

MICROWAVE SYNTHESIS OF SILVER NANOPARTICLES BY POLYOL METHOD AND TESTING THEIR SYNERGISTIC ANTIBACTERIAL ACTIVITY IN THE PRESENCE OF VANCOMYCIN

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ABSTRACT

Objective: The aim of this study is to prepare silver nanoparticles (AgNPs) and nanorods using polyol under microwave heating method.

Methods: The synthesized nanoparticles were characterized using UV-visible, infrared spectral studies (Fourier-transform infrared [FTIR]), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Finally, the antibacterial activity of polyethylene glycol (PEG) protected AgNPs, against a series of Gram-positive and Gram-negative bacteria, was studied.

Results: From UV-visible spectroscopy studies, the growth rate of AgNPs was confirmed by measuring the change in absorbance of plasmon peak shape at different time intervals. FTIR spectroscopy studies showed the changes in the metal and the capping agents. SEM and TEM images revealed that very stable colloidal solutions of AgNPs with almost spherical and rod shape along with high monodispersity can be obtained with PEG protected AgNPs. Finally, the antibacterial activity of PEG protected AgNPs was investigated, which showed a synergistic role for the AgNPs along with vancomycin.

Conclusion: Uniform and stable polymer protected AgNPs were synthesized using ethylene glycol, triethylene glycol, and PEG as reducing agent in the presence of polyvinylpyrrolidone.

Keywords: Polyol, Silver nanoparticles, Ethylene glycol, Triethylene glycol, Polyethylene glycol.

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INTRODUCTION

Nanotechnology is one of the fascinating areas of research in the last few decades. During the past two decades, considerable attention has been paid to the synthesis of metal nanoparticles due to their unique optical, magnetic, electronic, catalytic properties, and applications [1]. A number of methods have been developed for the preparation of nanoparticles including citrate method [2], radiolytic reduction [3], sonochemical method [4], microemulsion route [5], polyol process [6], alcohol reduction [7], microwave (MW) [8], and green synthesis [9]. Among these, MW synthesis approach is considered to be a green chemistry approach for the synthesis of metal nanoparticles because of its fast and large-scale synthetic capabilities. Polyol process involves the reduction of the soluble silver species by ethylene glycol and other polyhydric alcohols, nucleation of metallic silver, and growth of the individual nuclei in the presence of a suitable protective agent [10]. A variety of polymers can be used as protective agents such as polyvinyl alcohol, polyacrylic acid, polyvinyl pyrrolidone (PVP), and polyethylene glycol (PEG), which are used to stabilize the silver nanoparticles (AgNPs) [11]. The presence of this capping agent is also essential for preventing the coalescence of the nuclei during the growth steps. Some of the important considerations in this method include avoiding the particle-particle adhesion and sintering during the nucleation and growth of the AgNPs and prevention of sintering of the particles by adding a required amount of protective agent. It has been reported that PEG is an excellent protective agent for the synthesis of metal nanoparticles in aqueous medium without adding any external reducing agent [12]. The polymers are biocompatible, odorless, non-volatile, and non-irritating. It has been known that ethylene glycol and PEG are environmentally benign materials. They have been widely used in pharmaceutical industry as solvents, dispersing agents, ointments, etc. Ethylene glycol and its derivatives including PEG play a dual role as stabilizing agent as well as reducing agent because it is made of building

blocks of ethylene glycol units. In this paper, we have developed a novel synthesis route to reduce silver nitrate (AgNO_3) using polyol method under MW irradiation.

METHODS

Chemicals

AgNO_3 was used as a precursor of Ag particles. PVP was used as the stabilizing agent. PEG was used as reducing as well as protective agent. Potassium chloride and ferric chloride were used as solvents. All the chemicals were used as received from the suppliers without further purification.

Instrumentation

The UV-visible absorption spectrum was recorded on a Perkin-Elmer UV visible spectrophotometer Lambda 35 in the range of 190–1100 nm at a scan rate of 480 nm/min. Infrared spectra of the complexes are taken in potassium bromide disc using Shimadzu Fourier transform infra-red (FTIR) Spectrometer. Scanning electron microscopy (SEM) was carried out using JOEL-JSM-6360 instrument. The transmission electron microscopy (TEM) was recorded on ZOEL (100) instrument. The antibacterial studies were carried out using Kirby-Bauer test.

