



POLYVINYL ALCOHOL - MELAMINE FORMALDEHYDE FILMS AND COATINGS WITH SILVER NANO PARTICLES AS WOUND DRESSINGS IN DIABETIC FOOT DISEASE

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Globally there is a large population of people suffering with diabetes. A large percentage of these patients develop foot ulcers at some point that heal very slowly and can worsen very rapidly. The use of silver nanoparticles in silver release dressings and in management of infected wounds is important, as several pathogenic bacteria have developed resistance against various antibiotics. Such dressings vary in technological nature of their silver content and release. Use of silver dressings in recent times has a considerable challenge of lacking cost effectiveness. In-vitro susceptibility of microorganisms causing foot ulcers to a silver nanocomposite of Polyvinyl alcohol (PVA) and Melamine formaldehyde (MFR) resin both as films and coatings is being reported. AgNP solution was prepared by colloidal route and characterised. MFR was prepared and reacted with PVA and AgNP solution to obtain PVA-MFR composite and PVA-MFR-Ag composite. The antimicrobial composites were casted into films, also soaked into polyvinyl foam, and coated on Whatman paper. Antimicrobial efficacy of such prepared dressing materials was tested. They are stable and do not lose their antimicrobial activity with time. Tissues from the wounds of five diabetic patients with deep foot infections were collected to isolate and identify the microorganism responsible for causing foot ulcers in the diabetic patients. Their sensitivity towards various antibiotics was studied. *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* species isolated from wound samples, though were found resistant to many antibiotics are sensitive to PVA-MFR-Ag films. These silver composites can be cost effective for the reason that efficacy of nanosilver is superimposed on antimicrobial activity of cheaper PVA-MFR composite. Thus, AgNP immobilised on antimicrobial PVA-MFR, could probably be a promising wound dressing in diabetic foot disease management.

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AG¹⁰ (produced by Hartmann) and various others. These commercial applications in the Indian and third world scenario are prohibitively expensive. PVA-MFR composite and PVA-MFR-Ag nanocomposite are found to be antimicrobial.¹¹ Hence, the nanosilver immobilised on PVA -MFR was studied *in vitro* for antimicrobial activity in isolates from foot infections.

INTRODUCTION

Diabetic foot ulcers occur in about 25% of diabetics. India currently has a diagnosed diabetic population of about 60 million and another 60 million are considered to be pre-diabetics/ undiagnosed diabetics. Diabetic foot infections account for about 40% of hospital admissions worldwide due to diabetes. These infections are frequently polymicrobial and include organisms like *Staphylococcus Aureus* both methicillin susceptible and methicillin resistant, *Pseudomonas*, *Proteus*, *Escherichia Coli*, *Klebsiella* etc. Due to various reasons including misuse, many of these organisms are today resistant to conventional antibiotics and antimicrobial dressings.^{1,2} With the manifestation of more and more antibiotic resistant bacterial strains, another major healthcare related problem is bacterial infection from medical devices.^{3,4,5} Application of silver nanoparticles on the surface of medical devices is in use, to help decrease such complications. Silver has been used since time immemorial in the treatment of infected wounds. The use of nanoparticles of silver in the treatment of infected wounds is documented and has been studied.^{6,7,8} Currently the nanocrystalline silver is bound to a cloth base commercially and used for dressings, examples being Biatain AG⁹ (produced by Coloplast), Atrauman

EXPERIMENTAL

Materials and Methods

Silver nitrate (AgNO₃) from SD Fine Chemicals, starch from Merck, Mumbai and trisodium citrate dihydrate (AR grade), from Finar Chemicals, Ahmadabad were used. Poly(vinylalcohol) (PVA) M.W. 85,000 - 1, 24,000 (LR grade), formaldehyde (37 % w/v, LR) were purchased from S.D. Fine Chemicals Limited, Mumbai. Melamine powder (AR grade) was purchased from Gujarat Natural Fertilizers Limited (GNFC). All the solutions were made using double distilled water. TEM samples were prepared by 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. UV-Vis spectra were recorded on Systronics model 2201. FT-IR absorption spectra were carried out using the Fourier transform-infrared spectrometer (FTIR-Bruker Optics, Germany Model: TENSOR27). FT-IR spectra of the samples were obtained in the spectral range of 4000 – 400 cm⁻¹.

