Poly(vinyl pyrrolidone)-mediated synthesis of silver nanowires decorated with silver nanospheres and their antimicrobial activity

RUCHIR PRIYADARSHI* and YUVRAJ SINGH NEGI

Department of Polymer and Process Engineering, Indian Institute of Technology Roorkee, Saharanpur Campus, Saharanpur 247001, India

*Author for correspondence (ruchirpriyadarshi@gmail.com)

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Abstract. Pathogenic infectious diseases, like bacterial infections, are one of the most prevalent types of diseases all over the world. Although antibiotics are commonly used and widely available drugs for the treatment of bacterial infections, bacteria show multiple drug resistance, have a tendency to get genetically mutated and become resistant to commonly employed antibiotic drugs. This makes the invention of novel drugs essential. One of the possible approaches is the use of nanomaterials for this purpose. The present study attempts to synthesize silver nanospheres and silver nanowires and compare their antibacterial activity. Silver nanospheres were synthesized by chemical reduction of silver nitrate in the presence of a suitable stabilizer. For the synthesis of silver nanowires, polyol method was employed. Silver nanowires neatly decorated with silver nanospheres were obtained. The antibacterial activity was estimated by separately determining the minimum inhibition concentration of the two nanostructures on Gram-positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* cultured on Luria Bertani agar media. The comparison shows that the antibacterial activity of silver nanospheres is better than that of silver nanowires which is attributed to its higher surface area and the difference in uptake mechanism by the bacterial cells.

Keywords. Silver nanoparticles; silver nanowires; multiple drug resistance; *E. coli*; *B. subtilis*; minimum inhibition concentration.

1. Introduction

A plethora of infectious organisms like bacteria, viruses, fungi, protozoa, etc., are known to be pathogenic to humans. Out of these pathogenic species, bacteria constitute 38% of all the disease causing organisms [1]. Moreover, over 50% of the bacterial species known till date are pathogenic [1]. A large number of infectious microorganisms are also emerging regularly. Bacteria comprise 30% of these emerging pathogens [1]. The most common and most widely encountered pathogenic bacterium is Gram negative *Escherichia coli* (*E. coli*) which is responsible for many gastro-enteric diseases and urinary tract infections. These diseases caused by *E. coli* are mostly food-borne. Another common food-borne bacterium is Gram positive *Bacillus subtilis* (*B. subtilis*) which is one of the common food-spoiling bacteria.

Conventional antibacterial medicine is dependent on chemotherapeutic agents, known as antibiotics, for the treatment of diseases. Antibiotics either inhibit the growth of pathogenic microbes or destroy them without affecting the host in an undesirable way. But, excessive use of antibiotic drugs against bacteria leads to the emergence of multiple drug resistant strains. As the resistant population increases, the threat to successful treatment of microbial diseases increases proportionally. The mechanism of drug resistance may vary

in different classes of bacteria [2]. The drug resistance in bacteria is a consequence of modification in its genetic makeup and thus renders the development of new alternative strategies essential.

The use of nanomaterials is one of the possible alternatives as antimicrobial agents [3]. The antimicrobial nanomaterials are broadly classified into organic, inorganic and hybrid nanomaterials [4]. Substantial research has already been carried out on antimicrobial nanomaterials in the past few years. Tsao *et al* [5] demonstrated that malonic acid derivative of carboxy fullerene prevents *E. coli* induced meningitis in mice in a dose-dependent manner. The antimicrobial activity of carbon nanotubes (CNTs), specifically single-walled CNTs was first reported by Kang *et al* [6]. Akhavan and Ghaderi [7] produced graphene platelets deposited over TiO₂ thin films as a photo-induced antibacterial agent. Qi *et al* [8] synthesized Cu-ion loaded chitosan nanoparticles and studied their antibacterial characteristics against *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, etc. The antibacterial activity was found to be higher as compared to chitosan nanoparticles. Apart from these organic and hybrid nanomaterials, metal and metal oxide nanomaterials have also been the focus of researchers. The synthesis of Ag–Cu nanoalloys and their antibacterial characteristics against *E. coli* have also been reported recently by Taner *et al* [9]. The exposure of

ZnO nanoparticles and their composites to different Gram-positive and Gram-negative bacterial strains confirmed their growth reduction ability [10].

