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Enhancement of Sterilization Efficiency of Polymer Nanocomposite by Argon Plasma Irradiation

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Silver nanoparticles show excellent antibacterial and antifungal activity. Hence silver nanoparticles were used for synthesis of nanocomposites. Nano particles of silver were synthesized by psidium guajava (guava fruit) extract and characterized by using UV-Vis spectrophotometer, FTIR, XRD, SEM and TEM to ensure nano range and optical properties. Solution casting method was used to prepare nano composite polymeric membranes of 40 micron. Membranes were exposed with low temperature glow discharge argon plasma for 20 minute time to modify surface roughness of membranes. These membranes were characterized for surface and chemical modification by different techniques such as optical microscope, SEM, AFM and FTIR. The Ag NPs modifies antibacterial activity of polymers but plasma treatment enhanced antibacterial activity/sterilization efficiency of membranes by increasing surface content of NPs on membranes. Present study shows applicability of these surface modified nanocomposites polymer membranes for synthesis of antibacterial/sterilized surfaces for clinical and biomedical purposes.

Keywords: Sterilized Surface, Clinical Purpose, Polymer Nano Composites, Plasma Irradiation, Synthetic Membranes.

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1. INTRODUCTION

Polymers have become very important materials in modern manufacturing processes and offers wide varieties of chemical and mechanical properties applicable in electro-optical properties,[1] gas filtration,[2] electrochemical applications[3] and solar cell synthesis.[4] It is well known that permanent bonding,[5] coating,[6] printing,[7] etc. are difficult on many polymers without surface pre-treatment.[8,9] Therefore, after surface treatment of polymers they have significant benefits in specific requirements of surface properties while retaining bulk mechanical properties.[10,11] Physical and chemical surface modifications of polymeric materials without alteration of bulk properties are also of great interest in many biomedical applications.[12]

A number of experimental investigations on polymer nanocomposites have indicate that polymer nanocomposites exhibit new and sometimes improved properties that are not displayed by individual phases or by their conventional composite counterparts.[13–15] Polymer nanocomposites represent a new alternative to conventionally filled polymers.[16,17]

PMMA nanocomposites with high refractive index[18] can be made by incorporating Ag NPs into PMMA, with improve optical[19] and electrical properties.[20] PMMA-Ag composites were also used as additives,[21] digestion breathing through lungs and grills,[22] discharge of sweat from skin,[23,24] environment protection,[25] preservation[26] and biomedical applications.[27]

Silver NPs were also used to control infections and spoilages,[28] due to their well-known anti-bacterial,[28] anti-fungal,[20] anti-biotic properties[29] and high toxicity for microbes.[30] They can be used in microbial delivery vehicles targeting pest insects,[31] bioactive products development,[32] wound-healing,[33] water-treatment,[34] biomedicines,[35] anti-fungal drugs[36] and bio-sensors[37] etc.

Metallic nanoparticles synthesized traditionally by wet chemical method, where used chemicals are often toxic and flammable.[38] Since noble metal nanoparticles are widely used in biological applications shows a growing need to develop environmentally friendly processes for nanoparticle synthesis that does not use toxic chemicals or not generate toxic by-products.[39] Biological methods of nanoparticle synthesis using micro-organisms, plants including algae, fungi, bryophyte, pteridophyta etc. have been suggested as possible ecofriendly alternatives to

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chemical and physical methods.[40] Hence for present study Ag NPs were synthesized using psidium guajava (guava fruit) extract and characterized by various characterization techniques.

Complex nature of plasma due to presence of ions, neutrals and radiation in discharge makes low-temperature plasmas widely usable in a growing number of materials fabrication processes including etching of complex patterns of polymeric membranes.[41,42] Plasma surface treatment usually refers to a plasma reaction that either results in modification of molecular structure of surface,[43] or atomic substitution.[44] Plasma treatment is a useful tool to modify surface properties.[45] Accelerated ions and electrons from plasma have sufficient energy to induce cleavage of chemical bonds in membrane structure and to form macromolecule radicals, that can subsequently initiate graft copolymerization.[46] Plasma treatment can be done by either regular plasma treatment, or plasma graft copolymerization (PGC).[47] The use of low pressure plasma enables modification of polymer materials using various plasma components such as electrons, ions, radical etc.[20,28] These components react with exposed surfaces and if any part of surface were exposed to energies higher than characteristic bond energy of polymers,[48] those parts undergo etching.[49] Metal NPs have higher characteristic bond energy compared to polymer so their etching is in lesser amounts.[50] This makes glow discharge plasma technique is particularly useful to increase surface content/area of nanoparticles on nanocomposite surfaces and removal of surface contaminants.[51,52]