Experimental procedures

MW synthesis of AgNPs using ethylene glycol, triethylene glycol, and PEG 600

Various concentrations of PVP (20 mg, 30 mg, and 50 mg) were used with the same concentration of AgNO_3 (10 mg). A known amount of PVP was dissolved in 20 ml of ethylene glycol, triethylene glycol, and PEG 600. To synthesize polymer protected Ag cluster under MW mode, a domestic oven of frequency 2450 MHz was operated in a cyclic mode to prevent intense boiling of solvents as well as aggregation of metals.

Then, 10 mg of AgNO_3 was added to the hot PVP solution and irradiated by the MW oven. The temperature increased linearly and rapidly at a fast heating rate and reached 200°C after 1 min with the formation of yellow-colored silver colloids. Thus, the reaction was carried out only for 1 min. At the end of the reaction, the medium was rapidly cooled in running water until room temperature was reached. The prepared colloidal solution of Ag clusters was used for further characterization. Similarly, the above set of procedure was repeated for 5 times and characterized.

MW synthesis of AgNPs in PEG 600 in the presence of 0.1M KCl and 0.1 M FeSO_4

4–5 drops of KCl and FeSO_4 was added to 50 mg of PVP was dissolved in 20 ml of PEG and heated in the MW oven. Then, 10 mg, 20 mg, or 30 mg of AgNO_3 was added to the hot PVP solution and irradiated by MW oven. The temperature increased linearly and rapidly at a fast heating rate and reached 200°C after 1 min with the formation of yellow-colored silver colloids. Thus, the reaction was carried out only for 1 min. Similarly, the above set of the procedure was repeated for 5 times. The final colloidal solution was cooled to room temperature and used for further characterizations. Same concentrations of PVP (50 mg) were used with various concentrations of AgNO_3 (10, 20, and 30 mg).

Vancomycin AgNPs

4–5 drops of KCl and 20 mg of PVP were dissolved in 20 ml of PEG, and 10 mg of vancomycin was added. The mixture was heated in a MW oven. Then, 10 mg of AgNO_3 was added to the hot PVP solution and irradiated by MW for 1 min. The final colloidal solution was cooled to room temperature and further characterized.

RESULTS AND DISCUSSION

UV-visible spectral studies

It is one of the most widely used techniques for structural characterization of AgNPs. The formation of AgNPs was verified by UV-Visible spectroscopy where an intense band centered at about 415 nm was clearly detected and their intensity increased with increasing metal salt-to-polymer ratio and also with increasing units of polymer. This band was identified as a surface plasmon resonance is due to a collective excitation of the free electrons in the nanoparticles. The shape of the plasmon band is almost symmetrical, suggesting that the nanoparticles are well dispersed and spherical. When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, a strong absorption takes place, which is the origin of the observed color. Here, the color of the prepared AgNPs is yellow [13]. The change in UV-Visible absorption indicates that the size and dispersion of AgNPs were affected by both the concentration of polymer and AgNO_3 . Polymers are used as a protective medium for the colloids, and it affects the molecular motion of reduced silver and expected to be adsorbed on the surface of silver particles and subsequently limits the aggregation of colloids [14]. The formation of AgNPs could be achieved in a solution of PEG, and the time required for this reaction was only about 2 min. To achieve colloidal silver dispersions with a narrow size distribution and free of sintering, small amounts of PVP were required. When the number of glycol units increases, the viscosity increases and also reducing property increases. Figs. 1-5 compares the absorption spectra obtained for three different PVP concentrations with the same concentration of AgNO_3 . The binding of vancomycin antibiotic on PEG-protected AgNP surface which was confirmed by UV-Visible spectral studies is shown in Fig. 6. It is found that only one symmetric absorption peak around 420 nm was observed, which is mainly attributable to primary dipolar excitation. Therefore, PEG-protected AgNPs under MW irradiation were further confirmed by SEM and TEM studies.

