Advanced Electrochemistry
Vol. 1, 1–6, 2013

Antimicrobial Properties of Electro-Chemically Stabilized Organo-Metallic Thin Films

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In this study, *in situ* synthesis of silver nanoparticles (Ag NPs) in the polystyrene polymer matrix was carried out. TEM analysis revealed the average diameter of particles about 6 nm corresponds to 0.02 M concentration of $AgNO_3$ in colloidal. The absorption spectrum of the polystyrene and polystyrene with silver colloids showed an absorption band with a maximum of 230 nm. Microbial evaluations of Gram negative (*E. coli*) bacteria grown on Luria-Bertani media (neutral pH) were evaluated for silver nanocomposite polystyrene film. Results from this study provide a basis for the measurement of bacterial activity of Ag nanocomposite polymer membrane.

Keywords: Silver Nano Particles, Polystyrene, TEM, UV-Vis Spectrum, Bacterial Growth.

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1. INTRODUCTION

In the recent years, engineering of materials in nanoscale has become an emerging interdisciplinary field involving material science and biology. Nanocomposite materials consist of metallic nanoparticles incorporated in or with polymer matrix and there has been a growing interest for their potential applications in the fields of catalysis, bioengineering, photonics and electronics.[1–5]

Ag NPs due to some unique properties is widely incorporated in various materials and thus has become one of the most popular areas for research and biomedical applications. Several studies propose that Ag NPs may attach to the surface of cell membrane disturbing their permeability and cellular respiration. It is shown that smaller Ag NPs having the large surface area give more bactericidal effect than the larger Ag NPs.[6] It is also possible that Ag NPs not only interact with the surface of membrane but can also penetrate inside the bacteria.[7] Gram negative bacteria have a very thin peptidoglycan layer (≈3 nm) between cytoplasmic membrane and outer membrane[8] as compared to Gram positive bacteria which lack the outer membrane and have a peptidoglycan layer of 30 nm thick.[9]

The persistence of antibiotic-resistant bacteria has renewed interest in the use of silver and silver-based compounds, including silver nanoparticles (Ag NPs), as alternative antibacterial agents. Such compounds can be used medicinally to reduce infections in burns patients[10] and prevent bacterial colonization of prostheses,[11] catheters,[12] dental materials and human skin.[13] Due to their high thermal stability, low toxicity to human cells and effective broad-spectrum antibacterial activity[4] Ag NPs are being exploited in a range of commercial products.

Synthesis and characterization of nanoscaled materials in respect of their novel physicochemical properties is ideal in the formulation of bactericidal materials.[8] There are a wide range of methods for the preparation of metal nanoparticle–polymer composite films and have been reported in recent years.[14] The main fabrication approach is to disperse previously prepared particles in the polymer matrix.[15] This method commonly known as evaporation method involves evaporation of polymer solvent after nanoparticle dispersion from the reaction mixture. However, this often leads to irregular distribution of particles in polymer matrix. To address these challenges, it is essential to develop methods which can stabilize electro-chemically metallic nano-particles with the polymeric materials during the reduction process.

In this study, we used in situ synthesis of Ag NPs in the polymer matrix which involves the dissolution and reduction of metal salt or complexes into the polymer matrix. This method is easy to implement, yields a homogeneous distribution of nanoparticles in the polymer matrix and

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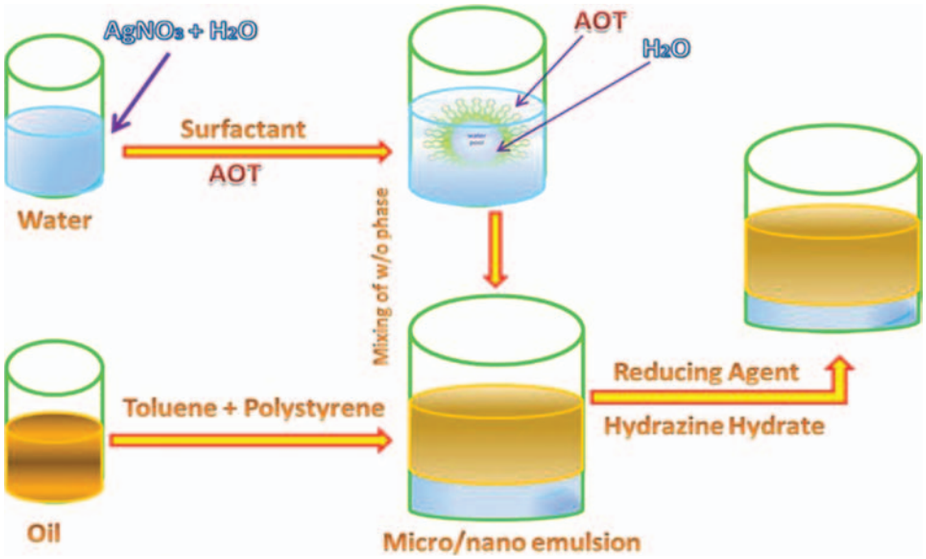