The noble metal silver has always been a focus of research considering its antimicrobial application and safety for human use. Since ancient times, silver serves the purpose of preservation of edible matter from the microbes. Silver vessels were used to store water for longer duration, which remained uncontaminated for days. Silver film is applied as edible coating on food items. There has also been a lot of research on silver nanoparticles in recent times. Silver nanoparticles were synthesized by several routes like chemical synthesis [11,12], green synthesis [13–17] and microorganism-mediated synthesis [18–20].

In the present study, the silver nanospheres were synthesized by wet chemical synthesis, while silver nanowires were synthesized by polyol method. The chemical and morphological characterizations of the nanostructures were carried out. Thereafter, the antibacterial activity of these nanostructures was compared by determining their minimum inhibition concentration (MIC) in the bacterial culture medium. The bacterial cultures of *B. subtilis* and *E. coli* were prepared using a serial dilution method for this purpose.

2. Materials and methods

2.1 Synthesis of silver nanostructures

Silver nitrate (AgNO_3 , 99%), ethylene glycol (EG, 99.8%) anhydrous, sodium borohydride (NaBH_4), tri-sodium citrate and poly(vinyl pyrrolidone) (PVP, MW 60,000) were procured from Sigma Aldrich. Luria Bertani (LB) broth and nutrient agar media were obtained from Hi-media Laboratories Pvt. Ltd., India.

For the synthesis of silver nanospheres, AgNO_3 was reduced using NaBH_4 with stabilizer tri-sodium citrate [11]. A solution of 10 mM NaBH_4 was prepared and was kept at 0°C in an ice bath. Another solution of 0.25 mM AgNO_3 and 0.25 mM tri-sodium citrate was prepared in deionized water. To 20 ml of this solution, 0.6 ml of NaBH_4 solution was added all at once while stirring it vigorously for about 30 s. The solution thus obtained was centrifuged at 10,000 rpm (equivalent to 16,770 g) for 30 min and the settled silver nanospheres were collected. The obtained nanospheres were washed several times with deionized water.

Polyol method was employed to synthesize silver nanowires. AgNO_3 was reduced with EG in the presence of PVP and Ag seeds [21]. In a typical synthesis, 500 μl of AgNO_3 solution (1×10^{-4} M, in EG) was added to 5 ml of EG and heated at around 160°C in a round-bottom flask (equipped with a condenser, thermo-controller and magnetic stirring assembly). After 5 min, 2.5 ml of AgNO_3 solution (0.10 M, in EG) and 5 ml of PVP solution (0.30 M, in EG) were added dropwise, simultaneously, to the hot solution over a period of 10 min. The heating of the reaction mixture was

continued at 160°C until complete reduction of AgNO_3 . Vigorous stirring was maintained throughout the process. The reaction mixture was diluted 10 times with deionized water and centrifuged at 3500 rpm (equivalent to 2050g) for 25 min. The supernatant layer could be easily removed using a pipette. This centrifugation procedure was repeated several times until the supernatant becomes colourless.

2.2 UV-visible spectrophotometry

UV-visible absorption spectra of the synthesized silver nanospheres and silver nanowires were obtained with the help of a Perkin Elmer UV-visible 3500 spectrophotometer. The nanostructures were suspended in distilled water by sonication before recording the spectra. The optical response was taken in a 1 cm path quartz cuvette at 300–900 nm range.

2.3 TEM

The estimation of the particle size of both the nanostructures was carried out using transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM). The samples were prepared by diluting the reaction solutions with distilled water and placing small droplets of the diluted samples on copper grids. The samples were dried in a desiccator connected to a vacuum pump. The images were taken using a JEOL JEM-2100F TEM operated at 200 kV.