In this study, we have synthesized Ag nano particle by biological root and prepared 3% Ag nano composite Poly methyl methacrylate (PMMA) membrane using solution casting method. These membranes were subjected to surface modification by glow discharge argon plasma irradiation. As an important application of this study antibacterial activity of plasma modified polymer membranes and results were compared with normal membranes. This study shows applicability for synthesis of antibacterial/sterilized surfaces for clinical and biomedical purposes.

2. EXPERIMENTAL DETAILS

Psidium guajava (guava fruit) extract was prepared by taking 25 g of thoroughly washed plant material in a 250-mL erlenmeyer flask with 100 mL of Mili-q water and then boil mixture for 10 min in water bath. The leaf broth was then cooled and filtered through Whatman No.1 filter paper (pore size 25 μm). For preparation of silver nanoparticles, 10 mL of the prepared plant extract was added to 90 ml of 1 mM silver nitrate solution and incubated in rotary shaker for 2 hours. Color of solution changed from light yellow to brown indicating the formation of silver nanoparticles.[33,34]

Poly methyl methacrylate (PMMA) granules (2 mm, 0.02 gm. Each, obtained as commercial grade from Loxim Polymers, Jaipur) were used to prepare flat sheet membranes by solution cast method. Dichloromethane of extra pure grade was used as a solvent for preparing 10% polymer solution. PMMA granules are weighed and dissolved in dichloromethane (CH_2Cl_2) to prepare a 10% solution. Agitation of solution is important, since solvent penetration is very slow for high molecular weight polymers and viscous coating is usually formed over each particle. Solution was stirred by magnetic stirrer to ensure uniform dissolution and to enhance rate of dissolution. Process is carried out at room temperature for around 2–3 hours till a clear solution was formed. Solution was then put into flat-bottomed Petri-dishes. Solvent was allowed to evaporate slowly over a period of 10–12 h. The films so obtained were peeled off using forceps.[35] For nanocomposite membrane PMMA solution was prepared in same way as mentioned above for pristine membranes. Ag nanoparticles were dispersed in dichloromethane using ultra-sonication. This dispersed solution was added to PMMA solution and stirred for

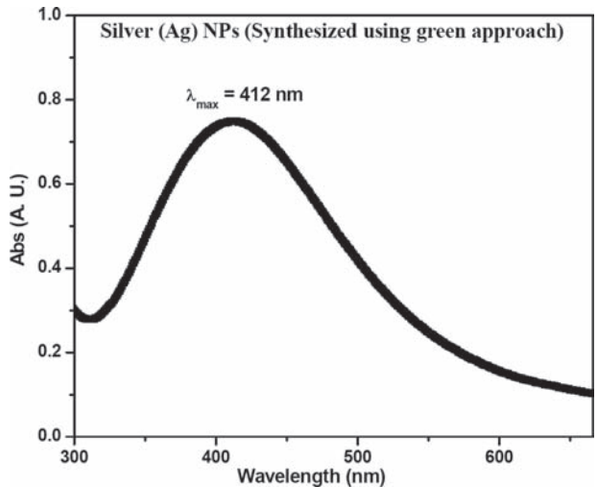

Fig. 1. UV-Vis absorption spectrum of silver nanoparticles.

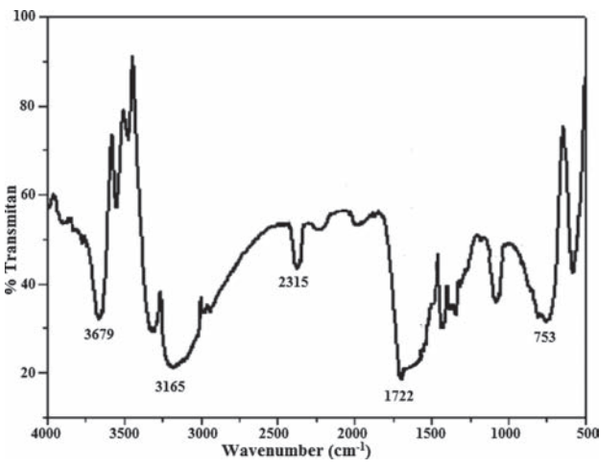

Fig. 2. FTIR spectra of vacuum dried powder of silver nanoparticles.