The antimicrobial activity of the samples was performed by Kirby-Bauer disc diffusion method¹¹ to check whether the samples are active or not. The Nutrient agar (Himedia) was prepared, sterilized, poured into petri dish and allowed to solidify. The bacterial culture was spread with the help of 'L' shaped glass rod. Then samples were added into wells and the petri dishes were incubated for 24 h at 37 °C. The antibacterial activity of the AgNP solution, PVA-MFR hereafter called composite, PVA-MFR-Ag, hereafter called Ag-composite were studied using the bacterial cultures of *Klebsiella pneumonia* (*K. pneumonia*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and *Proteus vulgaris* (*P. vulgaris*).

A piece of tissue from the depth of the infected wound was excised and passed through a tissue homogenizer to blend the tissue, so as to expose the organism present in the tissue. The homogenized tissue was then plated into various culture media for growth of bacteria and fungi. From the homogenized tissue, gram stains for bacteria and KOH stains for fungi were also done. The plates were checked for bacterial colony growth after 48 hours and for fungal growth after 7 days. Based on colony characteristics and culture media, gram staining was done to identify the organism as well as various identification reactions were run for the same. The colonies were then subjected to antibiotic susceptibility using disk diffusion technique to identify the susceptibility of the organisms to various antibiotics and antimicrobials.

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 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 was prepared by method¹² where formaldehyde and melamine are reacted under base catalyst and it's polymeric molecular weight increased by addition process. 57 g 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 2 N NaOH solution. The RB flask was kept on a magnetic stirrer and 50 g of melamine powder slowly added under stirring followed by 15 ml of distilled water. The reaction mixture was heated with continuous stirring till the temperature increased to about 60 °C and then very gradually allowed the temperature to rise to about 95 °C. Reflux 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 minutes,

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 were fixed by parameters like viscosity, water tolerance and gel time.¹³ Viscosity at 32 °C is 30 - 40 s; water tolerance at 30 °C is 1: 2 to 1:4, and gel time at 150 °C is 210 - 230 s. 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 and characterisation of silver nanoparticles

AgNP solution was prepared from AgNO₃ and trisodium citrate, by the colloidal route which involves not only reduction of Ag⁺ ion but also stabilisation of AgNPs by a lyophilic colloid. 50 ml of 0.008 M AgNO₃ 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 50ml of 0.08 M sodium citrate solution was added, under continuous stirring and heating for 2 h at 95 °C. The silver content of this solution is 2x10⁻⁶ mol ml⁻¹. The procedure was repeated three times, replacing starch with polyvinyl alcohol (PVA), polyethylene glycol (PEG), and Gum acacia.

Preparation of PVA-MFR-Ag nanocomposite as films and coatings

Preparation of these films and coatings has been carried out as by the procedure discussed in detail in our earlier work.¹¹ A (10 wt %) solution of PVA in 20 ml of AgNP solution, was prepared by stirring and heating over a water bath at 90 °C till all the PVA was dissolved. To this solution 2 ml of prepared Melamine formaldehyde (MFR) solution was added and blended, by using an electrical blender while heating at about 80 °C, over a water bath, taking care to stop the process just before it forms a thick ball like mass. At this stage the blend is ready to be cast into a film or a coating.

For making films the blend was poured and spread uniformly, on a plastic Petri dish and dried in hot air oven at about 70 – 80 °C for 2 h. After evaporation of the solvent at ambient temperature, film was peeled off and stored in Ziploc bags. To prepare blank PVA-MFR film, 20 ml of (10 wt %) PVA aqueous solution was used in the place of 20 ml of AgNP solution. The active composite blend could also be coated on a whatman paper or a thin strip of polyvinyl foam can be soaked into it. PVA-MFR-Silver films with varying concentration of AgNPs, labelled Ag0 to Ag6 shown in Table 1, were prepared by varying the dilution of AgNP solution taken. Film labelled Ag0 has no silver and is only a PVA-MFR blank film.

