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RESEARCH ARTICLE

WOUND DRESSING EFFECT OF SILVER-PEG NANOCOMPOSITE: SYNTHESIS AND ITS CHARACTERIZATION

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ABSTRACT

Silver (Ag)- Poly ethylene glycol-4000(PEG), Silver-PEG nanocomposite materials were synthesized by a microwave assisted method. The synthesized Silver-PEG nanocomposite contains weight percentage 50 (50 wt%) silver. The Silver-poly ethylene glycol nanocomposite was characterized using Transmission electronic microscope (TEM), UV– visible and Fourier transform infrared spectroscopy (FTIR). The UV absorption pattern indicated the presence of silver in the nanocomposite. The spherical morphology of silver nanoparticles was confirmed from the TEM image. FTIR spectroscopy was used for the structural elucidation. Silver- PEG nanocomposite exhibits good antimicrobial and antitumor properties. The reduction of silver ions into silver nanoparticles was achieved in starch solution and poly (ethylene glycol)-4000(PEG -4000). The incorporation of silver nanoparticles in the PEG material was confirmed by Fourier Transform Infrared (FTIR) spectroscopy analysis. In addition, the formed silver nanoparticles have an average particle size of ~ 16.5nm was observed by Transmission Electron Microscopy (TEM). The anti-microbial silver- PEG nanocomposite was demonstrated significant effects against Escherichia coli (E. coli), Pseudomonas, Staphylococcus. Therefore, the present study explained the in situ preparation of Silver-poly ethylene glycol nanocomposite material and it was used in biomedical applications as antimicrobial packaging and wound dressing and antibacterial materials.

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INTRODUCTION

In fact, nowadays it is still used in several commercially-available products such as composites with undergo a slow silver release and which act as preservatives, or products containing silver thiosulfate complexes which are introduced in packaging plastics for long-lasting antibacterial protection of packed products. (Gupta and Silver, 1998; Jose Ruben *et al*., 2005) Up to now, silver ions (Ag+) have demonstrated to be useful and efficient for antibacterial purposes, (Brown and Anderson, 1968) but, due to their unique properties, silver nanoparticles represent a reasonable alternative for boosting the development of new bactericides. Because of their high surface area to volume ratio and their high active surface, metal nanoparticles exhibit remarkable and outstanding properties, such as increased catalytic activity. Synthesis of metal nano particles may be carried out through various synthetic routes based either on bottom-up or top-down approaches, which have been summarized in recent publications. (Nanoscale Materials in Chemistry, 2004; Ajayan, 2004; Springer Handbook of Nanotechnology, 2004; Campelo *et al*., 2009; Macanás *et al*., 2011; Schmid, 2005) However, as it has been abovementioned, preventing their escape and controlling both their size and shape is decisive, so the presence of stabilizers is very often required. (Houk *et al*., 2009; Imre *et al*., 2000; Kidambi and Bruening, 2005) As a result, the development of stabilized polymer-metal nanocomposites containing metal nano particles is considered to be one of the most promising solutions. The broad spectrum antimicrobial properties of silver encourage its use in biomedical applications, water and air purification, food production, cosmetics, clothing, and numerous household products development. Besides, with the rise of the Nanotechnology, and as a result of reducing the scale of metals particles, new electrical, optical, magnetic, and chemical properties appear, so a new control on the behaviour of silver metal has been achieved. With increasing focus on green chemistry, natural compounds like glucose, chitosan, starch have attracted as considerable research interest as safer alternatives, reducing and stabilizing agents to synthesize the silver nanosphere (GAO Xianghua *et al*., 2011; Muhammad Amin *et al*., 2012). Starch has been shown as a good cppingagent for more work on inorganic nanoparticles such as gold and silver to form inclusion complexes (Li *et al*., 2011).

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Starch is one of the most abundant materials. It is widely-used in stabilizing and controlling size and shape of metal nanoparticles. Silver (Ag) nanoparticles have high therapeutic potential and exhibit good antimicrobial activity. Ag nanoparticles have a wide range of antimicrobial activities and exhibit high performance even at a very low concentration. Ag nanoparticles have been identified to possess good potential for the treatment of cancer. But the major disadvantage of using silver alone is that it is not specific at targeting the cancer cells and also it is toxic to the normal cells when exposed for a longer time when the size of silver used is >20 nm. Ag-PEG nanocomposite is one of the rare composite materials that is seen to possess a capability of being used as a biosensor as well as in the treatment of cancer as the PEG present in the nanocomposite is very specific to the cancer cells. It prolongs the action of silver on the affected cells while preventing the normal cell from the effect of silver. One more advantage of this nanocomposite is that it is biodegradable, i.e., it can be degraded by the enzymes present in the body making it suitable for the treatment of cancer. Apart from the treatment of cancer, the nanocomposite also possesses good antimicrobial and biosensing activity.