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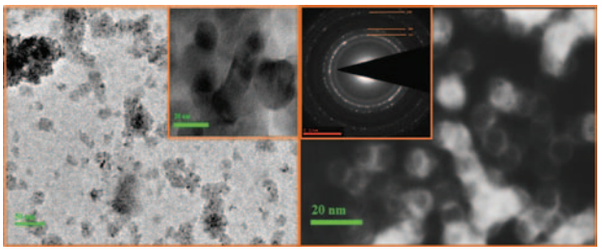

Fig. 3. TEM and diffraction pattern of Ag NPs (a) Ag NPs at 50 nm scale, (b) Ag NPs at 20 nm scale, (c) diffraction pattern and (d) dark field image of Ag NPs at 20 nm scale.

around 30 minutes. Solution was then poured into flat-bottomed petri-dishes floating on mercury to ensure a uniform thickness of membranes. Solvent was allowed to evaporate slowly over a period of 10–12 hours. The films so obtained were peeled off using forceps.

Argon plasma irradiation set up has a source chamber with power supply and vacuum system. Argon gas used as plasma gas is admitted into source chamber using a flow controller. Applied high voltage between two electrodes in magnetic field generates DC glow discharge. Chamber is evacuated to a base pressure of 10^{-10} torr and working pressure is maintained at 10^{-7} torr by admitting argon gas. Current in upper and lower electrodes is maintained at few mA and 3.2 KeV. The plasma is almost homogenous at this condition. Energetic species in this plasma includes ions, radicals, electrons and meta-stable photons in short-wave UV range.

UV-Vis spectrum of NPs was taken using UV-Vis spectrophotometer SHIMADZU 1800. FTIR Analysis was done using FTIR spectrophotometer (IR Affinity-1 Shimadzu) in the range of 4000–400 cm^{-1} for determination of possible functional groups present with synthesized Ag NPs. Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Morphology and particle size of Ag NPs are also determined using transmission electron microscopy (TEM) Technika TEM instrument operating at 200. Chemical modification before and after plasma treatment were recorded by Nicolet Megna IR 550 single beam FTIR spectrometer.

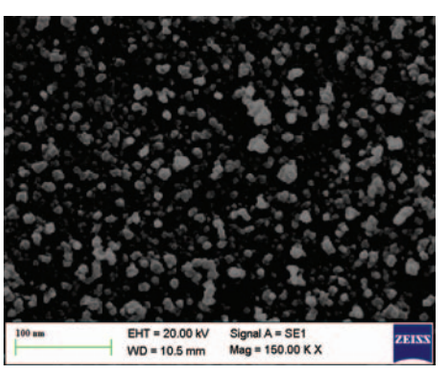

Fig. 4. SEM image of Ag NPs.

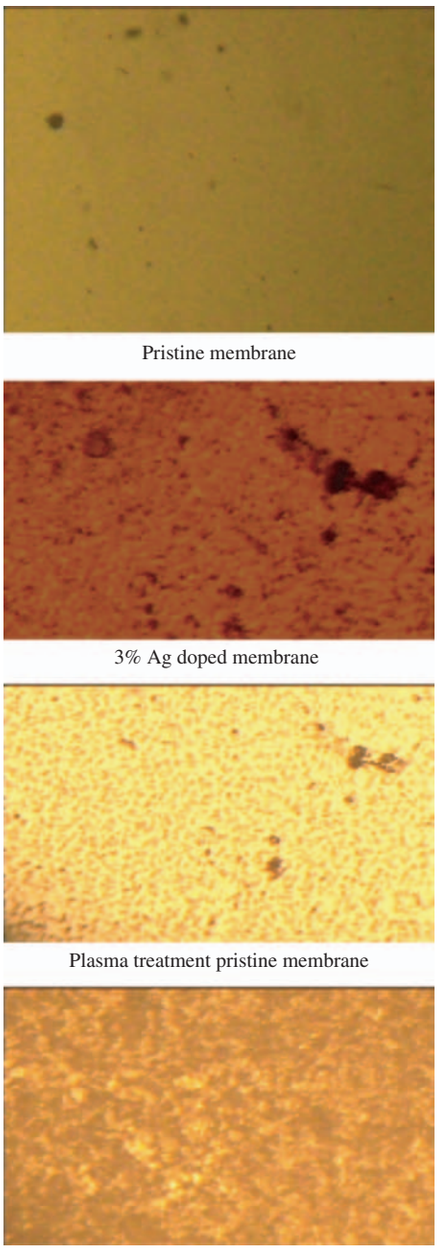

Fig. 5. Optical microscope images of pristine and Ag nanocomposite membrane before and after plasma irradiation.