2.4 XRD

For X-ray diffraction (XRD), the samples of the nanowires were dried in a hot air oven overnight. The powders thus obtained were characterized using a Philips X'Pert Pro Powder X-ray diffractometer using $\text{CuK}\alpha$ radiation ($\lambda = 1.5438 \text{ \AA}$) with the 2θ values between 10 and 70° at a scan rate of 4° min^{-1} .

2.5 SEM

Surface morphology of the nanowires was determined by scanning electron microscopy (SEM). The precipitate obtained after centrifugation was washed, dried and used directly for the SEM characterization. The images were taken using a Carl Zeiss LEO SUPRA 55 field emission SEM operated at 5 kV.

2.6 Study of antibacterial activity

Antibacterial activity of the synthesized nanostructures was determined by their MIC [22]. To study the antibacterial activity of silver nanospheres, two sets of LB broth was prepared for culturing the bacteria, one for *B. subtilis* and the other for *E. coli* and was incubated at 37°C overnight. The serial dilutions of these bacterial broths were carried out until the dilution of 10^7 is achieved. The diluted solutions were poured

into several test tubes, each having different concentrations of silver nanospheres from 0 (control) to $50 \mu\text{g ml}^{-1}$. The test tubes were incubated while stirring at 37°C for about 4 h and

Figure 1. UV-visible absorption spectrum of silver nanospheres. The sharp absorption peak obtained at around 410 nm which is the characteristic SPR band of Ag.

then $25 \mu\text{l}$ culture from each tube was spread inoculated onto agar plates. The plates were labelled according to the concentration of silver nanospheres and were incubated at 37°C for 24 h.

For the estimation of antibacterial activity of silver nanowires, the bacterial broths were prepared in the same way as for silver nanospheres. The test tubes of diluted bacterial broth having concentration of silver nanowires from 0 (control) to $400 \mu\text{g ml}^{-1}$ were prepared and the same procedure was followed as above.

3. Results

3.1 Synthesis of silver nanospheres and their characterization

The formation of silver nanospheres was confirmed by the UV-visible absorption spectra. A sharp absorption peak was obtained at around 410 nm as shown in figure 1 which is the characteristic surface plasmon resonance (SPR) band of the Ag. This confirms the successful formation of the silver nanospheres.

The size and dispersion of synthesized silver nanospheres were determined by TEM and the results are as shown in figure 2. The spherical silver nanoparticles can be clearly

Figure 2. HRTEM images of individual silver nanospheres.

Figure 3. Histograms for silver nanospheres were obtained for over hundred particles and the average particle size was found to be 3.6 nm.

seen in the HRTEM images. To obtain the particle size distribution, a histogram as shown in figure 3 was obtained for over hundred particles and the average particle size was found to be 3.6 nm.

3.2 Synthesis of silver nanowires and their characterization

UV-visible spectrum of the silver nanowires is obtained as shown in figure 4. The spectrum is in accordance with that already reported elsewhere [21]. The 380 nm peak which corresponds to the transverse SPR mode of nanowires accompanies the characteristic peak of nanosized silver at 410 nm. Moreover, the signature at 350 nm corresponding to the longitudinal SPR mode for bulk silver is also prominent. This confirms one-dimensional growth of silver, and hence, the formation of silver nanowires.

The XRD pattern as shown in figure 5, suggests that the synthesized silver nanowires are purely composed of face-centred cubic phase. The lattice constant was calculated from the XRD graph, and was found to be 4.079 Å which approximates the reported data ($a = 4.086$ Å, JCPDS file 04-0783) [23]. It was noted that the intensity ratio of [111] peaks to [200] peaks was around 2.7, which is higher than the theoretical value of 2.5. This indicates the enrichment of the [111] crystalline planes of the silver nanowires [21].