This work reports the use of starch as a template and reducing agent in the preparation of silver nanoparticles. The incorporation of silver nanoparticles into a polymer matrix has gained a lot of interest due to the possibility of these materials to combine properties from both organic and inorganic systems [20]. In this work, Silver-PEG nanocomposite was synthesized by the microwave chemical method with 50 wt% Ag. Silver-PEG was successfully synthesized and characterized with Fourier transform infrared spectroscopy (FTIR), UV –visible spectroscopic analysis (UV) and Transmission Electron Microscopy (TEM).

MATERIALS AND METHODS

Reagents

Silver nitrate, Poly ethylene glycol (4000), and starch were purchased from Aldrich USA, Loba Chemie and Merck India and were used without any purification. All the solutions were prepared in deionized water.

Preparation of Silver-PEG nanocomposites

Silver nitrate (5 mM) was added with 1% starch solution. The resultant solution was stirred for 30 min. The transparent solution was taken and the reaction was carried out under microwave irradiation, employing a domestic microwave oven. During preparation, the solution turned to the characteristic yellowish brown colour, then to grayish black, indicating the formation of silver nanoparticles. The silver nanoparticles thus prepared in starch using microwave wet chemical methods were stable for two months at room temperature, showing that the starch was a good reducing and stabilizing agent for the silver nanoparticles. The Silver-PEG nanocomposite was synthesized by freeze gelation method. Water was used as the solvent to prepare polymer solution. PEG was dissolved by using magnetic stirrer for 3h and the polymer solution was left overnight at room temperature to remove the air bubbles trapped in the viscous solution. Then suitable amount of Silver was dispersed in deionised water by 30 min ultrasonication. Ultrasonication was necessary to avoid agglomeration and to achieve proper dispersion. Nanosilver solution was mixed with polymer solution under agitation. The homogeneously mixed solution is immediately taken to microwave oven. After 10 minutes the samples were taken out and it was cooled. The homogeneously mixed solution is taken to deep freeze at –18 ^{o}C. After 48 h freezing the samples were freeze dried. The Silver –PEG nano composites were coded as Silver–PEG 50 where number denotes the wt% of PEG.

Characterization methods

The synthesized silver nanoparticles were characterized spectrophotometrically using Perkin Elmer UV-visible spectrometer. Surface morphology of Silver-PEG nanocomposite was observed using a HITACHI-SU 6600 TEM. Functional group confirmations were assessed by SHIMADZU IR-affinity FTIR spectrophotometer.

RESULTS AND DISCUSSION

FT-IR Spectroscopic studies

The FTIR spectra of the starch silver nanoparticles were recorded in order to identify the functional groups of starch involved in the reduction and capping/stabilization of the synthesized nanoparticles. Figure 1a showed the Fourier transform infrared spectrum for the synthesized Silver nanoparticle. An extremely broad band due to hydrogen bonded hydroxyl groups (O–H) appears at around 3333cm^{-1} for starch, which narrows down to 3334 cm^{-1} band in the case of silver nanoparticles. The characteristic broad and strong absorbance band at 1636cm^{-1} which could be assigned to the hydroxyl (O-H) group stretching vibrations. A shift from 3333 cm^{-1} to 3334 cm^{-1} is observed for stabilized silver nanoparticles. It can be found that the IR spectra of silver nanoparticles as well as that of starch are more or less similar. The peaks at 1152cm-1, 1080 and 1021 cm–1 attributable to the anhydro glucose ring O–C stretch indicating a possible coating of the silver nanoparticles with starch.