LABOMED optical microscope, Hitachi S-4500 SEM and Nanosurf easyScan 2 were used to identify surface modification before and after plasma treatment.

As polymers are receiving grate interest for synthesis of antibacterial (bacteria free) surfaces, blood purification systems and bags, environment protection and preservation and many other biomedical applications. Small addition of Ag NPs to polymers can improve efficiency of bacteria killing and antibacterial activity of polymers. Hence these membranes were tested for their bacteria killing

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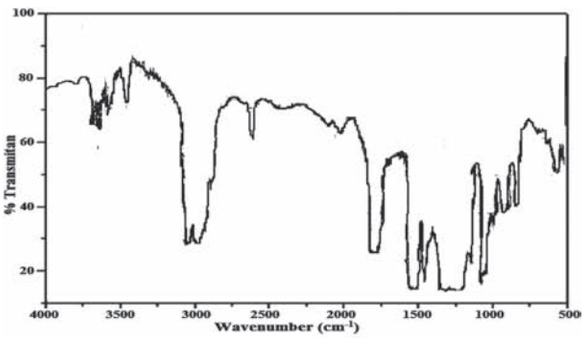
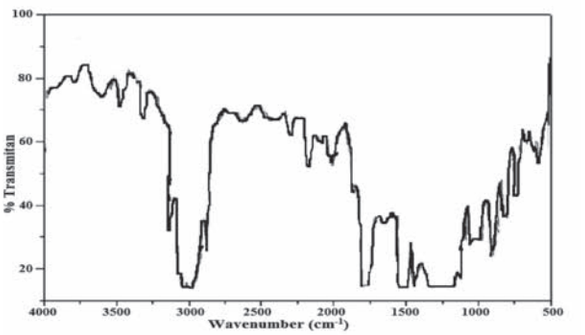
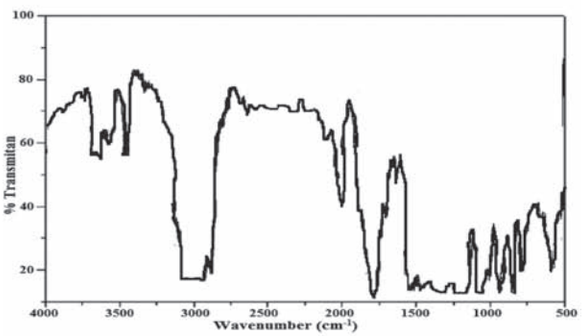


