POLYVINYL ALCOHOL - MELAMINE FORMALDEHYDE RESIN COMPOSITE AND NANOCOMPOSITES WITH Ag,TiO_2, ZnO NANOPARTICLES AS ANTIMICROBIAL FILMS, COATINGS AND SPRAYS

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Keywords: PVA-MFR composite films; Nanocomposites films, Nanoparticles of Ag, ZnO, TiO_2; Characterization; Antimicrobial activity

Melamine formaldehyde resin, MFR, is generally formed in a two stage process by the reaction of melamine and formaldehyde. The first stage reaction is carried out at about 70° - 80°C and pH 9-10; and the second stage involves subsequent polycondensation of the products in an acid medium. In this work, reaction is carried out till the first stage only. Thus, prepared MFR when blended in small proportion into PVA matrix, in-solubilises PVA, forming a well defined PVA-MFR composite. The blend can be cast into films of desired thickness and strength. Nanoparticles of Ag, ZnO and TiO_2 were prepared and characterised. Each of the prepared nanoparticles was first blended into aqueous PVA, then blended with MFR, and nanocomposite films obtained. The films were subjected to TGA, FTIR and antimicrobial studies. The PVA-MFR composite films are found to have a high level of antimicrobial activity. Nanocomposites have enhanced antimicrobial activity due to antimicrobial property of nano Ag/ZnO/TiO_2 in them. The activity is high, especially against highly resistant gram positive bacteria like *Staphylococcus Aureus and Bacillus*. The composite has good binding properties, forming stable, chemically resistant, coatings on fabric, paper, glass, polyester etc, rendering them antimicrobial. These films and coatings retain their antimicrobial activity over long period of time. Nano silver immobilised on antimicrobial PVA-MFR, could probably be an effective patch for wound dressings, and surgical mask, which are in great demand. A fine coating of the composite on medical devices can decrease the incidence of medical device related infections.

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INTRODUCTION

There has been a growing interest in developing polymeric materials with antimicrobial properties for biomedical, food packaging and storage applications. [1] As most of the types of commonly applied polymers have no antibacterial action, they have to be modified to obtain polymer materials with desired properties.[2] The modification of virgin polymer with a bioactive agent is a possible method where the polymer is a carrier, providing transport and controlled release of bioactive substances into the environment where they are needed.[3-5] Numerous methods have also been used to develop polymeric materials[6] with inherent antimicrobial properties. Poly (vinyl alcohol), PVA, belongs to the group of polymers which can be used in combination with other non-biodegradable polymers for tuning the required properties in the resultant composite blend. It is one of the synthetic, biodegradable, biocompatible, water-soluble polymers utilized in medical applications such as wound dressings,[7] artificial skin,[8] coatings,[9] Transdermal patches,[10] cardiovascular devices,[11-14] and drug delivery systems.[15] Moreover, it has good barrier properties against scents, oils, and fats. The physical characteristics of PVA are dependent on its method of preparation by hydrolysis or partial hydrolysis of Poly (vinyl acetate).[16] PVA complexed with iodine to get polymeric broad spectrum coatings working on the principle of slow release of incorporated iodine have been reported in U.S. Pat. No.5,071,648.[17,18] The patent also discloses effective antimicrobial polyvinyl acetal sponge wipes and coatings for non-wiping applications. Our emphasis is towards achieving water insoluble PVA films that show characteristic antimicrobial application which can help reduce complications, caused by bacteria commonly found in households, and resistant microbes acquired from hospitals. There are reports[20-22] of attempt, to in-solubilise PVA by treatment with sulphuric acid, formaldehyde, borax, boric acid, glutarldehyde. Other methods in the literature report highly in-solubilised polyamide epoxy cured PVA coating; methylated melamine formaldehyde cured PVA coating, ammonium chloride/formaldehyde cured PVA and in solubilised starch/PVA mixes.

We herein report in-solubilisation of PVA by reaction with Melamine-formaldehyde, MFR. The two component system (PVA-MFR) form well defined molecular composite with antimicrobial properties. The blend can be cast into antimicrobial films of desirable thickness and strength. The

composite has been found to have good binding properties on fabric, paper, glass, polyester etc and thus can render them antimicrobial. MFR a hard thermosetting plastic was prepared from melamine and formaldehyde. Gong and Zhang[19] have reported mechanical properties of MFR –PVA composites, where the matrix is MFR and effect of varying composition of PVA on the resulting composite is studied. In contrast, in the present study, MFR is blended in smaller proportion into PVA matrix to get water insoluble and chemically resistant composite films with antimicrobial properties.

Silver has been used as an antimicrobial since the 1800s. But since the discovery of systemic antibiotics in the early 20th century, the use of silver had declined. In the last two decades, with advent of nanotechnology, interest in silver for wound treatment resurged, since silver is efficacious for killing the antibiotic resistant bacteria strains. Silver releasing dressings and patches are increasingly in demand for treatment of infected wounds.[23] Along with Silver, ZnO and TiO_2 nanoparticles also have high level of antimicrobial activity and have been included in this study. Nanocomposites of PVA-MFR with ZnO/TiO_2/Ag as films and coatings that promise to be good polymeric material with wide range of antimicrobial applications are being reported. This gives us the advantage of using lesser concentration of nanoparticles in an inherent antimicrobial material PVA-MFR, so as to reach a desired level of antimicrobial efficacy, yet minimizing possibility of any toxicity by using lower concentrations of nanoparticles.[24] Bacterial infection from medical devices is a major problem in hospitals,[25] and so these polymeric antimicrobial coatings could probably be exploited to modify the surface of medical devices.

EXPERIMENTAL

Materials and Methods

Materials - Silver nitrate (extra pure-grade), Tri sodium citrate dihydrate (AR grade), Titanium trichloride (LR grade), Polyvinyl alcohol M.W. 85,000-1,24,000 (LR grade), Zinc acetate (LR grade), and Formaldehyde (37%w/v, LR) were purchased from S.D. Fine Chemicals Limited, Mumbai. Starch from Merck, Mumbai. Melamine powder (AR grade) was purchased from Gujarat Natural Fertilisers Limited (GNFC). All the solutions were made by using double distilled water. Nutrient broth and Nutrient agar was purchased from Himedia laboratories, Mumbai.