(FTIR spectroscopy)

FTIR spectroscopy was used to analyze the interactions between the metal and polymer. The wavelength of light absorbed is characteristic of the chemical bond present in the chemicals. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can

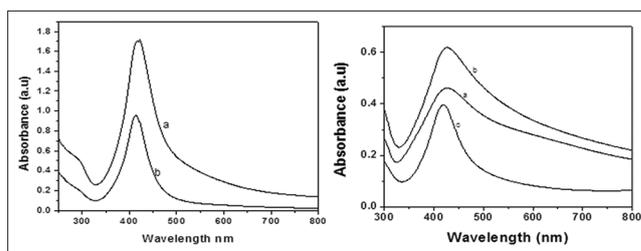


Fig. 1: (A and B) UV-visible absorption spectra of silver nanoparticles growth in 1 min and 5 min microwave irradiation: Ethylene glycol (20 ml) and silver nitrate (10 mg) with different concentrations of polyvinyl pyrrolidone (a) 20, (b) 30, (c) 50 mg

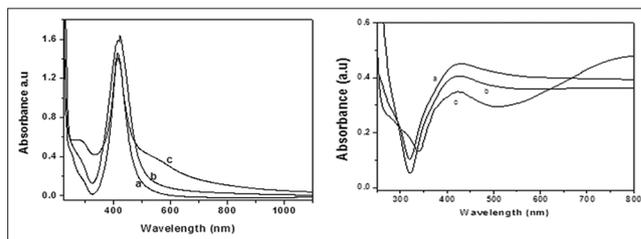


Fig. 2: (A and B) UV-visible absorption spectra of silver nanoparticles growth in 1 min and 5 min microwave irradiation: Triethylene glycol (20 ml) and silver nitrate (10 mg) with different concentrations of polyvinyl pyrrolidone (a) 20, (b) 30, (c) 50 mg

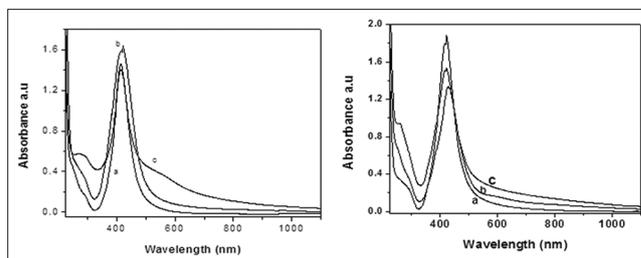


Fig. 3: (A and B) UV-visible absorption spectra of silver nanoparticles growth in 1 min and 5 min microwave irradiation: Polyethylene glycol (20 ml) and silver nitrate (10 mg) with different concentrations of polyvinylpyrrolidone (a) 20, (b) 30, (c) 50 mg (0.1M KCl)

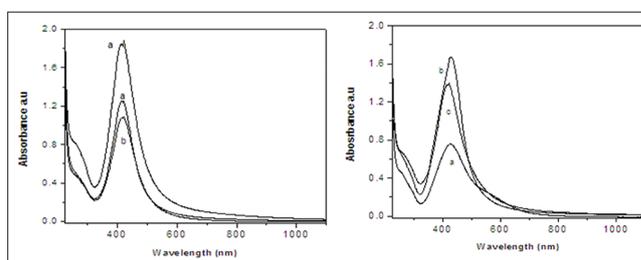


Fig. 4: (A and B) UV-visible absorption spectra of silver nanoparticles growth in 1 min and 5 min microwave irradiation: Polyethylene glycol (20 ml) and silver nitrate (10 mg) with different concentrations of polyvinylpyrrolidone (a) 20, (b) 30, (c) 50 mg (0.1M FeSO_4)

be determined. To further realize the role of PEG in the formation of AgNPs, in addition to that of a protective agent, the FTIR spectra for pure PEG and PEG-coated AgNPs were studied. For PEG spectrum in Fig. 7, the bands at $2872/\text{cm}$ correspond to C-H stretching region in alkyl ether. A peaks at 1351 and $1485/\text{cm}$ indicate CH_2 symmetric bending

vibrations, and peak at 949/cm indicates C-O stretching vibrations. The peak at 1107/cm corresponds to C-O-C stretching vibrations. The bands at 3430/cm are attributed to the stretching vibration absorption of -OH of adsorbed water.