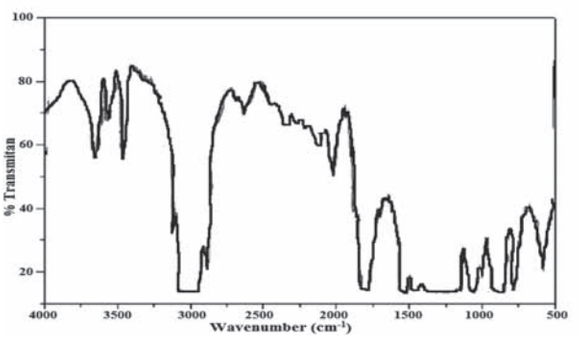
Pristine membrane



3% Ag doped membrane

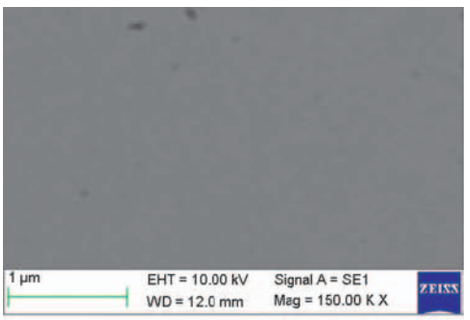


Plasma treatment pristine membrane

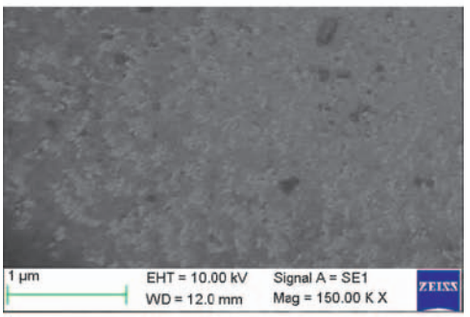


Plasma treated 3% Ag membrane

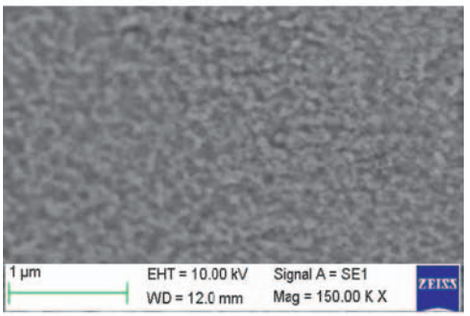
Fig. 6. FTIR spectra of pristine and Ag nanocomposite membrane before and after plasma irradiation.



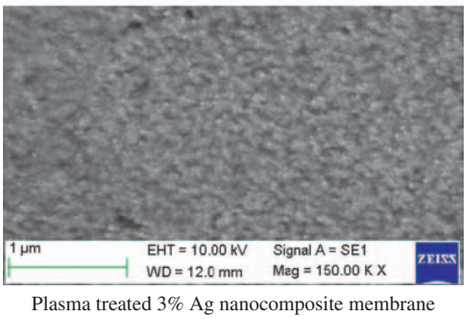
Pristine membrane



3% Ag nanocomposite membrane



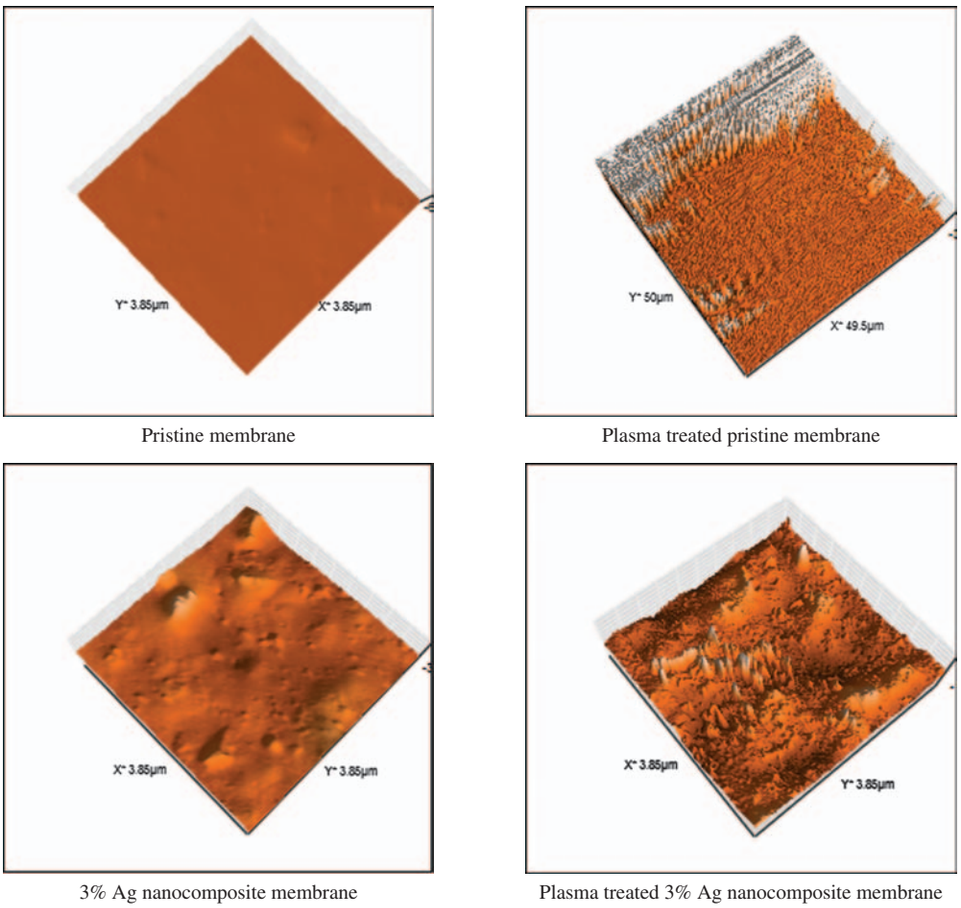
Plasma treated pristine membrane



Plasma treated 3% Ag nanocomposite membrane

Fig. 7. SEM images of pristine and Ag nanocomposite membrane before and after plasma irradiation.