Characterization - TEM samples were prepared by the placement of the sample mixture drops directly on Formvar polymer-coated grids with a micropipette. The morphology, size and shape distribution of the nanoparticles were recorded with a TECNAI FE12 TEM (Eindhoven, The Netherlands) instrument operating at 120 kV. All TGA thermograms were recorded on TGA/SDTA Mettler Toledo 851^e system (Zurich, Switzerland) using open alumina crucibles containing samples weighing about 8–10 mg with a linear heating rate of 10 °C min^{-1} between 25 to 800 °C. Nitrogen was used as purge gas for all these measurements. UV–Vis spectra were recorded on Systronics model 2201.

Antimicrobial studies

Disc diffusion method - Antimicrobial studies of PVA-MFR composite and PVA- MFR nanocomposite films were conducted by Kirby-Bauer disc diffusion method.[26] The nutrient agar media autoclaved at 15 lbs for 10 min and was poured in the petri dishes and allowed to solidify. 0.1ml of the innoculum of overnight cultures of the test organisms used such as *E.coli, Staphylococcus Aureus, Pseudomonas, Proteus, and Bacillus* was plated by spread plate technique on the nutrient agar plates. Different polymer composite films were cut into small pieces and were placed in their respective positions with the help of a sterile forceps onto the nutrient agar plates. The films were pressed gently with forceps to ensure contact with the agar surface. The plates were incubated at 37° C for 24 hours and later checked for antimicrobial activity by measuring zone diameter of inhibition.

Optical density method - Nanocomposite coatings inside glass beakers were subjected to turbidity method for their antimicrobial studies. Glass beakers coated with the nanocomposites were incubated with 20 ml of nutrient broth and 0.1 ml of microbial culture for 24 h at 37ºC. After incubation the media from the above beakers was taken under sterile conditions and absorbance measured turbidi metrically in a spectrophotometer at 450 nm.

Preparation of Nanoparticles

Zinc oxide Nanoparticles were prepared using 20 ml of 0.2 M Zinc acetate in dimethylsulphoxide[27] and stirred in a typical chemical reactor for 30 min. To this 1.2 M KOH in 10 ml ethanol was added drop by drop. Solution was stirred for 5 min and then 0.12 ml thioglycerol was added. Thioglycerol acts as a capping reagent and has been used to prevent the agglomeration of nanoZnO particles. The solution was stirred for 1 h and it turned milky white. The residue was collected and washed three times with methanol and allowed to dry on a Petri dish.

Titanium dioxide nanoparticles were prepared by mixing 20 ml of $TiCl_3$ solution with 60 ml of 0.1 M ammonium hydroxide solution and stirred for 48 h at room temperature on a magnetic stirrer[28]. A white coloured solution indicates the formation of titanium dioxide particles. The solution was centrifuged and the precipitate was washed with double distilled water, dried in isopropyl alcohol at room temperature. Organic molecules 'cap' the outer surface of core semiconductor[25] and prevent aggregation, oxidation, and also stabilize nanoparticles

Silver nanoparticles synthesis was carried with silver nitrate, starch and sodium citrate by following the procedure as reported in our earlier work.[29] 50 ml of 0.008M $AgNO_3$ was stirred for 15 minutes under reflux condition with a magnetic stirrer (Spinot Model MC_02), followed by addition of a solution of 200 mg starch powder dissolved in 100 ml of double distilled water, and then 50 ml of 0.08 M Sodium citrate solution was added, under continuous stirring and heating for 2 hours at 95ºC. The silver content of this solution is $2x10^{-6}$ mol mL^{-1}.

Preparation of Melamine Formaldehyde Resin (MFR)

These are primarily oligomers and are formed in a two stage process by melamine – formaldehyde reaction with a 1: (2–12) molar ratio of melamine to formaldehyde. The first stage reaction is carried out at about 70° - 80°C and pH 9-10; and the second stage involves subsequent polycondensation of the products in an acid medium. In our study the reaction has been carried to the first stage only. Melamine-formaldehyde used in the study is prepared by well-known industrial method, [30] where formaldehyde and melamine are reacted under base catalyst and it's polymeric molecular weight increased by addition process. 57g of (37%) formaldehyde was taken in a double necked RB flask and brought to pH 9.5-10 by the addition of few drops of 2N -NaOH solution. The RB flask was kept on a magnetic stirrer and 50g of melamine powder slowly added under stirring followed by 15ml of distilled water. The reaction mixture was heated with continuous stirring till the temperature increased to about 60°C and then gradually allowed the temperature to rise to about 95°C. Refluxation and stirring at this temperature was continued till a clear liquid was obtained. Further heating was carried while checking the water tolerance of the reaction mixture after every 10 min., until the water tolerance at 30°C dropped to 1:4. The mixture was allowed to cool to room temperature. Final properties of the resin like viscosity and water tolerance[31] were standardized to viscosity @ 32°C is 30 - 40 seconds; water tolerance @ 30°C is 1:2 to 1:4, and gel time @ 150°C is 210-230 sec. Under these conditions the reaction mixture is partially cured, and is a clear viscous liquid with the shelf life of 4-5 days at room temperature and about a month when stored in refrigerator. The prepared resin mixture however can be diluted with methanol if necessary for storage at room temperature.

Preparation of polymer films/coatings and sprays

PVA films - A very simple method was used to cast thin PVA polymer film.[32] A homogenous solution of PVA powder M.W.85,000 - 1,24,000 (10 wt %) in water as plasticizer was prepared under stirring and heated to 100 °C for about 30 minutes. Hot aqueous homogenous solution was poured on to a plastic Petri dish and spread uniformly with a glass rod and dried in a hot air oven at 70°C. Film could not be obtained for compositions below 5 wt % of PVA. The PVA films dried in a hot air oven at 70°C for 2 hrs could be easily lifted and stored in Ziploc pouches.