The IR spectrum of PEG-protected AgNPs is shown in Figs. 8 and 9. The reduction of silver ions in the solution can occur through the oxidation of hydroxyl groups of PEG to aldehyde groups ($\text{CH}_2\text{CH}_2\text{OH} \rightarrow \text{CH}_2\text{CHO}$). PEG has a clear stabilizing effect on the nanoparticles compared to shorter chain polymers. Silver colloids prepared using the monomer (ethylene glycol) need the addition of PVP to stabilize silver spherical nanoparticles. The presence of oxygen in the long chains can assist in the stabilization of the AgNPs. In Ag-PEG, increase in the intensity of the C=O peak was observed and the peak is observed at 1654/cm which is related to stretching vibration of C=O, and this implies the

weak coordination chemical bonding between C=O and Ag. This results indicate that PVP containing N-C=O molecules absorb on the surface of AgNPs through the formation of a coordination bond between silver and oxygen atom of the carbonyl groups in PVP. The peaks at 1956/cm related to the substituted alkyl group and allene group, respectively, which also might be the products of PEG oxidation. The bands at 1109/cm corresponding to asymmetric and symmetric stretching vibration of C-N shift in PVP interacts with silver. The peaks at 1457/cm indicate the carboxylate group of AgNPs. MW provides fast heating and thus accelerates the reduction of the metal cluster due to the rapid and homogeneous heating; a better and higher crystalline could be achieved. We suppose that both growth and nucleation phase occur simultaneously under MW irradiation condition because reaction time period is less. PVP, a protective polymer containing a N-C=O group, gets attached to the surface of silver. Different facets of silver will have different absorption ability. The facets containing fewer attached PVP molecules grow more rapidly than those with more PVP molecules. Hence, they tend to assemble one by one to fabricate a specific shape.

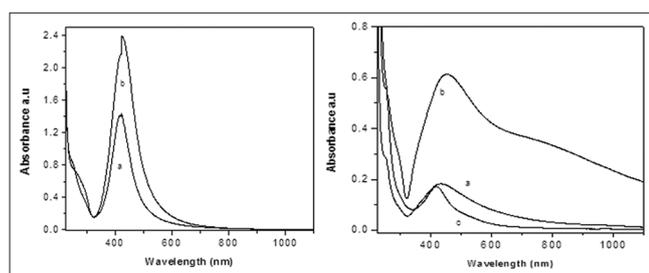


Fig. 5: (A and B) UV-visible absorption spectra of silver nanoparticles growth in 1 min and 5 min microwave irradiation: Polyethylene glycol (20 ml) and silver nitrate (10 mg) with different concentrations of polyvinylpyrrolidone (a) 20, (b) 30, (c) 50 mg

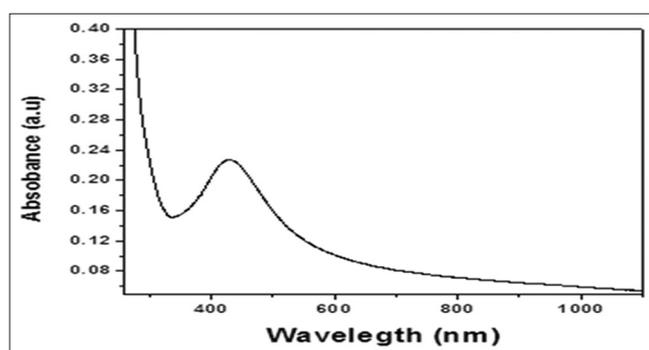


Fig. 6: UV-visible absorption spectra of silver nanoparticles growth in 1 min microwave irradiation: Polyethylene glycol (20 ml) and silver nitrate (10 mg) with 10 mg vancomycin

SEM

The surface morphology of the AgNPs was examined using SEM. Fig. 10 represents a SEM picture of PEG-protected AgNPs with 0.1M KCl synthesized using MW method. It can be seen that AgNPs have well-defined monodispersed spherical and triangular shapes. The size of the AgNPs studied through SEM had sizes ranging from about 40–200 nm.

TEM

The size and morphology of the nanoparticles were analyzed with a transmission electron microscope. The typical TEM micrograph and the size distribution of the AgNPs were obtained from 20 ml of PEG with 10 mg AgNO_3 and 20 mg of PVP and also with 0.1 M KCl (Fig. 11). The resultant particles essentially were very fine with the mean diameter of 100 nm. This revealed that PEG molecules indeed played the role of a protective agent for the formation of AgNPs. Further, it was observed that the morphology of the particles varied from rod, spherical, to triangular AgNPs when the reaction was changed from with to without addition of KCl. Therefore, the addition of KCl has a profound influence on the morphology of the AgNPs.