Table 1. PVA-MFR-AgNP films of different concentration of AgNP

Name	Ag0	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6
X 10 ⁻⁷ mol AgNO ₃ film area in cm ⁻²	0	0.4	0.8	1.2	2	3	4

RESULTS AND DISCUSSION

Characterization of AgNPs

The four AgNP solutions prepared under similar conditions but with different stabilisers were compared by UV-Visible spectral analysis carried out in the range 250-750 nm and the characteristic absorption (SPR) peaks obtained in the visible regions at 412 nm. Absence of peak at 560 nm in Figure 1 confirms no agglomeration of AgNP and they are highly dispersed in nature. Considering absorbance at wavelength 412 nm, is indicative of concentration of AgNPs formed, the yield of the AgNP is higher when starch or PVA are used as stabilisers. Further on comparison starch stabilised AgNP solution showed better antimicrobial efficacy than the rest of the stabilisers.

Thus AgNP solution prepared by combination of citrate and starch as described was preferred for use in further work. Very small concentration of AgNPs is formed with starch alone or with citrate alone. Our previous work¹⁴ on starch stabilised AgNPs discusses at length the role of starch as stabiliser as well as reducing agent.

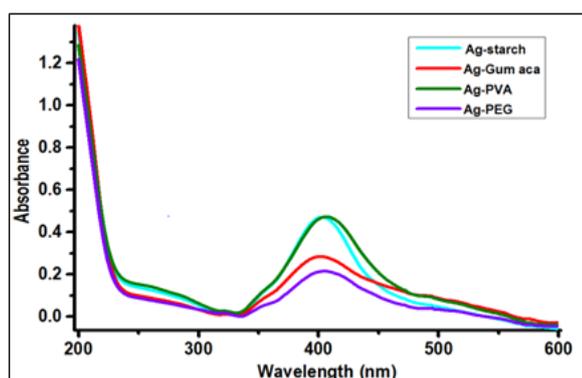


Figure 1. UV-Visible studies of AgNPs prepared using different protective colloids as stabilizers

Reaction parameters for the process have been standardized to obtain uniform size AgNPs that are stable for months at room temperature even after a year. Silver nanoparticles show a small gap between the conduction band and valence band where electron moves freely that are responsible for Surface Plasmon peak.^{15, 16} This absorption strongly depends on the particle size, dielectric medium and chemical surroundings.¹⁷ TEM studies were performed on the samples so as to access size and morphology. The TEM image of AgNP prepared using different protective colloids as stabilizers is shown in Figure 2 and as can be seen the size of the nanoparticles is less than 40 nm. The inset shows the SAED images and as can be seen the observed Debye Scherer concentric rings assigned to (111), (200), (220), and (311) planes consistent with the fcc phase of AgNPs.

FTIR spectrum of PVA film in Figure 3 shows typical strong hydroxyl bands for free alcohol (non bonded –OH stretching band at 3313 cm^{-1} and also PVA film reveals major peaks like C–H broad alkyl stretching band (2850–3000 cm^{-1}).¹⁸ An important absorption peak was verified

at 1245 cm^{-1} for –C–O stretching 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 PVA structure that is expected due to different process parameters.^{19, 20}

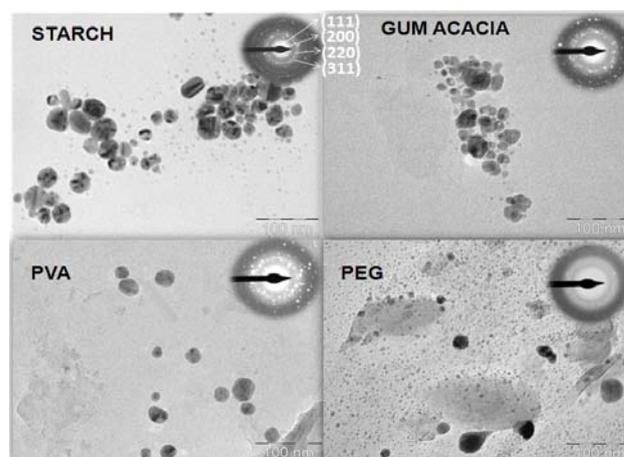


Figure 2. TEM images of silver nanoparticles prepared using different protective colloids as stabilizers

By cross linking PVA with MFR, the intensity of the –OH peaks reduced and became broad when compared to pure PVA that suggests hydrogen bonding becomes weak in cross linked 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 (1000–1300 cm^{-1}) as can be seen in Figure 3.