PVA-MFR composites films/coatings/sprays - Various methods of synthesizing PVA-MFR are well documented in US 4461858 Patent[16-18] wherein the material is primarily prepared to be used in paper industry as a binder as well as to improve the strength of paper because of its high absorption and binding capacity onto cellulose. The prepared PVA and melamine formaldehyde solutions were blended in various proportions as listed in Table.1, by using an electrical blender while heating at about 80°C. The PVA-MFR films with labels J1 to J8 were prepared by blending 20 ml of 10 wt% polyvinyl alcohol (PVA) and different volumes of melamine formaldehyde resin(MFR) i.e., 1 ml(J1), 1.5 ml(J2), 2 ml(J3), 2.5 ml(J4), 3 ml(J5), 3.5 ml(J6), 4 ml(J7) and 6 ml(J8).

Table 1. PVA–MFR films prepared with different composition by volume of MFR added to 20 ml of 10 wt.% aqueous PVA solution

Film label	J1	J2	J3	J4	J5	J6	J7	J8
MFR added in ml	1	1.5	2	2.5	3	3.5	4	6

The reaction mixture changed from colourless to white and its viscosity increased as the reaction proceeded. To cast a film the PVA-MFR - active blend was poured and spread uniformly on a plastic Petri dish before it forms a thick ball like mass, while for sprays and storage applications the blend was immediately diluted with methanol to the desired consistency and stored in air tight containers. The films were dried in hot air oven at about 70 – 80°C for 2 hours. After evaporation of the solvent at ambient temperature, film was peeled off and rinsed in benzene followed by water to remove any volatile unreacted materials and dried in oven and stored in Ziploc bags. It was noted that cross linking of PVA begins with even the smallest addition of MFR. As the concentration of MFR was increased the films were harder to tear and water resistance of the film increased. The opacity of the films obtained changed from transparent colourless to white opaque films with reduced elasticity as the composition changed from J1 to J8.

Active blend as prepared above was coated on paper strips, cloth strips, inner surfaces of plastic as well as glass beakers, and earthen clay pots each one dried in oven as described above. The glass beakers and clay pots had to be heated overnight to get hardened inner lining. The lining so prepared on a plastic beaker could be dislodged as a moulded cup. The paper strips had greater tear strength in one direction and retained shape even in boiling water. At the same time the PVA-MFR–active blend obtained was diluted four times with methanol and sprayed on food grade Aluminum foil and food wrapping films and dried by suspending freely in hot air oven for two hours to get thin antimicrobial coating on them.

Preparation of PVA–MFR nanocomposite films/coating and sprays - Repeat experiments showed that it is possible to get PVA–MFR composite films of desired thickness and strength and these films are antimicrobial. Synthesised ZnO, Ag, or TiO_2 nanoparticles were first blended into aqueous PVA and then allowed to condense with MFR. PVA–MFR-silver composite films were obtained using 20 ml of as prepared nano silver solution (silver content of 2 x 10^{-6} moles/ml or 2 milli moles/lit). 2g PVA powder was added directly and dissolved by heating over a water bath with constant stirring ensuring a homogeneous solution. To this 2 ml MFR was added and blended and films were prepared by the method similar to that of PVA-MFR composite films and about 100 sq.cm of the film were obtained. Thus, the film had 4 x 10^{-7} moles of silver/sq.cm of film area. These silver nanocomposite films obtained was brownish in colour. Similar nanocomposite films were obtained by incorporating TiO_2 or ZnO nanoparticles, the difference being the prepared nanoparticle powder was first dispersed in water using a sonicator, followed by addition of PVA and MFR in same proportion and following the same method as for nanosilver composites. The concentration of ZnO and TiO_2 in the composite was 25 mmol/lit of each. Nanocomposite sprays and coatings were made in the same manner as the corresponding composites.

Reactivity of the films - PVA-MFR-polymeric films were found to be un-reactive towards solvents like boiling water, dilute acids, sodium hydroxide, benzene, chloroform, methanol, ethanol and hexane etc., though it disintegrated on heating in concentrated hydrochloric acid and charred in sulphuric acid. The material is thus very stable towards various applications.

Results and discussion

FTIR studies of pure PVA and crosslinked PVA-MFR films

The interaction of PVA-MFR was indicated by the FTIR spectral data[33] as reported, where the disappearance of 1000 cm^{-1} peak for methylol ($-CH_2OH$) group in MFR indicating its reaction with –OH group of PVA leading to the formation of C-O-C linkage between PVA and MFR.

This is further supported by the suppression of –OH absorption band at 830 cm^{-1} in PVA suggesting reaction of some of the free –OH groups of PVA with pre-polymerised MFR.

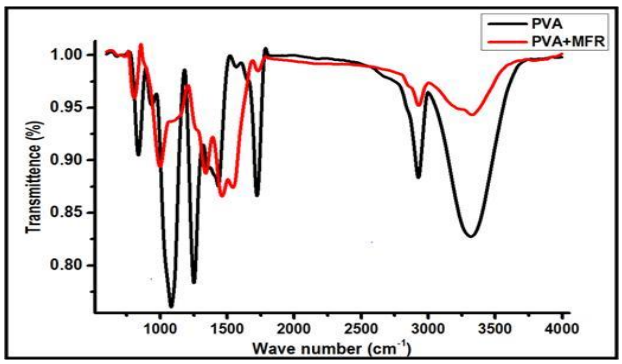

Figure 1. FTIR spectra of pure PVA and crosslinked PVA-MFR films

FTIR spectrum of PVA film shows typical strong hydroxyl bands for free alcohol (non bonded –OH stretching band at 3313cm-1 and also PVA film reveals major peaks like C–H broad alkyl stretching band (2850-3000 cm^{-1}).[34] Intramolecular and intermolecular hydrogen bonding are expected to occur among PVA chains due to high hydrophilic forces, where strong intramolecular hydrogen bonded band 3200-3570 cm^{-1} may occur. An important absorption peak was verified at 1245 cm^{-1} for –C-O str bond and 1090 cm^{-1} for –C-O-H bending vibration. These bands have been used as characteristic bands for assessing the semi-crystalline nature of poly(vinyl alcohol) structure which is expected due to different process parameters.[35-36]

By crosslinking PVA with MFR (synthesized and restricted to oligomeric form only 1st stage, where there is only conversion of methylol groups to primary amine)[37] the –OH peaks have been reduced and became broad when compared to pure PVA that suggests hydrogen bonding becomes weak in crosslinked PVA as shown in the FTIR spectrum of PVA-MFR. In addition to that, the C-O stretching at 1090 cm^{-1} is reduced in cross linked PVA to a broader absorption band PVA-MFR (1000-1300cm^{-1}) as can be seen in Figure 1. The peak of 813 cm^{-1} exists in all these IR spectra that is characteristic of triazinyl ring[38] of melamine moiety.