efficiency before and after plasma treatment. Nutrient agar (autoclaved) media were speared on each autoclaved membrane. Standard *E. coli* inoculums were prepared by method as described by Agrawal et al.[28] having *E. coli* (≈115 cfu/mL). Few ml of this E. coli inoculum was speared on agar (Autoclaved) media containing membranes. These membranes were kept at 37 °C in incubation chamber (i.e., favourable condition for bacteria growth). Optical density was recorded after each two hours, using spectrophotometer to study bacteria killing efficiency of Plasma irradiated Ag nanocomposite polymer membranes.



Pristine membrane | Plasma treated pristine membrane

3% Ag nanocomposite membrane | Plasma treated 3% Ag nanocomposite membrane

Fig. 8. AFM images of pristine and Ag nanocomposite membrane before and after plasma irradiation.

3. RESULTS AND DISCUSSION

Figure 1; UV-Visible absorption spectrum shows characteristic surface plasmon resonance (SPR) peak of Ag NPs at 412 nm ($E_g = 3.01$ eV), indicating reduction of silver nitrate and formation of Ag nanoparticles.

After complete reduction of Ag^+ ions by plant extract and formation of Ag NPs, they was analysed by FTIR spectrophotometer (IR Affinity-1 Shimadzu) in the range of 4000–400 cm^{-1} to identify possible functional groups responsible for the formation of silver nanoparticles (Fig. 2). The absorption bands as obtained are classified as (a) 753 cm^{-1}, stretching vibration of Ag NPs (b) 1686 cm^{-1}, C═C unsaturated (c) 1722 cm^{-1}, C═O stretching vibration (d) 3165 cm^{-1}, CH_3 stretching vibration and (e) 3679 cm^{-1}, C—H stretching vibration of aromatic compound.

Ag NPs were dispersed in water by ultra-sonication and used for particle size analysis by TEM (Fig. 3). Image clearly shows spherical shape NPs with particle size ranging from 10–15 nm. Grain boundaries can be easily identified from TEM images showing no aggregations in Ag NPs, especially particle size and grain boundaries can more clearly identified form dark field image of Ag NPs (Fig. 3(d)). For this study, TEM image is best indicator of particle size. But to determine aggregation present in NPs at higher sample amounts, we have also recorded SEM images of synthesized Ag NPs (Fig. 4). As individual Ag NPs can easily be identified form SEM image, showing no aggregation in NPs even at higher sample amount.

These Ag NPs were used for synthesis of Nanocomposite Polymer Membranes and Argon ion plasma irradiation was done and characterized using different techniques before and after plasma treatment.[15]

LABOMED optical microscope was used for recording optical micrographs. Micrographs were stored in computer through CCD camera, attached to computer with standard Pixel View software. Images shows that pristine membrane have very smooth surface but plasma treatment increase its porosity and roughness. Nanocomposite membrane is comparatively having high porosity but plasma treatment further increases their porosity and roughness (Fig. 5).

FTIR is used to gather information about chemical structure of compound and chemical modification after

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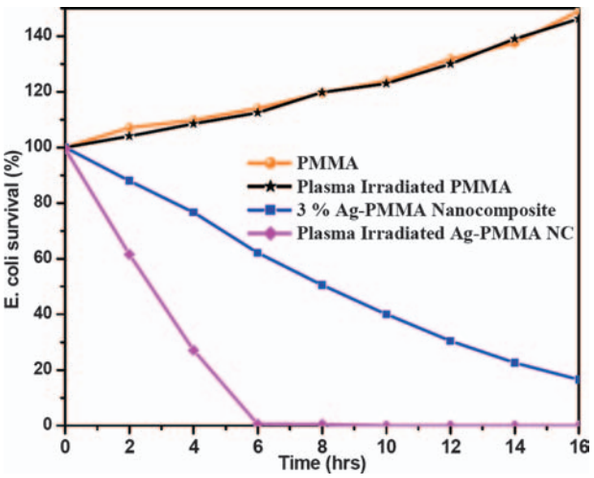