Fig. 1. Schematic block diagram of Ag nanoparticles polymer nano composite synthesis.

formation of free-standing films. Microbial evaluation of Gram negative (*E. coli*) bacteria on silver nano particle composite polystyrene film is tested. Transmission Electron Microscopy (TEM) is used to calculate the size and distribution of nanoparticle and optical microscope is used to study the mechanism of Ag nanoparticle interaction with these bacteria.

2. MATERIALS AND METHOD

2.1. *In Situ* Synthesis of Ag-Polystyrene Composite

In situ Ag nanoparticles embedded in polystyrene matrix were synthesized by the microemulsion technique. Silver nanoparticles were prepared through the reduction mechanism of $AgNO_3$ by $NH_2NH_2H_2O$ in the presence of sodium bis(2-ethylhexyl) sulfosuccinate (AOT) as a surfactant. Solution A was made with $AgNO_3$ and AOT under constant ultrasonication stirring for 15 minutes. Aqueous solution of $AgNO_3$ (0.2 M) was prepared in deionized water while AOT (0.05 M) is dissolved within equal volume of deionized water and ethanol. Solution B was prepared by dissolving polystyrene (PS) [Mw.280, 000] 5% in toluene while solution C was prepared by dissolving $NH_2NH_2H_2O$ (0.6 M) in DI water.

For the synthesis of *in situ* Ag nanoparticle embedded in PS matrix, solution A was injected into solution B in the ratio of water/oil (w/o) emulsion (1:4) at room temperature and the magnetic stirring carried out until formation of its milky colloidal solution. Then solution C injected in milky colloidal solution and stirred for further 15 minutes that results a light yellow colour of the emulsion. It shows the precipitation of Ag NPs within the micro emulsion. The colloidal solution was very stable and could be kept for week at room temperature without precipitation embedded in polymer matrix was achieved by w/o emulsion solutions are given below:

$$4AgNO_3 + N_2H_5OH \longrightarrow 4Ag + 4HNO_3 + N_2 + H_2O$$

Figure 1 shows schematic block diagram of Ag nanoparticles polymer nano composite synthesis is given below:

The colloidal solution was very stable and could be kept for week at room temperature without precipitation. The bifurcations of micro and submicron level particles within the colloidal solution were carried out by the centrifuge at 10 K RPM for 10 minutes and filtered through a 0.2 micron filter before film casting. Then the films were subjected to vacuum drying at 60 °C for 6 h.

2.2. TEM Analysis of Ag Nanoparticle

The sample for TEM analysis was obtained by placing a drop of the colloidal solution onto a carbon-covered copper grid and evaporating it in air at room temperature. A HRTEM FEI Technai 20 U Twin Transmission Electron Microscope (TEM) was used for observing the morphology and analyzing the size of nanoparticles. The particle sizes were determined by TEM at 120 kV.

2.3. UV-Vis Spectroscopy of Colloidal Solution

UV-Vis absorption spectra of Ag NPs were acquired in the 300–800 nm wavelength range using an UV-Vis spectrophotometer (Varian) operating at a resolution of 2 nm for the analysis of optical properties of colloidal solution.

2.4. Bacterial Strains and Growth Conditions

Escherichia coli (E. coli) bacteria were obtained from IMTECH, Chandigarh India (MTCC-40). The culture was

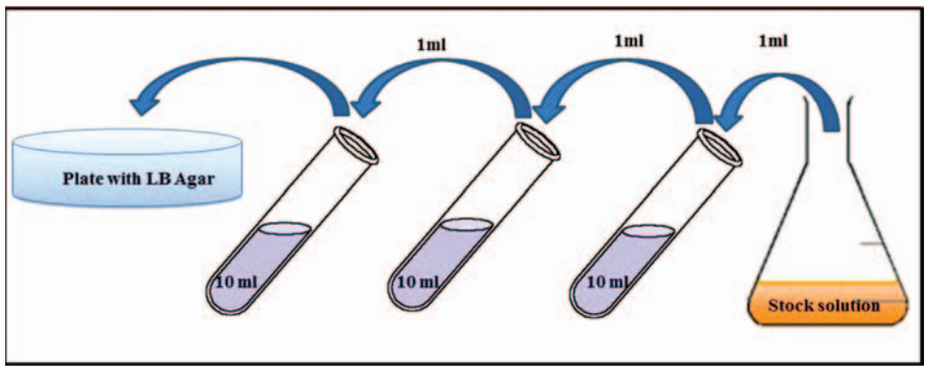

Fig. 2. Schematic diagram representing serial dilution of stock broth solution for cell count.