Characterisation of nanoparticles

The as-synthesized ZnO nanoparticles were characterized by the UV-Visible Spectroscopy and TEM. The size of the nanoparticles plays an important role in changing the entire properties of materials. Thus, size evolution of semiconducting nanoparticles becomes very essential to explore the properties of the materials. UV-visible absorption spectroscopy is widely being used technique to examine the optical properties of nano-sized particles. The absorption spectrum of ZnO nanopowder is shown in Figure 2. It exhibits a strong absorption band at about 336 nm. It is also evident that significant sharp absorption of ZnO indicating the monodispersed nature of the nanoparticle distribution.[39, 40]

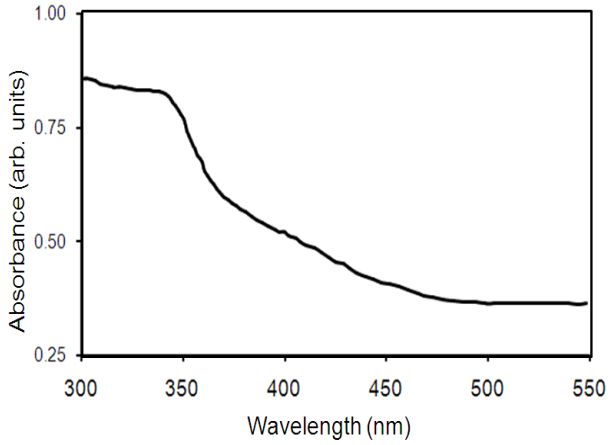

Figure 2. Absorption spectra of as-synthesized zinc oxide nanoparticles

To access the size and morphology of the samples, we performed TEM and the images are presented in Figure 3. As can be seen from the image, the size of the ZnO particles are below 5 nm with uniform morphology.

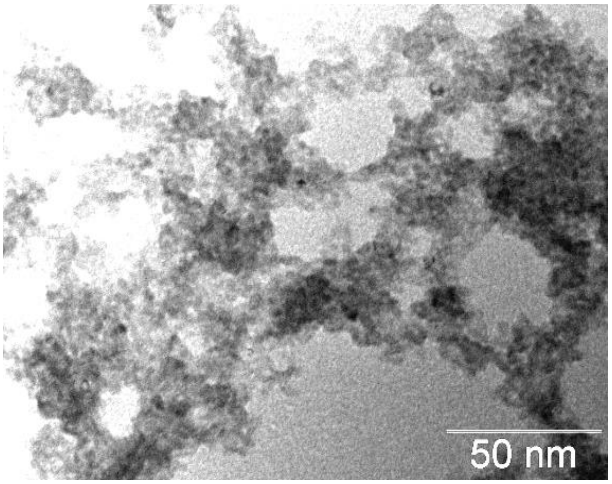

Figure 3. TEM image of the as synthesized zinc oxide nanoparticles

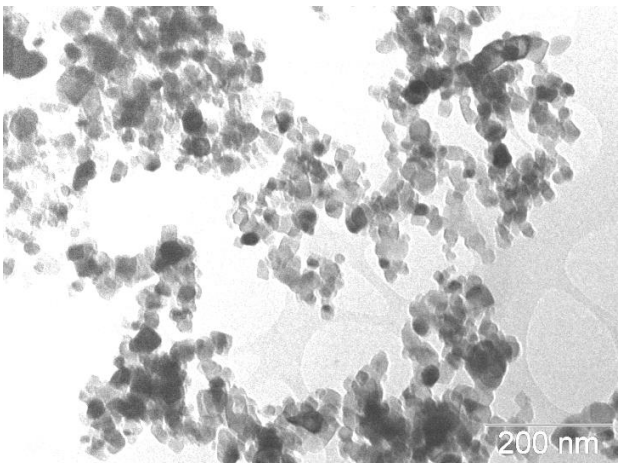

Figure 4. TEM image of as synthesized Titanium dioxide nanoparticles

The particle size of the TiO_2 prepared was found to be 15-20 nm by TEM (Figure 4).

Highly stabilised silver nanoparticles were characterised by TEM (Figure 5) and UV-Visible measurements (Figure 6).

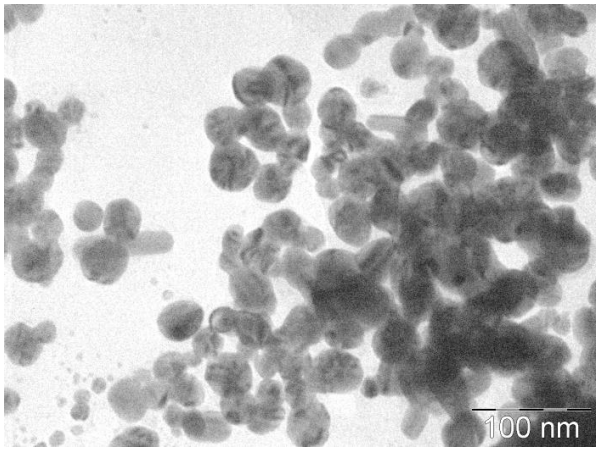

Figure 5. Characterization of silver nanoparticles by TEM

Silver nanoparticles show a small gap between the conduction band and valence band where electron moves freely that are responsible for Surface Plasmon peak.[41,42] This absorption strongly depends on the particle size, dielectric medium and chemical surroundings.[43] The reduction of Ag+ ions was monitored by measuring the UV-Visible spectrum by diluting a small aliquot of the sample into distilled water shown in Figure 6.