Fig. 9. Efficiency of bacterial survival on Ag nanocomposite polymer membranes before and after plasma irradiation applicable in bacterial resist films formation or bacteria-sterilization.

plasma treatment. FTIR images of pristine and doped (untreated and plasma treated) membranes are as shown in the Figure 6. We notice certain characteristic differences in peaks at certain wavelengths. These wavelengths are indicators of change in chemical bonding and structure of samples due to plasma treatment. Peaks at around 3000–2750 cm^{-1} are characteristic of $–CH_3$ bonds. Similar peaks in FTIR spectra of treated and untreated samples show that there is no change in basic structure of nanocomposite membranes. Increments in absorption bands of C—O at 1030 cm^{-1} and C=O at 1770 cm^{-1} has been attributed to creation of unsaturated –C=C– bonds at 1645 cm^{-1} after plasma treatment. It is also observed from FTIR spectra that C—C and C—H bands have been decreased after plasma treatment. It indicates that polymer material get etch out after plasma irradiation.

We have investigated surface morphologies of this nanocomposite membrane using SEM and AFM. SEM and AFM images of nanocomposite membrane before and after plasma irradiation are shown in Figures 7 and 8 respectively. Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine while AFM was done using Nanosurf easyScan 2. Plasma treated nanocomposite membrane can be compared with non-irradiated one shows improve in porosity and roughness after plasma treatment.

Effect of plasma irradiation on Ag nanocomposite polymer membrane for bacterial resist film formation/surface sterilization was determined by measuring Optical density after each two hours, using spectrophotometer. Figure 9 shows % bacteria survival or bacterial growth on samples, calculated with respect to initial concentration of bacteria on membranes. 3% bacterial growth per hour for PMMA membrane and almost similar bacterial growth was found on plasma treated PMMA membranes i.e., they support bacteria growth. But -5.00% (negative) per hour bacteria growth was found for Ag nanocomposite PMMA i.e., almost all the bacteria placed on these membrane were killed after 20 hour. After plasma treatment the rate enhanced to -20% i.e., all the bacteria were killed with in 5 hour. This is due etching of polymer by plasma irradiation and it enhances surface amount/concentration of Ag NPs on nanocomposite surface. Typical sterilization efficiency of these nanocomposite membranes makes them suitable for synthesis of bacteria free surfaces for clinical/biomedical purposes as amount of bacteria in environment is very less then bacterial culture (≈ 115 cfu/mL) used here.

4. CONCLUSIONS

Color of solution changes from yellow to black as plant extract was mixed in aqueous solution of silver ion complex, this gives primary indication of formation of silver nanoparticles, which is further confirmed by analysing of these NPs by different technique like UV-Vis spectrophotometer, FTIR, TEM and SEM. UV-Visible spectrum obtained in visible range of 300 nm to 800 nm. A typical absorbance SPR peak at 412 nm of silver nanoparticles was obtained due to surface plasmon vibrations of silver nanoparticles. Particle size of biosynthesized silver nanoparticles were further confirmed by TEM and SEM measurements which comes around 10–15 nm. FTIR measurement carried out to identify possible interaction between biomolecule and silver NPs. The FTIR measurements of biosynthesized silver nanoparticles show absorption bands at around 783 cm^{-1}, 1686 cm^{-1}, 1722 cm^{-1}, 3168 cm^{-1} and 3679 cm^{-1}.

Pristine PMMA membranes and Ag nanocomposite membrane (3 weight%) were prepared by solution cast method. Argon Plasma irradiation technique applied here has shown considerable modulation in chemical and surface properties of polymer membranes. Optical microscope, SEM and AFM images shows high porosity, roughness and etching of polymer material after plasma treatment. The increments in absorption bands have been attributed to creation of unsaturated bonds and etching of polymer after plasma treatment.

Plasma treated Ag nanocomposite membranes were further texted for bacterial sterilization and found that plasma treatment enhanced the bacterial killing efficiency/sterilization on Ag nanocomposites to be used for bacterial resist films formation.

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RESEARCH ARTICLE

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