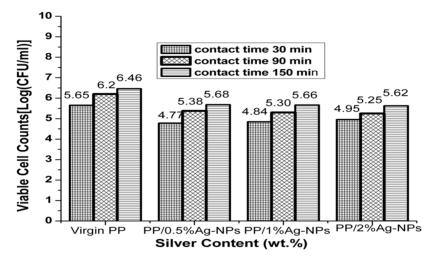


Fig. 2 Viable cell count for virgin PP and PP/Ag-NPs nanocomposite film at various contact times against Staphylococcus aureus

It was revealed that the viable cell count increased to higher value for nanocomposite film with higher concentration of Ag-NPs with the increase in contact time against the bacteria *Escherichia coli*, but the same showed a decreasing trend in case of the bacteria *Staphylococcus aureus* except for the contact time of 30 min. The dispersion of Ag-NPs in the nanocomposite film is the reason behind showing antimicrobial activity and efficacy. Higher the concentration of Ag-NPs in nanocomposite films appearance of particle clusters were seen in the composite which give rise to larger agglomerations of Ag-NPs. These agglomerations were believed to worsen the antibacterial performance [33]. The claim was in line with the work done by Dowling and co-workers [34]. They revealed that silver with larger particle size showed the lower antibacterial performance.

Figure-3 and 4 show the percentage of bacterial reduction shown by virgin PP film and PP/Ag-NPs nanocomposite films at different contact times *against Escherichia coli* and *Staphylococcus aureus*. It was observed the percentage reduction of *Escherichia coli* and *Staphylococcus aureus* increased with the increase in the concentration of Ag-NPs loading in the polymer matrix. Further, the percentage of bacterial reduction is outstanding in case of 0.5 wt. % and 1 wt. % loaded PP/Ag-NPs nanocomposite film due to higher degree dispersion of Ag-NPs particles against *Escherichia coli* and *Staphylococcus aureus*.

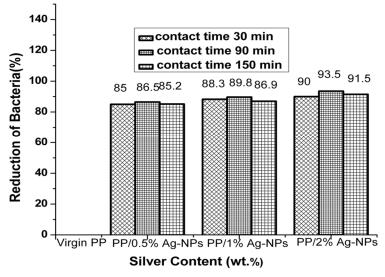


Fig. 3 Percentage reduction of Escherichia coli for Ag-NPs containing PP nanocomposite films at various contact times

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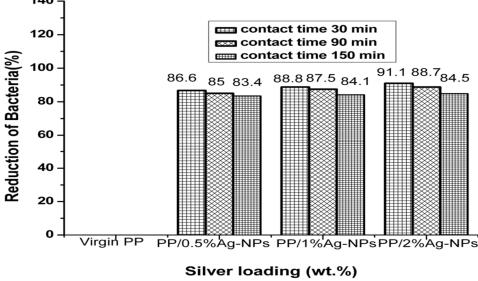


Fig. 4 Percentage reduction of Staphylococcus aureus for Ag-NPs containing PP nanocomposite films at various contact times

In order to evaluate the antimicrobial efficacy of the PP/Ag-NPs nanocomposite film containing different loading of Ag-NPs viable cell count method was performed. The rate of bacterial reduction or bacterial survival (% viability) was determined as a function of elapsed time during 6, 10 and 24 h period. The results of the antimicrobial properties are shown in Figure-18 for *Escherichia coli* bacteria. It is noted that after a 24 hour period 86% of the bacterial colony had died. The results indicated that a low proportion of Ag-NPs (2 wt. %) in the nanocomposite film makes it effective against the bacteria. The similar result was observed by Morones et al. reported that small size Ag-NPs may pass through cell membranes generating cell breakdown [35]. It can be concluded that all the nanocomposite film prepared are effective against *Escherichia coli* and *Staphylococcus aureus*, with clear dependence on Ag-NPs concentration and on contact time. The similar observation was also obtained for *Staphylococcus aureus* (Figure-6). It was seen that the effect of bacterial reduction is slightly less up to 83 %.The number of the surviving bacterial colonies decreases with increasing filler content. Figure-5 also shows the survival bacteria (*Escherichia coli*) after 24 h of surface exposure for PP/Ag-NPs nanocomposite film containing 0.5 wt. %, 1 wt. % and 2 wt. % of Ag-NPs, and their comparison with virgin PP. It is seen that the best results were obtained with the higher (2 wt. %) amount of Ag-NPs, showing its efficacy against *Escherichia coli* (86% bacteria killed).

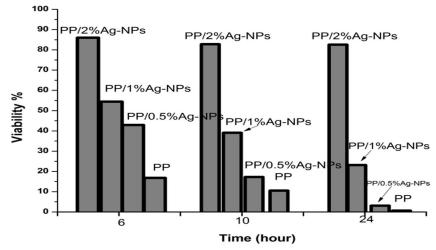


Fig. 5 Percentage of survival of *Escherichia coli* bacteria (viability %) after 6, 10 and 24 hour for virgin PP and PP/Ag-NPs nanocomposite films



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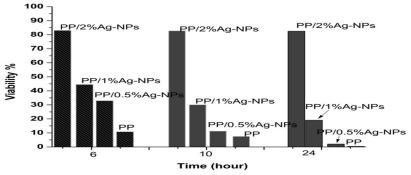


Fig. 6 Percentage of survival of Staphylococcus aureus bacteria (viability %) after 6, 10 and 24 hour for virgin PP and PP/Ag-NPs nanocomposite films

V. CONCLUSION

Virgin PP film and different wt. % of Ag-NPs loaded nanocomposite films have been prepared using the melt intercalation method and their mechanical, thermal, rheological, morphological, barrier and antimicrobial properties have been investigated. Incorporation of Ag-NPs into the PP matrix resulted in the significant increase in the tensile strength and modulus of the virgin PP. Among the PP/Ag-NPs nanocomposite films, the sample containing 1 wt. % Ag-NPs exhibited maximum tensile strength and modulus, which can be explained due to increased Ag-NPs per surface area of contact with the virgin matrix. Further increase in Ag-NPs showed the reduction in tensile properties as compared to 1 wt. % loaded Ag-NPs filled nanocomposite film. The presence of compatibilizer helped the batter dispersion of Ag-NPs in the nanocomposite structure and increased the tensile properties of the virgin PP. The PP/Ag-NPs nanocomposite films showed outstanding antimicrobial efficacy as documented by the percentage of the viable count reduction of the growth of Escherichia coli and Staphylococcus aureus. The virgin PP film did not inhibit the bacterial growth. However, the degree of antibacterial activity of the nanocomposite films was dependent on the percentage of Ag-NPs loading within the polymer matrix. Among all the films, the PP/PP-g-MAH/Ag-NPs (94:5:1) nanocomposite film showed a strong bactericidal effect against both Escherichia coli and Staphylococcus aureus. This is because of accumulation of Ag-NPs accumulate in the bacterial cytoplasmic membrane causing a significant increase in membrane permeability and leading to cell death. Hence, PP/Ag-NPs nanocomposite film with strong antimicrobial activity against both Gram-positive and Gram-negative bacteria may have good potential for using as antimicrobial food packaging materials

VI. ACKNOWLEDGEMENT

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