UV-Vis spectral analysis was done in the range of 250-750 nm and the absorption (SPR) peaks obtained in the visible regions at 412 nm, that are identical to the characteristics UV-visible spectrum of metallic silver.[44]

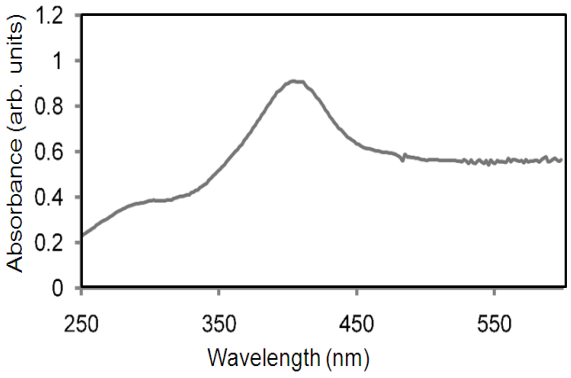

Figure 6. UV Visible spectra of nanosilver

TGA studies of PVA –MFR films - The polymeric PVA-MFR films labelled J1 to J8 listed in Table. 1 were prepared using different composition of PVA and MFR. Representative films J1, J3, J5, J6, were selected and subjected to thermal degradation for TGA studies as shown in Figure 7. The thermal stability of polymeric films plays an important role in determining the final film properties and is greatly influenced by the structure, chemical composition, monomer distribution, and different interaction parameters. The thermal properties of these films depend primarily on network structure and the stability is a function of more than one variable than just the extent of crosslinking with MFR, both the kind and concentration of remaining groups, cohesive energy between molecular chains, molecular chain rigidity, and other chemical structural factors such as, for example, steric strain and conformational arrangements of groups.

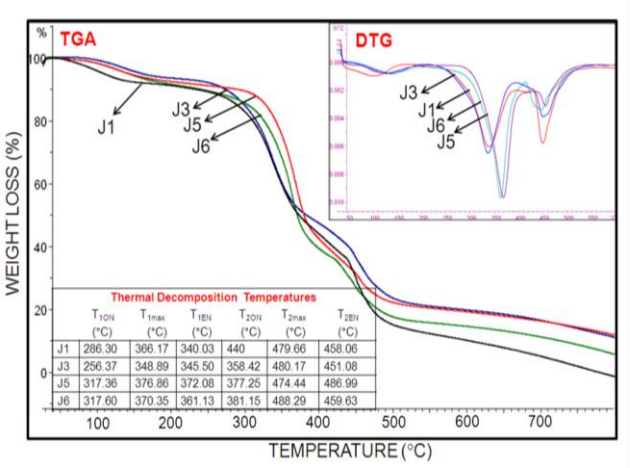

Figure. 7. TGA Studies of polymer composite PVA-MFR films prepared with changing concentration of MFR in 10wt% PVA

Thermogravimetric (TG) curves of all the polymer composites show mainly two step decomposition. The initial step at temperature less than 200°C can be attributed to loss of loosely bound solvents that are accompanied by the formation of volatile disintegrated products. The decomposition at this temperature, which is closer to decomposition temperature of fully hydrolysed PVA at 230°C, is also due to loss of PVA component in the composite films.[45,46] The weight loss is comparatively more

in J1 where lesser composition of MFR is used, as the concentration of MFR is increased the loss at this step is reduced indicating all the PVA is cross-linked with MFR, hence loss in weight from J3 to J6 is the same at this temperature. The residue in all the films after this initial step is predominantly PVA-MFR composite and shows a similar TGA profile. As can be seen from Figure 7, the onset of decomposition T_{1ON} of the first step at lower concentrations of MFR i.e., in J1and J3 is at 285.3 and 256.37° C, respectively that is much lower than that observed for higher concentrations i.e., J5 and J6, at 317.36 and 317.60 °C showing an increase in thermal stability. Similar trend was observed in the temperature of maximum decomposition T_{2max} for the synthesized PVA-MFR films, whereas the second step has a temperature of maximum decomposition T_{2max} in a narrow range i.e. 474 – 488 °C.

TGA studies of PVA–MFR-Nanocomposite films - PVA-MFR composite corresponding to composition J3, (Table1) were used to prepare organic–inorganic hybrid nanocomposites. In Figure 8, the film label J3 - stands for PVA-MFR composite film of composition J3. Labels ZnO, TiO2, and Ag stand for corresponding nanocomposite films at nanoparticle concentration of 25, 25, 2 mmol/L, respectively and PVA-MFR composition corresponding toJ3.

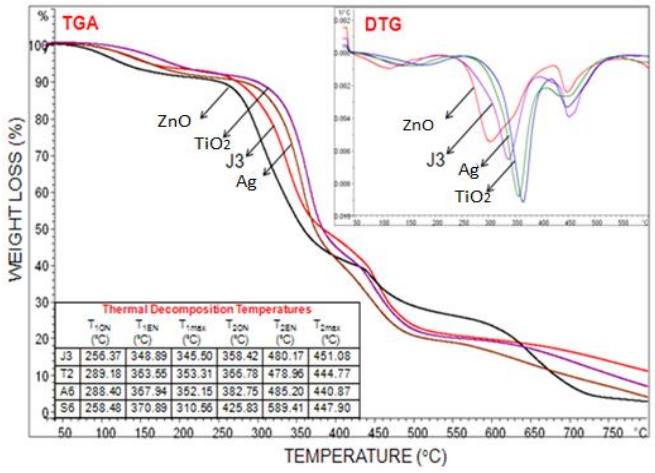

Figure 8. TGA Studies of PVA-MFR nanocomposite films. The film label J3-stands for PVA-MFR composite film of composition J3. ZnO, TiO_2, and Ag, stand for films at nanoparticle concentration of 25, 25, 2 mmol L^{-1}, respectively and PVA-MFR composition corresponding to J3.