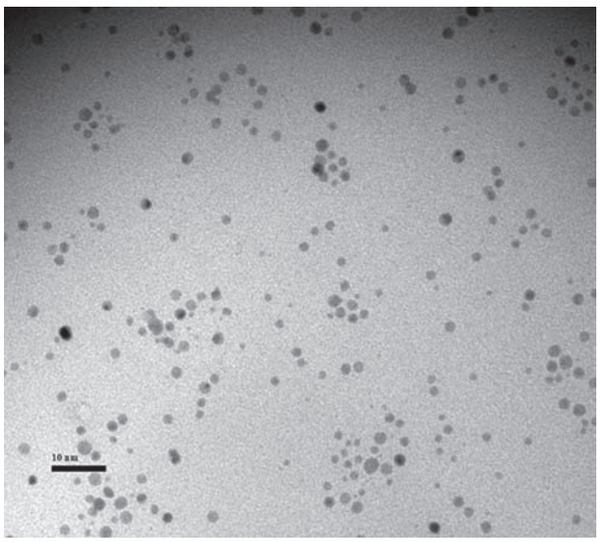

Fig. 3. TEM micrograph and average size distribution of Ag nanoparticles in AOT reverse micelles.

revived, subcultured and maintained on Luria-Bertani agar (Himedia) and stored at 4 °C. For the experiment, a single colony of *E. coli* was inoculated into 10 mL of Luria-Bertani broth (LBB) and incubated overnight at 37 °C with shaking at 120 rpm. The cultured broth was then subjected to three serial dilutions and from the final dilution, LB Agar plate, Pristine, PS doped with 0.2% Ag NPs and 1% Ag NPs were inoculated and bacterial colonies were observed after their overnight growth at 37 °C (Fig. 2).

2.5. Antibacterial Activity Assay

E.coli bacteria from the stock plate was inoculated on separate LB-Agar plate containing Pristine, PS doped with 0.2% Ag NPs and 1% Ag NPs respectively and optical micrographs of microbial growth on Ag nano polymer composite system were captured with a CCD camera attached to a LABOMED microscope. All the experiments were performed and repeated at ambient temperature of 25 °C.

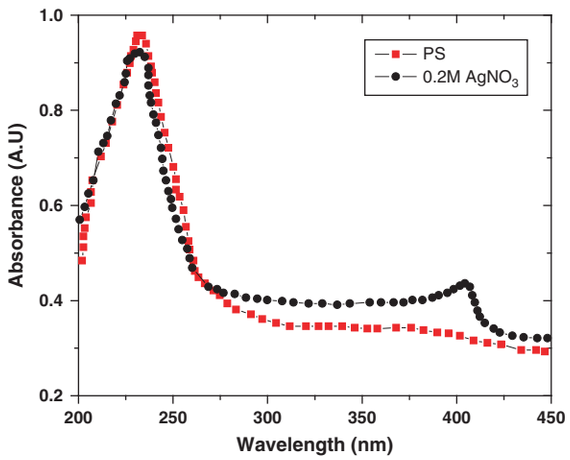

Fig. 4. Absorption spectra of *in situ* synthesized Ag nanoparticles in AOT reverse micelles.

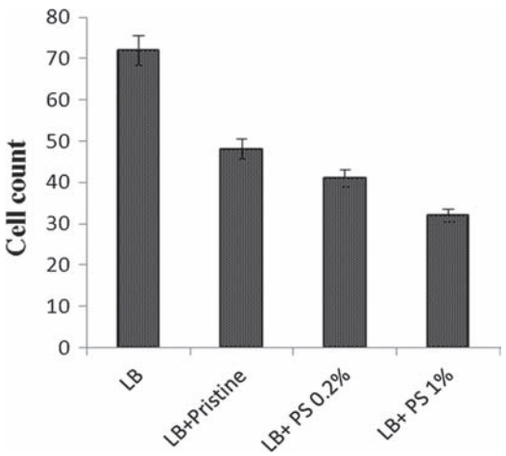

Fig. 5. Bacterial cell count on Agar plate after serial dilutions.