The microscopic studies of silver nanowires were carried out using SEM and TEM as shown in figure 6. The Ag nanowires thus formed are found to have a very high aspect ratio having a nanosized diameter with micron-sized length. The TEM image (figure 6b) shows neat one-dimensional Ag nanowires with fine spherical Ag particles decorated along the length which can be attributed to the process of partial

Figure 4. UV-visible spectrum of the silver nanowires. The 380 nm peak corresponds to the transverse SPR mode of nanowires which accompanies the characteristic peak of nanosilver at 410 nm. The signature at 350 nm corresponds to longitudinal SPR mode for bulk silver.

Figure 5. XRD pattern suggests that the synthesized silver nanowires existed in the FCC phase.

Ostwald ripening taking place due to the depletion of Ag precursors in solution. The confirmation of this was obtained from the HRTEM image of one of the bulged portion seen on the nanowire surface (figure 6d). The lattice fringe width corresponding to the [111] plane of silver matches with the HRTEM image of silver nanowire (figure 6c).

Figure 6. Electron micrographs: (a) SEM and (b) TEM micrographs showing Ag nanowires with a high aspect ratio. The HRTEM images of (c) Ag nanowire and (d) silver nanoparticle decorated on nanowires, show neat lattice fringes corresponding to the (111) face of Ag.

3.3 Comparative antibacterial activity of nanostructures

To estimate the MIC of silver nanospheres on *B. subtilis* and *E. coli*, 10 culture plates with nanosphere concentration of 5–50 $\mu\text{g ml}^{-1}$, with an increment of 5 $\mu\text{g ml}^{-1}$ was prepared for each bacterial strain. Along with this, a control plate of both the bacteria with no nanospheres (0 $\mu\text{g ml}^{-1}$ conc.) was also prepared which showed 100% growth. For *B. subtilis*, the bacterial colonies were obtained up to 30 $\mu\text{g ml}^{-1}$ concentration, while for *E. coli*, the growth was obtained until 40 $\mu\text{g ml}^{-1}$ concentration. These MIC values of 30 and 40 $\mu\text{g ml}^{-1}$ are for *B. subtilis* and *E. coli*, respectively, as seen in table 1.

For the MIC estimation of silver nanowires, 10 culture plates with nanosphere concentration of 50–400 $\mu\text{g ml}^{-1}$ were prepared for each bacterial strain, at an increment of 50 $\mu\text{g ml}^{-1}$. Along with this, control plates with no nanospheres (0 $\mu\text{g ml}^{-1}$ conc.) were prepared which showed 100% growth of bacteria. The bacterial colonies were obtained up to 200 $\mu\text{g ml}^{-1}$ concentration, but not beyond that. To further enhance the precision, plates of nanosphere concentration between 200 and 250 $\mu\text{g ml}^{-1}$ were prepared at an increment of 10 $\mu\text{g ml}^{-1}$ and the process was repeated as before.

The MIC was obtained at 230 $\mu\text{g ml}^{-1}$ for *B. subtilis* and at 250 $\mu\text{g ml}^{-1}$ for *E. coli*, as seen in table 2.

4. Discussion

It is hypothesized that the silver nanoparticles have an antibacterial mechanism similar to that of silver ions (Ag^+). However, the antimicrobial activity of silver nanoparticles is far better than that of silver ions. The reason for this is that a large number of surface-associated Ag^0 in silver nanoparticles are oxidized on attachment with biomolecules present on the cell surface. This renders silver nanoparticles far more effective against microorganisms as compared to equal amount of Ag^+ ions [24]. This fact was supported by an earlier study according to which silver nanoparticles are effective in nanomolar range unlike silver ions which are effective in micromolar quantity [25].

Several mechanisms have been proposed for the antimicrobial activity of silver nanoparticles. Sondi and Salopek-Sondi [26] reported that the bactericidal activity of silver nanoparticles is a consequence of their interaction with the bacterial

Table 1. Colony forming units per millilitre (cfu ml⁻¹) of the bacterial culture when exposed to different concentrations of silver nanospheres.