Figure 8 compares the effect of inorganic nanoparticles on the decomposition profile of PVA-MFR composite, J3. The T_{1max} is lowered by the presence of ZnO, while it is increased with silver and TiO_2 in corresponding films. Except for film with ZnO all the samples show a similar thermogravimetry profile with mainly two steps of decomposition after 200°C. Film with ZnO on the other hand has a third decomposition peak at 600°C, with a reduced second step resulting to the third step, resulting in only 4 wt% of final residue mass, as compared to 6, 8.5, 10.5% for Ag, TiO_2 and J3 films, respectively. Generally during the initial stages of the decomposition, the strained bonds or the weakest bonds with low dissociation energy break resulting in the strain-free cross-linked intermediate (after the first stage of decomposition) that decompose at higher temperatures and correspond to second and third stages of degradation as in film ZnO.

Antimicrobial Studies

Antimicrobial properties of PVA-MFR films with varying MFR concentration - A series of these composites were prepared by taking 20 ml of 10 wt % PVA solution and adding varying volumes (x - ml) of MFR liquid. Films ranging from KI-K10 (Table 2) are similar to films J1 to J6 in Table 1, except a wider range of compositions have been explored. Antimicrobial property of the films was studied by disc diffusion method. A zone of inhibition was seen around the polymer films to which the organism is sensitive. All the organisms were sensitive to the polymer films and hence, found to be having antimicrobial activity. The zone diameter of inhibition was measured and tabulated (Table 2). Film labelled K0, the blank, had no MFR and is only a plain PVA film and showed zero zone diameter of inhibition against all organisms, ascertaining that PVA by itself is not antimicrobial. All the PVA –MFR films from K1 to K10 are antimicrobial. Highest antimicrobial activity 3.9 cm of zone diameter of inhibition was observed with K5 against *S.aureus* and the least activity observed with *P.mirabilis* against K2. Table 2 shows an increase in the activity in films K1 to K5 with increase in MFR concentration, there after it levels off, so K4 can be taken as a representative composition, which is 20 ml of 10% PVA solution blended with 3 ml of MFR. The fact that antimicrobial activity does not continue to increase with increased concentration of MFR used in films K5-K10, shows that the activity is not brought in merely by addition of MFR to PVA. The antimicrobial activity is also not due to any free formaldehyde in the prepared MFR resin as the films were heated in oven for two hours at 70° C whereby the entire volatile component would have evaporated. The films have been found to retain the antimicrobial efficacy when studied even after a period of eight months, as can be seen from the results in Figure 9.

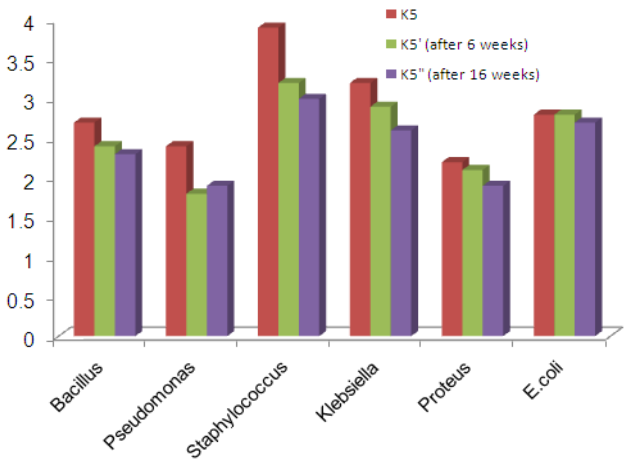

Figure 9. Antimicrobial activity – variation with time of PVA-MFR film composition K5. Zone diameter of inhibition in cm on immediate use, after six and sixteen weeks

PVA-MFR-Silver nanocomposite films - effect of increasing silver concentration -.Silver releasing dressings and patches are increasingly in demand for treatment of infected wounds. A small concentration of nano silver immobilised in antimicrobial PVA-MFR, can be a far cheaper version of silver dressing as far lesser quantity of nanosilver would be required to reach the required level of antimicrobial activity. Concentration of silver, MFR, and film thickness can be varied easily and studied.

Table 2. Antimicrobial properties of PVA-MFR films - varying MFR concentration composition of the films is - x-ml of MFR in 20 ml of 10 wt.% PVA solution

FILM LABEL	K_0	K_1	K_2	K_3	K_4	K_5	K_6	K_7	K_8	K_9	K_{10}
Composition (X ml MFR)	**0**	**1**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**
ORGANISM	**Zone of inhibition in diameter in cm**										
Bacillus.subtilus	0	0.9	2.4	2.6	2.2	2.7	2.4	2.3	2.4	2.3	2.8
Pseudomonasaeruginosa	0	0.4	1.9	1.7	2	2.4	2.2	2.1	3	2.9	3
Staphylococcus aureus	0	1	2.0	2.5	3.2	3.9	2.1	2	2	2.4	2.7
Klebsiella pneumonia	0	0.8	1	2	2.8	3.2	2.4	3	2.8	2.4	2.8
Proteus mirabilis	0	0	1.5	1.6	1.9	2.2	2.3	2.2	2.4	2.1	2.5

Table 3. Antimicrobial studies of PVA-MFR-silver nanocomposite films- effect of increasing silver concentration blank E0 with no silver and E1-E6 with increasing silver concentration

Nano-Ag film	10^{-7} mol $AgNO_3$ cm^{-2} of film area	*S. aureus* / Zone inhibition diameter, cm	*P.Aeruginosa* Zone inhibition diameter, cm
E0	0	2	1
E1	0.4	2.5	1.8
E2	0.8	3	2
E3	1.2	3.2	2.2
E4	2	3.3	2.3
E5	3	3.3	2.2
E6	4	3.4	2.3