Antibacterial activity of PEG-protected AgNPs

The antimicrobial action of AgNPs was evaluated using the disc diffusion or Kirby-Bauer method. Two Gram-positive strains of bacteria (*Staphylococcus aureus* and *Micrococcus luteus*) and two Gram-negative strains of bacteria (*Escherichia coli* and *Klebsiella pneumonia*) were used to study the antibacterial effect of the prepared nanoparticles as shown in Table 1. The antimicrobial activity of vancomycin increased in the presence of AgNPs against the strains. In the different Gram-positive and Gram-negative strains tested, vancomycin showed a synergistic enhancement in its antibacterial activity in the presence of AgNPs. The results further showed that the synergistic effect was more pronounced in *K. pneumonia*, *S. aureus*, and *M. luteus*. The enhanced activity of

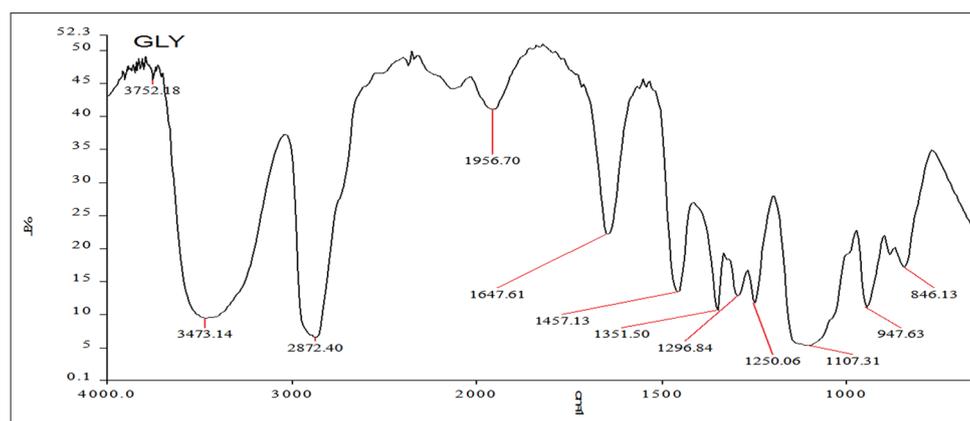


Fig. 7: Fourier-transform infrared spectrum of pure ethylene glycol

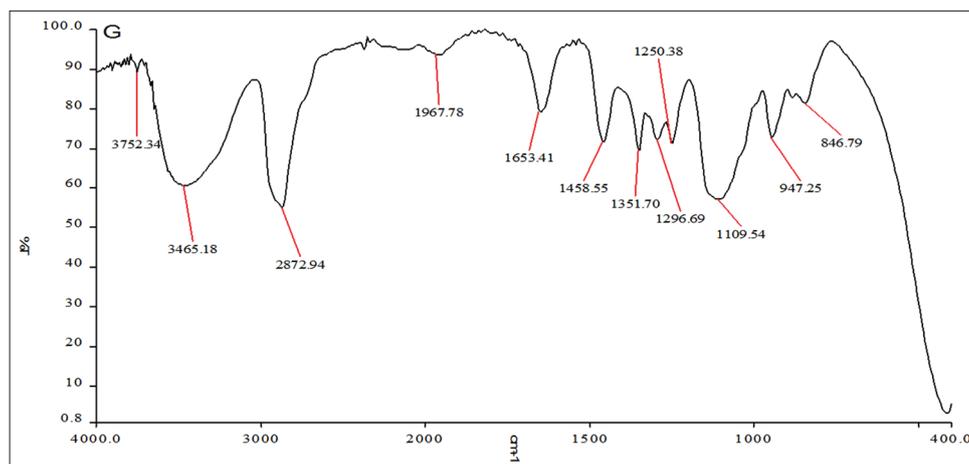


Fig. 8: Fourier-transform infrared spectrum obtained after mixing polyethylene glycol (20 ml) with silver nitrate (10 mg) and polyvinylpyrrolidone (20 mg) - Microwave for 5 min