The absorption bands at 3368 cm−1 was due to the O–H stretching band, 2901 cm−1 was due to the aliphatic C–H stretching, 1456, 1374 and 1339 cm−1 were due to C-H bending vibrations, and also the combination band of O–C–H and C–O–H deformation is calculated from 1456 to 1259 cm^{-1}. Then the in plane C–H and O–H deformation from 1259 to 1018 cm^{-1} can be observed. In the region from 1145 to 554 cm−1, the C–O and C–C groups vibration modes are present and the carbohydrates generally show their characteristic bands. The interaction of silver nanoparticles obtained with PEG and gluconic acid products by reduction of starch compound were confirmed by FT-IR spectra (Figure 1b). Intense absorptions are observed at 1731, 1652 and 1018 cm−1. The IR band at 1730 cm−1 is characteristic of the C=O stretching mode of the carboxylic acid group for gluconic acid. The bands due to C–O stretching mode were merged in the very broad envelope centered on 1259 and 1018 cm^{-1} arising from C–O, C–O–C stretches and C–O–H bends vibrations of silver nanoparticles in PEG. Also, the aliphatic C–H stretching, in 1456 and 1259 cm−1 were due to C–H bending vibrations. After the bio-reaction of starch with the $AgNO_3$ in the PEG matrix, the created peak in 1731 cm^{-1} certified to the binding of –C=O for carboxylic acid in gluconic acid, and the shift in the peak at 1018 cm^{-1} towards lower frequency compared to peak in 1094 cm^{-1} for PEG is attributed to the binding of C–C–O and C–C–H groups with nanoparticles. The broad peaks in

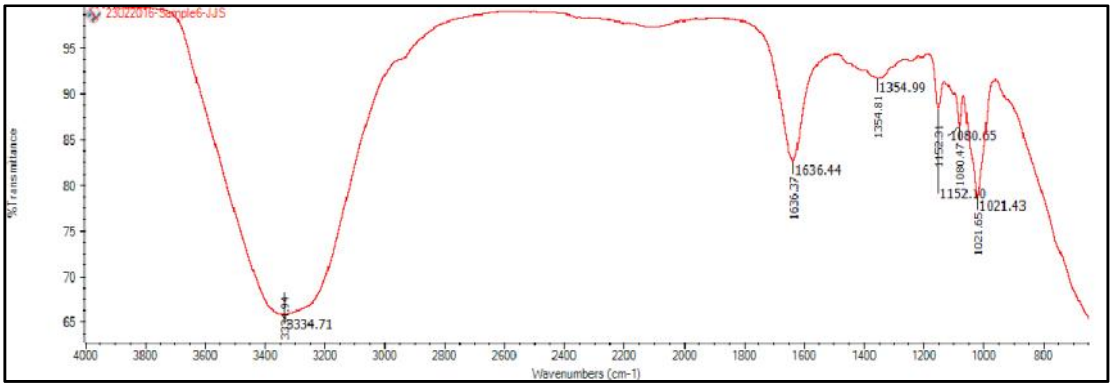


Figure 1a FT-IR spectrum of Silver nanoparticle

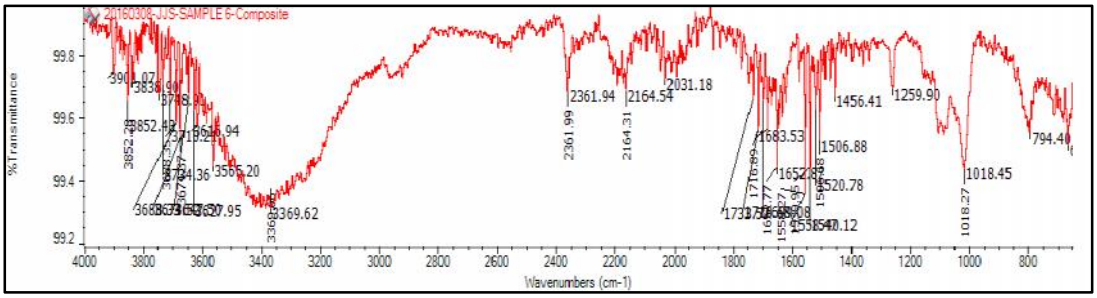


Figure 1bFT-IR spectrum of Silver-PEG nanocomposite

503cm^{-1} related to silver nanoparticles banding with oxygen from hydroxyl groups of PEG chains. Therefore, the FT-IR spectra showed the existence of van der Waals interactions between the chain of PEG and silver nanoparticles in the polymeric media.