As prepared nanosilver solution was diluted with double distilled water and PVA-MFR-silver nanocomposite films with different concentrations of silver were made. Listed in Table 3 are films E1 to E6, with different silver concentrations. The film E0 was a blank PVA-MFR film and contained no silver. All the films E0 to E6 had composition, 2ml of MFR in 20 ml of 10 wt. % PVA. This composition was chosen so as to use minimal amount of MFR yet get its antimicrobial contribution, while rest is to be contributed by silver. Concentration of silver, MFR, and film thickness can be varied easily and studied. Antimicrobial studies on the films were conducted in similar conditions. Results in Table 3 show antimicrobial property of the films and it is found to increase with increasing concentration of silver. All organisms studied for K-series showed activity, but for convenience only results *S. aureus* and *Pseudomonas* of are shown here. Antimicrobial activity for both the organisms in all the films containing silver (E1-E6) was more compared to the film E0 containing no silver, where the activity is only due to PVA-MFR. Antimicrobial activity due to silver is thus clearly superimposed over the activity due to PVA-MFR. Activity increased with increasing concentration of silver used in films E1 to E3 and levelled off thereafter in E4-E6. Thus, antimicrobial property of the film can be increased by using more of nanosilver concentration and less of MFR if so desired for any medical applications. At the same time if cost is a criteria and MFR has no harmful effect, concentration of MFR in the film can be increased with lesser or no silver at all. In this study, ideal example is E3, with a composition which was achieved by using 6 ml of as prepared nanosilver solution, containing 0.012 mmol of silver, diluting it to 20ml with double distilled water, and then dissolving 2g of PVA powder to get 10 wt. % PVA, 2 ml of MFR, and proceeding as described earlier.

This composite so prepared gave about 100 sq cm of film, having about 1.2 x 10^{-7} mol of silver cm^{-2} of film so prepared.

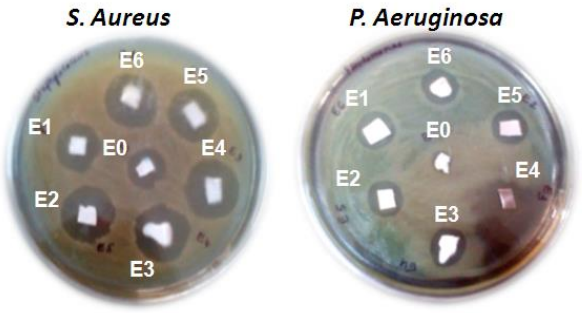

Figure 10. Antimicrobial studies of PVA-MFR-silver nanocomposite films- effect of increasing silver concentration blank E0 with no silver and E1-E6 with increasing silver concentration in the case of *S. aureus* and *Pseudomonas/P. aeruginosa*

A study was conducted to ascertain the level of depletion of antimicrobial activity of the films after the first study, if the films have to find usage as a medicinal patch. The piece of film material which was used for the first round of antimicrobial studies was picked, thoroughly washed with water, dried in oven and resubjected to antimicrobial studies. All the samples used in Table 3, for *S. Aureus* studies were restudied in this way. *S. Aureus* was picked for reusability studies as it had shown maximum antimicrobial effect. Figure 11 shows a fall in activity in each film, yet a good level of activity is still found in each of the films on reuse, an encouraging result.

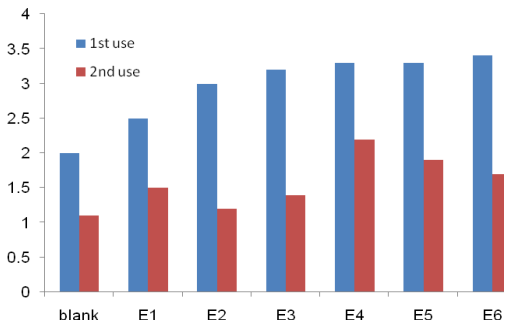

Figure 11. Reusability of silver nanocomposite films - Antimicrobial studies with *S. aureus* (Zone diameter of inhibition in cm) films

Although research indicates that silver nanoparticles are more effective to gram negative than gram positive bacteria, involving the charge of peptidoglycan molecules in the bacterial cell wall, our results show that they are equally effective against both gram-positive bacteria and gram negative bacteria, and showing highest activity against *S. Aureus*. The mechanism of the bactericidal effect of silver nanoparticles is not very well-known. It is believed that cellular proteins become inactive after treatment with silver nanoparticles.[44] Silver nanoparticles after penetration into the bacteria in-activate the enzymes, generating hydrogen peroxide and causing bacterial cell death.[43] Silver nanoparticles can be used as effective growth inhibitors in various micro-organisms, making them applicable to diverse medicines and antimicrobial control systems. It is thought that silver atoms bind to thiol groups (-SH) in enzymes and subsequently cause the deactivation of enzymes. The silver-catalyzed formation of disulfide bonds could possibly change the shape of cellular enzymes and subsequently affect their function.

Antimicrobial efficacy of films - variation with time - Since the PVA-MFR composite has shown good adhesion property on paper, fabric, jute, as well as strong antimicrobial film making property, it can find application in dry grain storage and other packaging applications. A study was conducted to see the stability of film and how its antimicrobial property is affected over time period. A representative PVA-MFR film K5 from Table 2 was chosen and compared its antimicrobial properties on immediate use, after six and sixteen weeks. Very encouraging trend is that a slight fall in the activity is seen, for all the microbes and all the films, in first six weeks and then there is negligible change in next ten weeks, as seen in Figure 9. We recommend jute, raw cotton bags to be given a thin PVA – MFR film lining or else directly coat them with PVA-MFR, which will not only protect them from moisture but also from microbes, making a better grain storage, packaging material.

Similar antimicrobial studies were done on PVA-MFR and corresponding Ag, ZnO, TiO_2 nanocomposite films, observed in Figure 12. All the films with nanoparticles have higher antimicrobial activity than the plain PVA-MFR films. The nanocomposite films are stable and have retained a good level of antimicrobial activity even after eight months of their preparation. For convenience, only S. Aureus work is being reported here.