The peak at 813 cm^{-1} is characteristic of triazinyl ring of melamine moiety. A slight change in the IR spectrum of the PVA-MFR composite was observed for the band peaking at 1300–1200 cm^{-1} . These changes are more pronounced for the Ag-PVA-MFR nanocomposite with the blending of inorganic phase. The decrease in the ratio of intensities of this band upon incorporation of the Ag nanofiller indicates interaction of Ag nanoparticles and the free OH groups of cross linked polymer and decoupling of corresponding vibrations. This is further noticed^{21,22,23,24} by the decrease in intensity of symmetric CH bend in the region 1000–850 cm^{-1} .

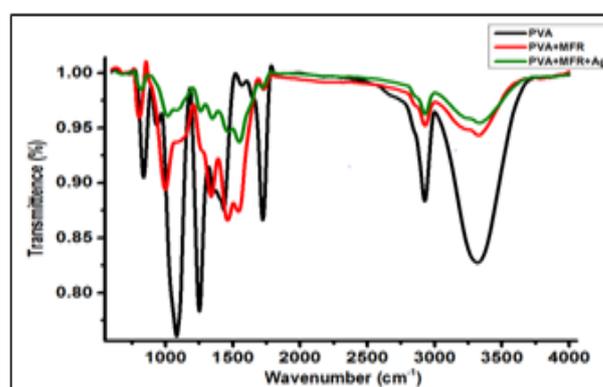


Figure 3. FTIR spectra of plain PVA film, PVA-MFR composite film and PVA-MFR-Ag nanocomposite film

Results of antimicrobial studies done on prepared PVA-MFR composite and PVA-MFR-Ag composite films, in Table 2, show that the PVA-MFR composite film is antimicrobial but efficacy is more in comparison for the Ag composite film.

Antimicrobial activity due to silver is thus clearly superimposed over the activity due to the polymer. Concentration of silver, MFR, and film thickness can be varied easily and studied.

Studies on films prepared with different concentration of AgNP from Table 1 show antimicrobial property of the films increases with increasing concentration of silver. All organisms studied in Table 2 were studied to see the trend, but for convenience only *S. Aureus* results are shown here.

Table 2. Zone of inhibition diameter in cm for composite and Ag composite film

Organism	AgNP-composite film	Composite film
<i>E.coli</i>	1.8	1
<i>K.pneumonia</i>	2	1.2
<i>P.aeruginosa</i>	2.5	1.2
<i>P.vulgaris</i>	2.7	1.3
<i>S. aureus</i>	3.3	1.1
<i>B.subtilis</i>	3	1.3

Antimicrobial activity in all the films containing silver (Ag1-Ag6) was more compared to the film Ag0 containing no silver, where the activity is only due to PVA-MFR, as is seen in Figs 4 and 5. Activity increased with increasing concentration of silver used, in films Ag1 to Ag3 and levelled off thereafter in Ag4-Ag6.

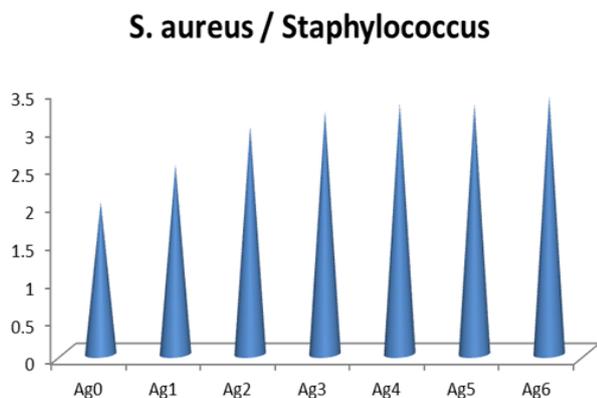


Figure 4. Zone of inhibition diameter in cm. Increasing *S. aureus* antimicrobial activity of Ag-Composite films with increasing concentration of silver in film