3. RESULTS AND DISCUSSION

The particles size analysis and percentage particles loading with in polystyrene matrix are carried out by TEM and UV spectroscopic techniques. Figure 2 shows the variation of

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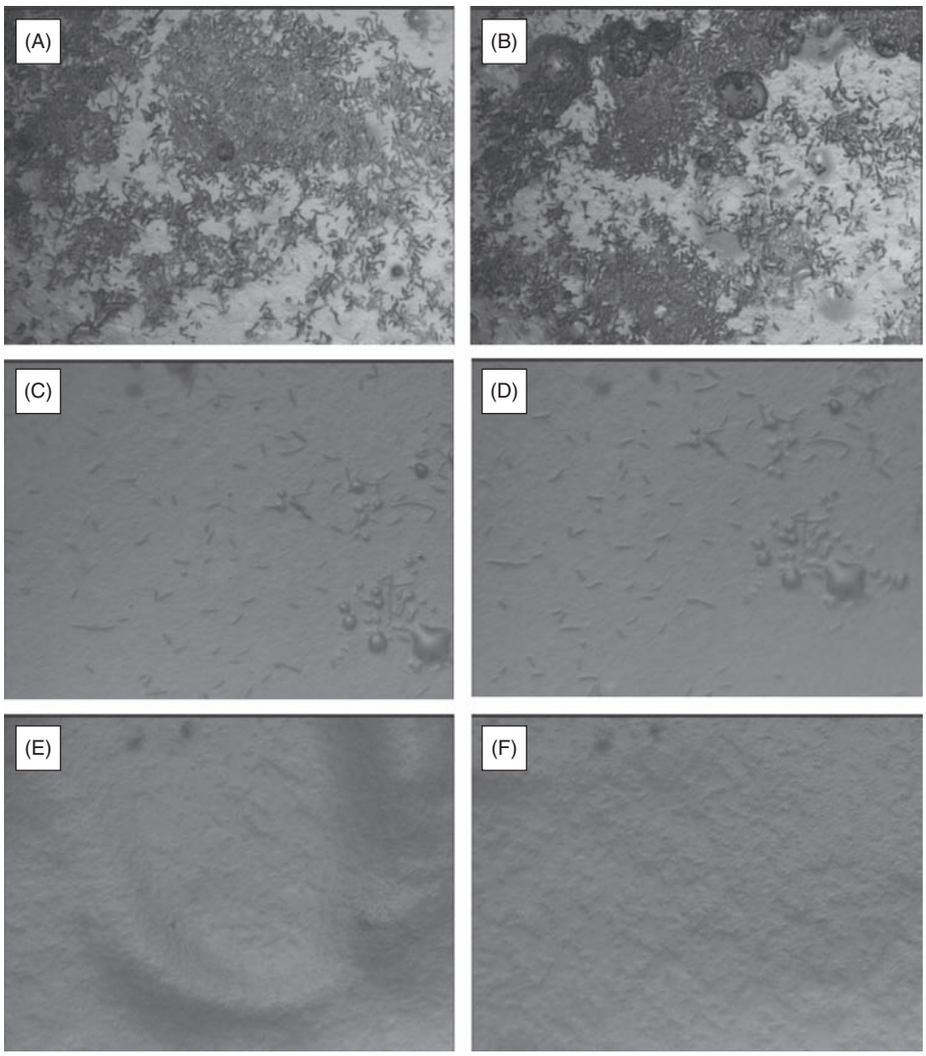

Fig. 6. Bacterial growth on PS film (a & b), 0.2% Ag composite PS (c & d) and 1% Ag composite PS (e & f).

particles size with variation $AgNO_3$ concentration within oil phase of reverse microemulsion. The average diameter of particles, as shown in Figure 3, are found to be 6 nm corresponds to 0.2 M concentration of $AgNO_3$ in colloidal.

One of the important factors to synthesize nanoparticles in reverse microemulsion is the intermicellar exchange rate which is denoted by the value of intermicellar exchange rate coefficient (k_{ex}). The polystyrene carbon chain makes the oil phase of toluene molecule more coiled and hence its penetration in the surfactant layer becomes more difficult. As a result, the interaction between surfactants and solvent molecule will decrease when toluene dissolved polystyrene is used as oil solvent to prepare Ag NPs. Hence, at given water content, the decrease of the intermicellar exchange rate constant induces a decrease in particles size. According to the common dynamics rule of the effect of intermicellar exchange rate on the particle size, a rapid exchange among materials at high k_{ex} will form a larger number of micelles containing silver atoms than that of the critical nucleation. This will result in smaller particle size due to the increase in polystyrene content in toluene. Figure 4 shows the absorbance band corresponds to polystyerene and 0.2 M concentration of Ag nanoparicles in colloidal solution.