Table. 4. *S. Aureus* - antimicrobial activity by turbidity method coating inside glass beaker and a suspended film

Sample	Absorbance	
	Composite coated inside glass beaker	**Suspended composite as film**
PVA polymer	0.21	0.21
PVA-MFR	0.16	0.06
PVA-MFR-ZnO nano	0.05	0.02
PVA-MFR-Ag nano	0.02	0.01
PVA-MFR-TiO_2 nano	0.01	0.01
PVA-MFR-TiO_2 commercial	0.1	0.1

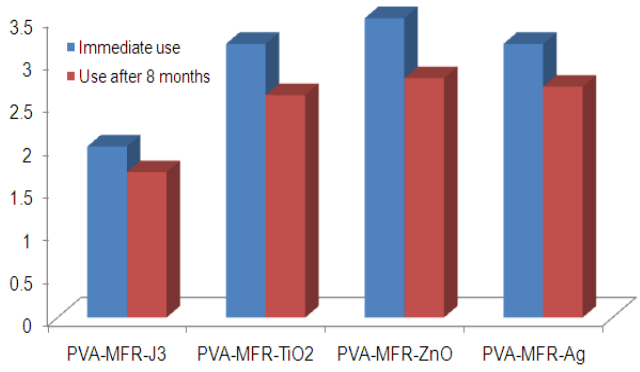

Figure 12. Antimicrobial activity (*S.Aureus*) – variation with time - Zone diameter of inhibition in cm - on immediate use and after eight months PVA-MFR, and nanocomposite films with TiO_2, ZnO, and silver nanoparticles.

PVA–MFR–nanocomposite coatings inside glass beakers - The nanocomposite coatings inside glass beakers, PVC beakers and clay pots were subjected to turbidity method for their antimicrobial studies. Though all the three coatings showed antimicrobial activity, only work on glass beakers is being reported. Corresponding films were also studied by the turbidity method by suspending them in the culture medium in the same manner. Near zero absorbance for all the nanocomposite coatings seen in Table 4, shows a very good level of antimicrobial property in these coatings, whereas the blank, a plain PVA coating showed maximum growth of microorganism and thus high absorbance value, indicative of negligible antimicrobial activity.

Similar results are seen with the suspended films in Table 4. It is thus concluded that MFR in solubilises PVA, with good adhering properties as well as renders it antimicrobial .The nanoparticles in the nanocomposite, superimpose the antimicrobial properties of the nanoparticle to the film or the coatings. Comparison of absorbance values for coatings with PVA-MFR-TiO_2 (commercial), and PVA-MFR- TiO_2(nano) particle showed large difference in absorbance indicating the efficacy of nanoparticle as an antimicrobial agent and confirm the presence of nanoparticles in the PVA-MFR–nanocomposites.

Spray coatings of PVA-MFR nanocomposite on food grade aluminium foil - It needs to be mentioned here that food grade foils, and the foil used in packaging industry are essentially a thin film of aluminium coated with a polymer, and hence it is possible to spray coat them or laminate them with PVA-MFR.

Antimicrobial studies on these films reported in Table. 5, show the aluminium foil by itself has zero activity, while films coated with PVA-MFR are antimicrobial and a higher level of enhancement of the antimicrobial activity when foil is coated with PVA-MFR- nanocomposites as can be seen in Figure 13.

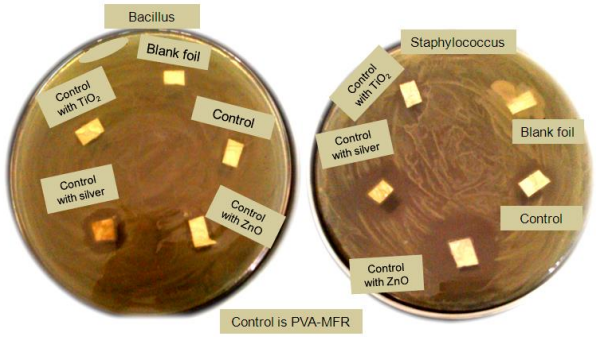

Figure 13. Antimicrobial studies of PVA-MFR Nanocomposite Coatings on food grade Aluminium foil - *Bacillus and Staphylococcus.*

Table 5. Antimicrobial studies of PVA-MFR Nanocomposite Coatings on food grade Aluminium foil- Zone of inhibition diameter in cm.

Composite	Microorganism	
	Bacillus / B .subtilus	***Staphylococcus/ S.aureus***
PVA- MFR-Ag	2	2
PVA- MFR-TiO_2	1.6	1.6
PVA- MFR-ZnO	2	2.3
PVA-MF blank	0.6	1
Blank foil	0	0

These were essentially very thin coatings, obtained by diluting the active composite with methanol before it is cured, hence have very low concentration of nanoparticle, yet antimicrobial efficacy is good with all the nanoparticles. Such prepared coatings can be explored for antimicrobial food packaging applications.

Conclusion

PVA -MFR films are antimicrobial, especially in the case of gram positive bacteria like *S. Aureus* and *Bacillus* that are generally persistent in hospitals, and are a major health hazard, these films sprays and coatings could probably make a cheap and effective material in the hospitals and diagnostic labs where the cultures are prepared. Since the material has good adhesion properties clay pots, glass, polypropylene lab containers, can be coated with PVA-MFR to render them antimicrobial. Paper, jute, raw fabric grain storage bags can be sprayed with or given PVA-MFR coating or else film lining which will not only protect them from moisture but also from microbes, making a better grain storage and food packaging material. The prepared films and coating are stable and retain their activity over a long period. The active composite can be diluted before curing to be used as a spray for uniform, fine antimicrobial coating on food grade aluminium foil and polyester films etc.

Though PVA-MFR films are antimicrobial but films where nanoparticles are immobilised in PVA-MFR matrix, have a large surface to volume ratio and more effective antimicrobial activity. These can be explored for surgical face masks and other medical applications. A very minimal concentration of silver was used in making these films as It remains to be determined if silver nanoparticles will be safe for patients in the long run. In the mean time, due to the evolution of antibiotic resistant bacteria silver nanoparticles remain a hot item and these are incorporated in a number of products ranging from device coatings and wound dressings to commercially available deodorants and cosmetics. Application of PVA-MFR- silver nanocomposite films and coatings as an antimicrobial patch for treatment of highly resistant wounds of diabetic foot diseased patients has been studied, and is being reported separately.

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