UV-Visible Spectroscopic studies

The formation of silver nanoparticle in the polymeric media was further determined by using the UV-visible spectroscopy. It was observed that the spherical silver nanoparticles contributed to the absorption bands at around 400 nm in the UV-visible spectra. This was confirmed by the TEM results also. Also absorption spectra of larger metal colloidal dispersions can exhibit broad peaks in the lower absorbance in the UV-visible range

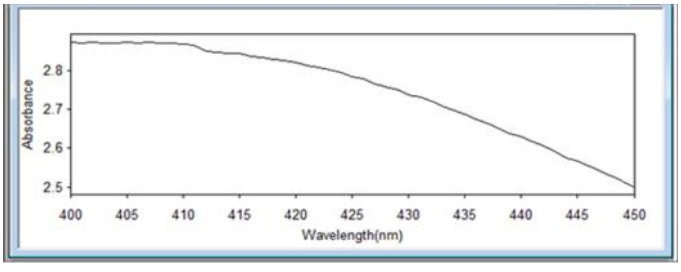


Figure 2a UV-Vis absorbance spectrum of Silver nanoparticle

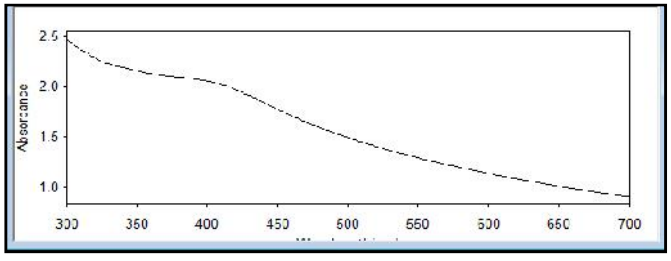
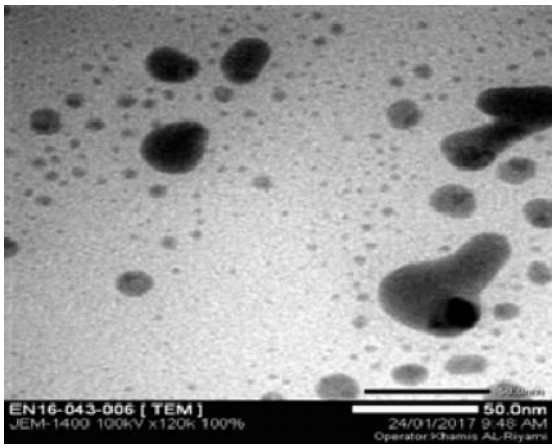


Figure 2b The UV-Vis absorbance spectrum of Silver- PEG nanoomposite

Figure 2a and 2b showed the absorption occurred at 420 nm for starch stabilized silver nanoparticles and silver –PEG nanocomposites, indicating the formation of silver nanoparticles.

TEM studies



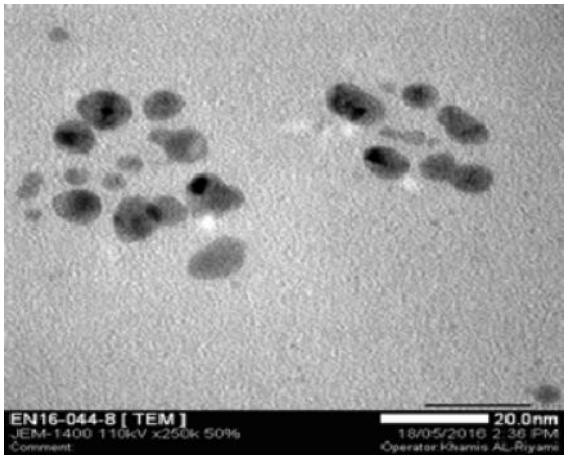


Figure 3a. TEM image of Silver nanoparticle

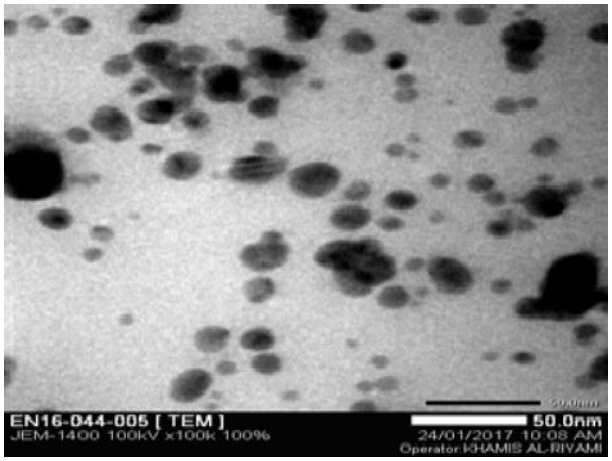


Figure 3b. TEM image of Silver-PEG nanocomposite

TEM images clearly confirm that the silver naoparticles formed under microwave irradiation have an average size of 20- 50 nm (Figure 3a). The TEM image of silver-PEG nanocomposites is presented in Figure 3b. TEM image clearly showed the spherical morphology of nanoparticles.