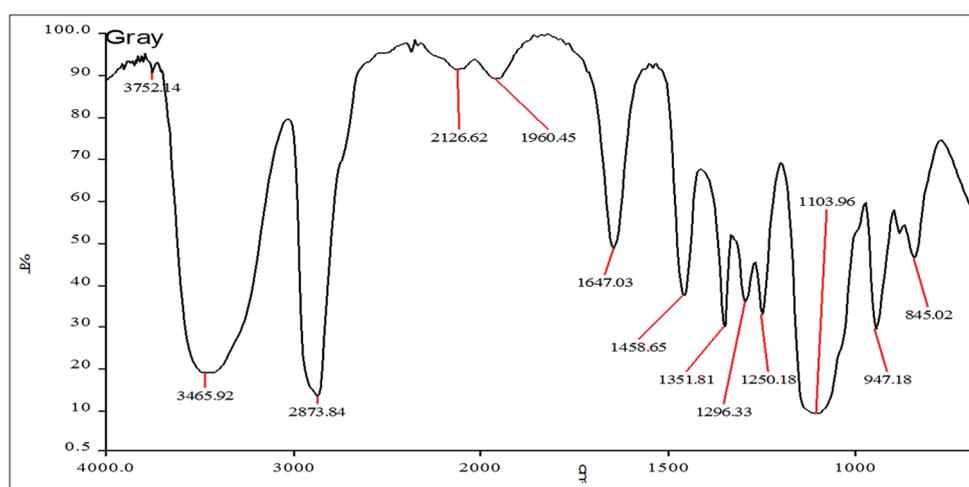


Fig. 9: Fourier-transform infrared spectrum obtained after mixing polyethylene glycol (20 ml) with silver nitrate (10 mg) and polyvinylpyrrolidone (20 mg) in KCl-Microwave for 5 min

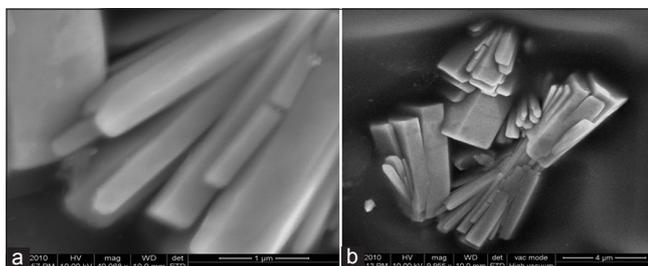


Fig. 10: (a and b) Scanning electron microscopy images of polyethylene glycol-protected silver nanoparticles in the presence of KCl (0.1 M) under microwave heating and subsequent UV irradiation

the drug along with the AgNPs could be due to any of the different mechanisms that silver uses against the bacterial pathogens. AgNPs may attach to the surface of the bacterial cell membrane and disturb its power functions such as permeability and respiration by impairing ATP synthesis [15]. Silver ions have also been shown, to penetrate into the bacterial cell and thereby cause damage to phosphorus and sulfur containing compounds such as DNA and proteins [16]. This is achieved predominantly by the process of denaturation. The release of silver from the nanoparticle could additionally contribute to the bactericidal properties of the nanoparticles. Bacterial cell lysis and modulation

of the protein profile of the bacteria have also been shown to be the reasons for the bactericidal activity of silver [17]. In the present study, the results show a synergistic effect of vancomycin in the presence of AgNPs.

The average size of the synthesized silver colloid particles ranged from 40 to 200 nm, depending on the concentration of PVP and on the type of reducing agents in the reaction system. It is reported that the bactericidal effect of AgNPs decreases as the size increases and is also affected by the shape of the particles. Thus, the increased activity of the AgNPs could be attributed to the small size of the nanoparticles [18]. Pal *et al.* demonstrated that AgNPs had increased antibacterial activity against *E. coli* compared to Ag^+ ions in the form of AgNO_3 and proposed that nonmaterial such as Ag(III) crystals synergistically promoted the antibacterial effect against *E. coli* [19]. Similar results were shown by others also. Recently, many research groups have worked to synthesize the silver functionalized vancomycin for targeted drug delivery. Then, the functionalized drug easily forms a monolayer assembly when the drug was allowed to equilibrate the AgNPs. These methods are exploited for drug delivery of controlled and relaxed antibiotic to avoid side effects of overdose [20] (Fig. 12).