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 if MFR has no harmful effect, concentration of MFR in the film can be increased with lesser or no silver at all. The film Ag6 in this study, for example was prepared by using 20 ml of as prepared nanosilver solution, dissolving 2g of PVA powder into it, to get 10 wt. % PVA solutions with the silver containing 0.04 millimol of silver.

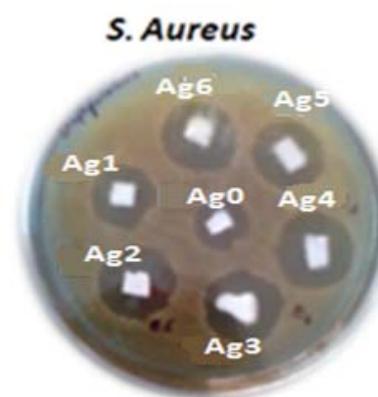


Figure 5. Increase in zone of inhibition for *S.Aureus* studies, with increase in silver content in silver composite films from Ag1 to Ag6

To this 2 ml of MFR was added and proceeded as described earlier. This composite so prepared gave about 100 sq cm of film, having about 4×10^{-7} mol of silver cm^2 of film area. The films are found to be stable and do not lose their efficacy with storage. The composite and Ag-composite films have been studied, in immediate use and eight months after they have been prepared.

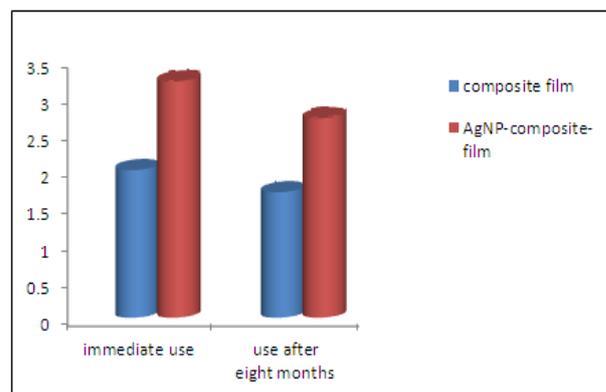


Figure 6. Zone of inhibition diameter in mm; *P. Aeruginosa* studies demonstrating the stability and antimicrobial efficacy of film over time period

Results in Figure 6, represent antimicrobial activity with *P. aeruginosa* only, though trend is same with other organisms also. Within experimental errors there is a marginal fall in activity of both the films over period of eight months. In fact, the films are stable and effective even two years after they have been prepared.

Bacterial Identification of microorganisms from foot infections of patients

Tissues from the wounds of five diabetic patients with deep foot infections were collected to isolate and identify the microorganism responsible for causing foot ulcers in diabetic patients. The samples from patients labelled as case 1 to 5, were subjected to tissue culture and the organism present in them identified.²⁵ The organism identified in the wound of case 1 to 5 proved to be in case 1-*Staphylococcus aureus*, case 2-*Proteus vulgaris*, in case-3 and 5-*Pseudomonas aeruginosa* and case 4 - *Enterobacter cloacae* (Table 3).

Antibiotic susceptibility of microorganisms from foot infections of patients

Antibiotic susceptibility testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*. World Health Organisation (WHO) has recognised the spread of multiple antimicrobial-resistant pathogenic as a serious global human and animal health problem. The development of bacterial antimicrobial resistance is not a new phenomenon, but a troublesome situation because of the frequency with which it is emerging. Selection of an appropriate agent from a variety of available antimicrobial agents has made doctors more dependent on *in-vitro* antimicrobial susceptibility testing. Antibiotic susceptibility in all five cases (Table 4) was checked using disk diffusion technique to identify the susceptibility of the organisms to various antibiotics.²⁵