UV-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. The absorption spectrum (Fig. 4) of the polystyrene and polystyrene with silver colloids showed a absorption band with a maximum of 230 nm, indicating the presence of lone spherical or roughly spherical Ag NPs, and TEM imaging confirmed this (Fig. 3).

The UV-visible spectra of the solution containing the particles with larger aspect ratios (Fig. 4) showed three main absorption peaks at 230 and 417 nm. These absorption peaks may be due to the in-plane dipole resonance mode associated with nanoplates with edge lengths greater

than 100 nm and the strong coupling between the nanoparticles arising from aggregation of Ag NPs. The optical absorption spectra of metal nanoparticles are dominated by surface plasmon resonances (SPR), which shift to longer wavelengths with increasing particle size. The position and shape of plasmon absorption of silver nanoclusters are strongly dependent on the particle size, dielectric medium, and surface-adsorbed species.

The interaction of Ag NPs was analyzed by growing *E. coli* bacteria on pristine PS, 0.2% and 1% Ag nanoparticle doped PS films. The bacterial colony grown on LB Agar plate after serial dilutions were counted and Figure 5 shows cell count on pristine, Ag NPs doped on PS at 0.2% and 1%. The graph clearly depicts the reduced number of cell counts in the Ag NPs doped samples. The cells numbers are found to reduce gradually with increase in Ag NPs.

Figure 6 shows the number of bacterial colonies grown in the presence of different amount of Ag nanoparticles in polystyrene films and pristine polystyrene film. As expected, bacterial growth was found to be dependent on concentration of Ag NPs embedded in the polymer film. Figures 6(c–e) shows that polystyrene silver nanoparticles composite film exhibited significantly superior antibacterial property at a given nanoparticle concentration when the initial *E. coli* concentration was reduced. Figure 6 depicts the killing activity for *E. coli* in polymer matrix, containing different amounts of Ag NPs. The killing activity was found to be sustained, as no bacterial growth was seen even after 24 h of incubation. The Figures 6(a–b) shows the dynamics of *E. coli* growth as observed in the pristine polystyrene film without any modification.

Several hypotheses explaining the antibacterial activity of Ag NPs have been reported. The ability of Ag NPs to release silver ions by rapid breakdown is the key to their antibacterial activity.[16] The ionic silver released interacts with the thiol group of bacterial enzymes thereby inactivating them. The high surface area of Ag NPs increases their contact with bacteria, promoting the dissolution of silver ions, thereby improving biocidal effectiveness. Further inhibition of bacterial DNA replication, damage of cytoplasmic membranes leads to damage of adenosine triphosphate (ATP) and thus causing cell death.[17]

Recently many research articles on the bactericidal activity of silver nanoparticles of either simple or composite nature have been reported.[18] Elechiguerra and coworkers showed the size-dependent interaction of Ag NPs with human immunodeficiency virus type 1, preferably via binding to gp120 glycoprotein knobs. The size dependent interaction of Ag NPs with Gram negative bacteria has also been reported by the same group.[19] The selective bacterial growth was observed on the nonporous polymer membrane of polycarbonate with thickness 25 μm. The electron surface modification of polymer membrane can increase the cross linking density at the surface whereas plasma and neutral beam treatment can change the surface properties of these membrane to improve the bio-adaptability.[20]

4. CONCLUSION

In conclusion, we have found that silver nanoparticles undergo interaction with the Gram-negative bacterium *E. coli* in a concentration dependent manner. It may be speculated that silver nanoparticles with the same surface areas but with different shapes may also have different bactericidal properties. Though at present we are not able to relate the bactericidal capability of silver nanoparticles with their effective surface areas or to give an estimation of how the surface areas of different nanoparticles influence their killing activity, however our results provide a basis for the measurement of bactericidal activity of silver nanoparticles. The interaction of silver nanoparticles with biosystems is just at the beginning, these particles could be used as microbicidal agents in various fields.

Acknowledgments: We acknowledge the support and facility provided by DST Unit on Nanosciences, Indian Institute of Technology, Kanpur. One of author (KA) is thankful to Department of Science & Technology, Govt. of India for financial support under INSPIRE Faculty Project.

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Received: 12 December 2012. Accepted: 10 April 2013.