CONCLUSION

Polyol process is a simple and efficient method for synthesizing AgNPs. We have synthesized AgNPs and nanorods using polyol method under

Table 1: Antibacterial activity of silver nanoparticles on various microorganisms

Microorganisms	Zone of inhibition (mm)		
	Vancomycin (standard) 10 ug/ml	Silver nanoparticle 10 ug/ml	Vancomycin+Silver nanoparticle 10 ug/ml
<i>Klebsiella pneumoniae</i>	21	19	39
<i>Escherichia coli</i>	24	20	40
<i>Staphylococcus aureus</i>	23	21	41
<i>Micrococcus luteus</i>	21	20	40

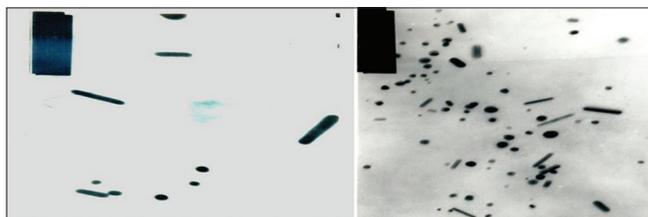


Fig. 11: Transmission electron microscopy images of polyethylene glycol-protected silver nanoparticles in the presence of KCl (0.1 M) synthesized under microwave heating and subsequent UV irradiation

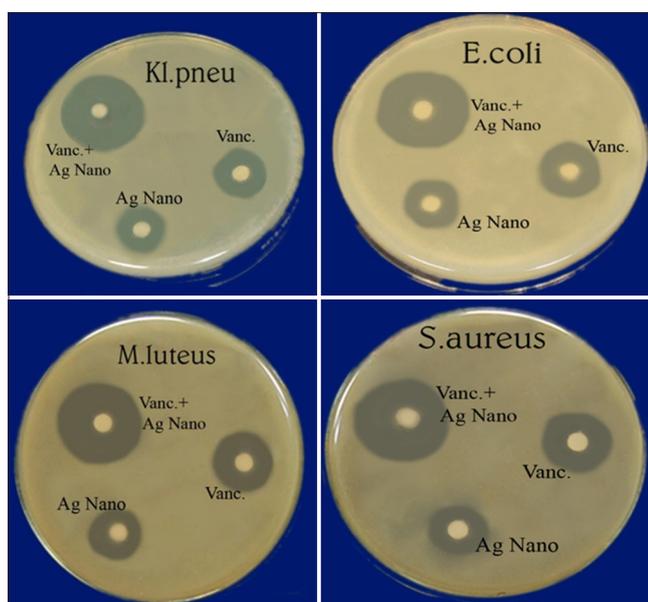


Fig. 12: Photograph images of the zones of inhibition of polyethylene glycol-protected silver nanoparticles by Kirby-Bauer test with standard antibiotic vancomycin

MW heating. Furthermore, we have observed that a catalytic amount of KCl and Fe^{2+} greatly influences the growth of AgNP size and shape. Uniform and stable polymer protected AgNPs were synthesized using ethylene glycol, diethylene glycol, triethylene glycol, and PEG as reducing agents in the presence of PVP under MW mode of heating. In the case of long-chain polyhydric ethylene glycol and PVP as the reaction medium, both PVP and PEG played a dual role as a reducing as well as shape directing agents, and thereby, the AgNPs were stabilized by both the polymers. From UV-visible spectroscopy studies, the growth rate was confirmed by measuring the change in absorbance of plasmon peak shape at different time intervals. The binding of vancomycin antibiotic on PEG-protected AgNP surface was confirmed by UV-visible spectral studies. FTIR spectroscopy studies showed the changes in the metal and the capping agents that result in the formation of PEG-protected AgNPs. SEM and TEM images revealed that very stable colloidal solutions of AgNPs with almost spherical and rod shape along with high monodispersity can be obtained with PEG-protected AgNPs. Finally,

the antibacterial activity of PEG-protected AgNPs was investigated, which showed a synergistic role for the AgNPs along with vancomycin. Thus, this study shows the potential synergistic role that AgNPs play along with established drugs; however, further studies are needed to functionally characterize their antibacterial activity.

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AUTHOR'S CONTRIBUTIONS

D. Malathy conducted the study, Meyappan Revathi planned and designed the study, and M. Letticia supported the conduction of the study.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest. All authors have equally contributed to this article.

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