In-vitro testing of susceptibility of microorganisms from foot infections to PVA-MFR-Ag composites

The microbes isolated from the foot ulcers of five different patients identified above are, case 1-*Staphylococcus aureus*, case 2-*Proteus vulgaris*, case-3,5-*Pseudomonas aeruginosa* and case- 4 *Enterobacter cloacae*. The antibiotic studies above showed the expected pattern, that the microbes often are found to be resistant to many antibiotics.²⁶ The polymer sensitivity was studied with PVA-MFR and PVA-MFR-Ag composites to establish the sensitivity of isolated microbes in cases 1 to 5. Composites are found to be antimicrobial against *S. Aureus*, *P. Vulgaris*, *Pseudomonas* and *Enterobacter* species in these samples. In each case, antimicrobial activity is found in PVA-MFR and the activity is enhanced in the corresponding PVA-MFR-Ag composite. Tests were done to study the composite in solution, films, coatings on paper and foam and each one found to be antimicrobial towards the isolated microbes.

Susceptibility to Composites in solution

The solutions tested were (1) on as prepared solution of AgNP (2) 10 Wt% solution of PVA in as prepared AgNP solution (3) PVA-MFR-Ag solution that was obtained by diluting the active composite blend with an equal volume of water before casting into films. The dilution of the composite needs to be done to avoid solidification, and the dilution can be done with methanol also.

Figure 7 shows zone of inhibition diameter in mm for different microorganisms from foot infections of the five cases studied. It can be seen from Figure 7, AgNP solution in water and AgNP solution in PVA are equally effective. This goes to prove that Ag in Ag/PVA solution is as active as in pure AgNP solution. In comparison the antimicrobial activity in all the cases is enhanced in the case PVA-MFR-Ag solution which is due to antimicrobial contribution from MFR, over and above the contribution from AgNPs. This novel composite offers the flexibility of increasing the contribution from MFR or from AgNP if we so desire by merely increasing their concentration in the reaction mixture.

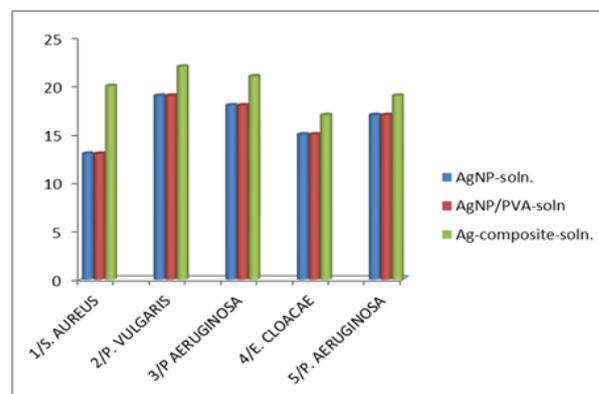


Figure 7. Zone of inhibition diameter in mm. Susceptibility of microorganisms from foot infections of patients to AgNPs, Ag/PVA, PVA-MFR-Ag in solution form

Susceptibility to composite films

Susceptibility of all the five cases with PVA-MFR and PVA-MFR-AgNP composite films was studied. PVA-MFR is antimicrobial in all the cases. Figure 8 shows a relative increase in the antimicrobial efficacy of PVA-MFR-AgNP composite as compared to PVA-MFR only, due to contribution by AgNP in the film. This clearly corroborates the role of AgNP and MFR in the film as PVA by itself has absolutely no antimicrobial activity. *Enterobacter* species is not as sensitive as the rest of the microbes. The polymeric silver films are far more effective as compared to the solution form.