Concentration ($\mu\text{g ml}^{-1}$)	<i>B. subtilis</i>		<i>E. coli</i>	
	Colonies on plate	Colony forming units (cfu ml ⁻¹)	Colonies on plate	Colony forming units (cfu ml ⁻¹)
Control	124	4.69×10^{10}	258	1.03×10^{11}
10	32	1.28×10^{10}	140	5.6×10^{10}
20	15	6.0×10^9	63	2.52×10^{10}
30	0	MIC	19	7.6×10^9
40	—	—	0	MIC

Table 2. Colony forming units per millilitre (cfu ml⁻¹) of the bacterial culture when exposed to different concentrations of silver nanowires.

Concentration ($\mu\text{g ml}^{-1}$)	<i>B. subtilis</i>		<i>E. coli</i>	
	Colonies on plate	Colony forming units (cfu ml ⁻¹)	Colonies on plate	Colony forming units (cfu ml ⁻¹)
Control	Dense growth	Dense growth	Dense growth	Dense growth
210	204	8.16×10^{10}	406	1.62×10^{11}
220	67	2.68×10^{10}	84	3.36×10^{10}
230	0	MIC	55	2.2×10^{10}
240	—	—	12	4.8×10^9
250	—	—	0	MIC

cell membrane. The interaction leads to disruption of the cell membrane leading to cell death. Another mechanism as explained by Bragg and Rainnie [27] was the inactivation of respiratory enzymes leading to the formation of reactive oxygen species resulting in cell damage. A further explanation in this context was given by McDonnell *et al* [28] that silver has a great affinity towards sulphur and phosphorous containing molecules. Due to this, there is an interaction between sulphur containing amino acids like methionine and cysteine and silver, the result of which is the inactivation of several enzymes in the microorganism's metabolic machinery. Also, the interaction between silver and phosphorous containing DNA (deoxyribonucleic acid) in bacteria leads to the inactivation of this genetic material. This leads to inhibition of the synthesis of proteins important for normal cellular functioning [29].

The difference in antibacterial activity of silver nanowires and silver nanospheres has been speculated to the difference in uptake mechanism of the two nanostructures. The nanospheres being spherical in shape show dimensional symmetry and are taken up by the bacterial cells by simple endocytosis, as carried out by bacterial cells for food particles. On the other hand, since nanowires do not have shape symmetry, they are taken up by a more complex mechanism as explained by Yi *et al* [30]. The receptor-mediated endocytosis not only depends on the particle shape and size, but also on the membrane tension and bending stiffness. The uptake by bacterial cell membrane follows near perpendicular entry mode at small membrane tensions, while a parallel entry mode

enhances membrane tensions. Hence, it can be presumed that the difference in uptake mechanism is an important factor responsible for low uptake of nanowires as compared to that of nanospheres, resulting in an enhanced antibacterial action of the latter. Also, the surface to volume ratio is another important factor which comes into play at the nanoscale dimension. Since properties of nanoparticles is a surface phenomenon, the nanospheres have a higher surface to volume ratio as compared to that of nanowires, which makes them have a better interaction with the intracellular molecules, leading to a better antimicrobial activity.

5. Conclusions

In the present study, attempts were made to synthesize Ag nanospheres and nanowires. Ag nanospheres of size around 4 nm were obtained. The nanowires obtained by seed techniques have a unique feature of Ag nanoparticles decorated along the Ag nanowires. The antibacterial activity of these nanostructures was studied on Gram-positive food-borne bacterium *B. subtilis* and Gram-negative pathogenic bacterium *E. coli*. The overall antibacterial activity of silver nanostructures was better against *B. subtilis* as compared to that against *E. coli*. The Ag nanospheres show better activity in terms of MIC as compared to Ag nanowires. This may be attributed to a better surface to volume ratio of Ag nanospheres compared to Ag nanowires on the bacteria.

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