Electrical properties

Table 1. Electrical properties

Samples	Conductvitiy Values
Silver nano particles	84.3 µs/cm
Silver- PEG nanocomposite	36.5 µs/cm

Encapsulated silver nanoparticle showed the lowered conductivity value. Table 1 shows the overall electrical behaviour of polymeric nanocomposite. However, in order to prove that the localization of charge carriers in starch –PEG based polymer may prevent the growth of bacteria, further research such as *in-vitro studies* is required.

Antibacterial Application of Silver Nanocomposites

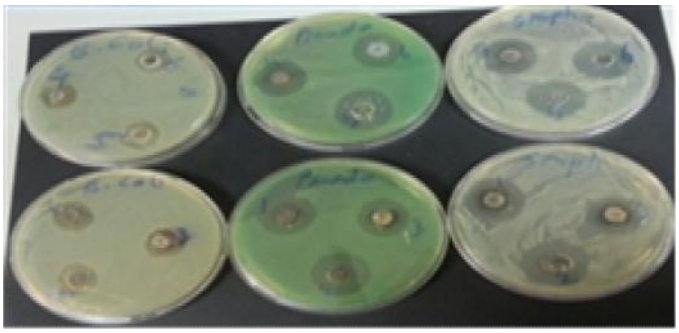


Figure 5. Antibacterial activities of silver nanoparticles and Silver – PEG nanocomposite

The use of Ag-containing PEG films as functional wound dressings is assessed by observing their antimicrobial activity (based on the disc diffusion method) against some common bacteria found on burn wounds: *E. coli, P. sudomonas, Staphylococcus.* It exhibited the typical antimicrobial test results of films by the disc method. It was found that the exhibited an inhibition zone. The nutrient agar was spread into the Petri dish over the whole film and, the *E. coli* culture is streaked on the solid surface of the media. A significant difference was observed in the plates. The results clearly indicated that the Silver–PEG nanocomposite was exhibited excellent antimicrobial activity.

In vitro bioresorbability test

Figure 6 shows the in vitro bioresorbability of pure silver – PEG nanocomposite. A considerable variation in pH value is observed with respect to soaking period in silver-PEG 50 composition. It can be seen that the pH value of SBF solution does not exhibit much variation in pH value by the addition of silver-PEG nano composite. After the addition of silver-PEG 50 an appreciable change in the pH value was noticed. There is no evidence for the decrease in pH with increase in soaking period. Biological mechanism can be studied in future research work. It was concluded that the samples have no adverse bioresorbability response.

Table 2. *In vitro* bioresorbability test

Samples	1 hour	24 hours	72 hours	96 hours	144 hours	168 hours	192 hours
Silver nano particle	5.69	5.74	5.96	5.88	6.12	5.92	6.00
Silver-PEG nanocomposite	4.65	4.52	4.57	4.51	4.46	4.60	4.71

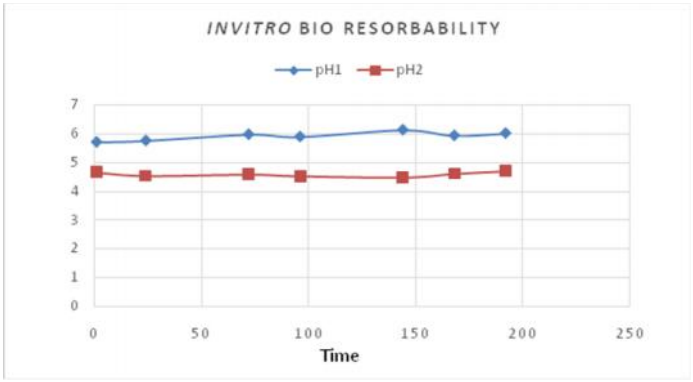


Figure 6. Invitrobioresorbability studies of silver nanoparticles and Silver– PEG nanocomposite

Conclusion

In this study, silver nanoparticles were synthesized by chemical reduction method and embedding them into PEG matrix to form Silver-PEG nanocomposites. Various characterisation techniques also confirmed the synthesis of silver-PEG nanocomposites. Results showed that antibacterial activity of PEG nanoparticles was significantly enhanced when loaded with silver nanoparticles. It was concluded that the silver-PEG nanocomposite have no adverse bioresorbability response. In future work the biological mechanism of *In-vitro* bioresorbability studies can be studied in detail. Therefore, the present study clearly provides novel antimicrobial material which is potentially useful in food packaging and wound dressing.

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