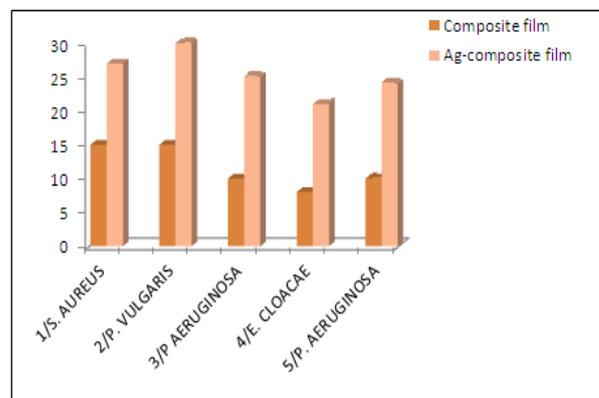


Figure 8. Zone of inhibition diameter in mm. Susceptibility of microorganisms from foot infections of different patients to composite and Ag-composite films.

Susceptibility to nanocomposite coatings

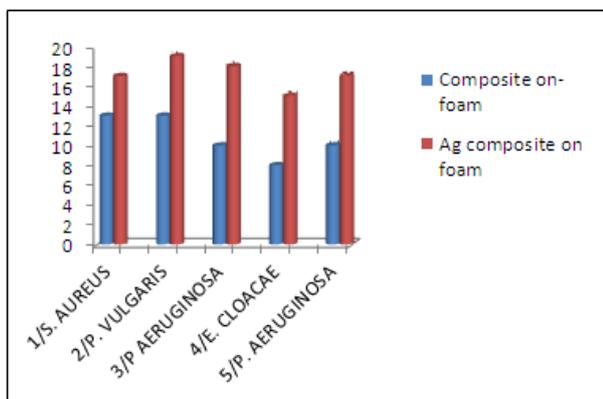
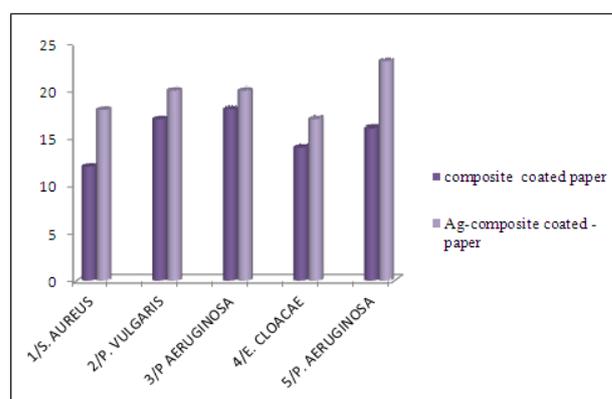
The polymeric composite has good binding properties forming stable chemically resistant coatings on fabric, paper, glass, polyester etc, rendering them antimicrobial. These films and coatings retain their antimicrobial activity over long period of time. The composite absorbed in polyvinyl foam and coated on paper have been tested in similar manner as the films. Effectiveness of the prepared nanocomposite foams towards different organisms is well demonstrated in Figure 9. The zone of inhibition diameter in mm is plotted. It is possible to soak more nanocomposites in the foam and increase its antimicrobial efficacy.

Table 3. Bacterial identifications and the observations from various test performed

Case 1 <i>Staphylococcus aureus</i>		Case 2 <i>Protens vulgaris</i>	
Coagulase positive	Catalase positive Mannitol fermentative	Motile bacilli Urease positive	Indole positive Phenyl alanine deaminase positive
Beta Haemolysis on blood agar	Golden yellow colonies	Swarming growth on blood agar	
Case 3, 5 <i>Pseudomonas aeruginosa</i>		Case 4 <i>Enterobacter cloacae</i>	
Motile bacilli Citrate positive	Oxidase positive Non lactose fermenting colonies	Motile bacilli MR negative	Indole negative VP positive
		Citrate positive Arginine dihydrolase positive	Aesculine hydrolysis negative

Table 4. Antibiotic susceptibility of microorganisms from foot infection of patient cases 1 to 5

Case 1 <i>Staphylococcus aureus</i>			Case 2 <i>Protens vulgaris</i>		
Sensitive			Sensitive		
Cefazolin	Cefotaxime	Amoxicillin clavulanic acid	Amoxicillin clavulanic acid	Piperacillin tazobactam	
Oxacillin	Linezolid	Amikacin	Imipenem		
Vancomycin				Resistant	
Intermediate Sensitive			Ciprofloxacin		Ofloxacin
Ciprofloxacin	Ofloxacin	Levofloxacin	Levofloxacin		Ceftazidime
Resistant			Cefepime		Cefotaxime
Azithromycin	Erythromycin	Co Trimoxazole	Amikacin		Gentamicin
Gentamicin			Tobramycin		
Case 4 <i>Enterobacter cloacae</i>			Case 3, 5 <i>Pseudomonas aeruginosa</i>		
Sensitive			Sensitive		
Imipenem			Amikacin		Tobramycin
Resistant			Piperacillin tazobactam		imipenem
Ciprofloxacin		Ofloxacin	Resistant		
Levofloxacin		Ceftazidime	Ciprofloxacin		Ofloxacin
Cefepime		Cefotaxime	Levofloxacin		Ceftazidime
Ceftriaxone		Amikacin	Cefepime		Gentamicin
Gentamicin		Tobramycin			
Piperacillin tazobactam		Amoxicillin clavulanic acid			

**Figure 9.** Zone of inhibition diameter in mm. Demonstrating effectiveness of foams coated with composite to microorganisms from foot infections of patients case 1 to 5**Figure 10.** Zone of inhibition diameter in mm. Demonstrating susceptibility of microorganisms from foot infections of patients case 1 to 5 towards composite and Ag-composite coated paper

Results of studies on paper coatings in Figure 10 corroborate the results seen with foams. The efficacy in the case of foams and paper coatings is low as compared to that with corresponding films. The antimicrobial efficiency can be improved by making thicker coating of PVA-MFR-Ag composite if the need be. What is common with solution, film and coatings is that all the forms are antimicrobial towards all the organisms.

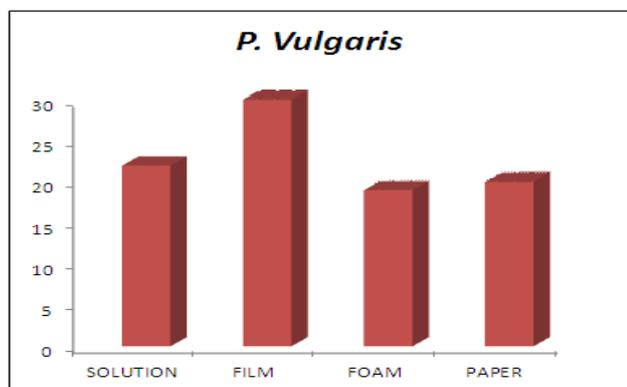


Figure 11. Zone of inhibition diameter in mm for organism *P. Vulgaris* Comparison of effectiveness of different forms of PVA-MFR-AgNP Composite - in Solution, on Paper, Foam and Films

The PVA -MFR is less effective while PVA-MFR-Ag has higher level of antimicrobial efficacy due to contribution from nanosilver immobilised on antimicrobial PVA-MFR. Comparative studies on solution, film and coatings in Figure 11 show that microorganisms are most sensitive to the Ag-composite in films than the corresponding solution or coatings.

CONCLUSION

The study shows that the PVA-MFR-Ag composite films are able to kill microorganisms isolated from foot ulcers in case 1 to 5, where they were resistant to many antibiotics. Considering the results, it is obvious that as compared to solution form, it is PVA-MFR-Ag film which is most effective form of the Ag - composite. Further it is possible to increase the efficacy of the films and the coatings either by increasing the concentration of MFR in the acceptable limits or else by increasing the loading of AgNPs. An interesting observation with film is that it does not leach out all its silver in one use. A piece of film once used for antimicrobial studies was washed, dried in oven and tested again, was found to be antimicrobial though with reduced efficacy. The films, coatings and solution form of the composite are stable over months and do not lose the antimicrobial efficacy. We hope that the PVA-MFR-Ag films can possibly become safe, effective and affordable wound dressing material for patients with foot ulcers, that are resistant to conventional antibiotics and antimicrobial dressings. Also modification of surface of medical devices by giving them a coating with this silver Nanocomposite, so that no bacterial adhesions may occur, could probably be a step in the direction to help decrease the incidence of medical device related infection.

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