

## Preparation and characterization of poly(ethylene glycol) stabilized nano silver particles by a mechanochemical assisted ball mill process

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**ABSTRACT:** Nano silver particles with an average mean crystallite size of between 10 and 12 nm were synthesized from different molecular weights of poly(ethylene glycol) as a stabilizing agents, through solid state oxidation of silver nitrate using a higher energy planetary ball mill. Ultraviolet-Visible spectra were used to confirm the synthesis of nano silver particles. The surface plasmon resonance bands were observed around 410 nm. Fourier transformed infrared spectrum, X-ray diffraction, and transmission electronic microscopy techniques were used to characterize the nano silver particles synthesized. Thermal stability was determined using thermogravimetric analysis and the elemental composition of the sample was determined by energy dispersive X-ray analysis. The nano silver particles synthesized, exhibited very good antibacterial activity against Gram-positive bacteria (*Bacillus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*). Based on the obtained results, it was additionally explored that the size and the stabilization of the nano silver particles synthesized, strongly depend on the molecular weight of poly(ethylene glycol). © 2015 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2016**, *133*, 43027.

**KEYWORDS:** bioengineering; biomaterials; biomedical applications; nanoparticles; nanowires and nanocrystals

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### INTRODUCTION

Nanotechnology has been used immensely in the synthesis of noble metallic nanoparticles for advanced technological applications.<sup>1,2</sup> These noble metallic nanoparticles contribute the significant role in a wide range of fields such as biomedical, pharmaceutical, cosmetic, drug delivery, antimicrobial, energy and electronic devices etc.<sup>1,2</sup> Among the noble nanoparticles, nano silver particle has gained very good prominence as antimicrobial agent and it has also been used in the clothing, food industries, sunscreens, wounds dressing, and cosmetics.<sup>3–5</sup> Furthermore, silver nanoparticles with polymers have gained popularity in an ointment, wound dressing, and other applications.<sup>6</sup> Therefore, nano silver particle is set to be the future generation antimicrobials agent.

In the literature, a number of chemical methods have been employed in the synthesis of nano silver particles<sup>7–12</sup>; however, some issues remained still challenging. One of them is associated with the size, shape and surface morphology of nano silver particles in solid state.<sup>13–16</sup> Silver nanoparticles with large particle size and low specific surface area, significantly decrease its anti-

microbial activity.<sup>17–19</sup> In order to increase the antimicrobial activity, the size of nano silver particles should be lessened to extremely nano level. Furthermore, the synthesis of these nanoparticles is expensive and restricted to the laboratory scale. Therefore, there is an increasing need for high-yield, low cost, a non-toxic and eco-friendly procedure for the synthesis of nano silver particles in large (or industrial) scale.

Among the various techniques, mechanochemical ball milling process is one of the widely used techniques in order to synthesize nanoparticles in the solid state.<sup>20,21</sup> Moreover, by using this process, plenty of nanomaterials can be synthesized at ambient temperature, resulting in the absence of residues and with good control of particle size.<sup>5,22,23</sup> These features are more suitable and important in the antimicrobial field. This technique is an eco-friendly and cost-effective physical process and it is an attractive technique for large-scale industrial production in the solid state. Recently, various nanomaterials, such as magnetite, zinc oxide, cuprous oxide and nitrogen doped carbon nanoparticles were synthesized using this

procedure.<sup>20,24–26</sup> However, according to literature, there has been limited reports for the synthesis of nano sized silver particles by this process.<sup>27</sup>

Poly(ethylene glycol) (PEG) is a synthetic polyether polymer frequently used medical applications, owing to its wide range of molecular weight, excellent solubility in aqueous medium, low toxicity, chain flexibility and its biocompatibility properties and in addition, it is biodegradable *via* its excretion from the body to form non-toxic metabolites.<sup>28–32</sup> PEG is used to modify biomaterial surface in order to prevent the foreign body reactions.<sup>33,34</sup> Further, PEG is playing an important role in the field of nanotechnology where PEG is being used as reducing and stabilizing agent. By using PEG nano inorganic particles were prepared without agglomeration.<sup>35,36</sup> Lately, researchers have reported that increase in the molecular weight of PEG will exhibit higher stability in the formation of nano silver particles from silver nitrate.<sup>37,38</sup>

Based on the above, we report on the synthesis of nano silver/PEG (6000, 8000) *via* a green mechanochemical (ball milling) process, using two different molecular weights: PEG 6000 and PEG 8000, as stabilizing agents. The results indicate that by appropriate choice of milling time, the nanoparticle size, as well as the composition of the powder, can be adequately controlled. Thus, a very eco-friendly and cost-effective physical process (a solid state phenomenon) on a large and industrial scale synthesis of nano silver particles are practicable and hence the antibacterial activity of the nano silver particles synthesized has been studied and reported herein.

## MATERIALS AND METHODS

### Materials

Two different molecular weights poly(ethylene glycol)s (PEG = 6000, 8000) and silver nitrate ( $\text{AgNO}_3$ ) were obtained from Merck (Bombay, India). The Department of Biochemistry (University of Johannesburg, Johannesburg, South Africa) provided standard cultures of the organisms.

### Synthesis of PEG Stabilized Silver Nanoparticles

PEG stabilized nano silver particles were synthesized by ball milling silver nitrate (0.5 g) in a planetary ball mill (RETSCH PM-200, Germany), in the presence of PEG (5 g) powder. Milling was done at room temperature in a polyethylene (PE) vial (volume  $\sim 35 \text{ cm}^3$ ) using ten hardened steel balls with 10 mm diameter and keeping sample-to-ball mass ratio at about 1:10 throughout the experiment. The milling process lasted for 2 h, with a rotation speed 200 rpm. The powder was milled for a period of 24 h and a small amount of milled powder were taken at time intervals of 6 h, 12h, 18 h, and 24 h for characterization.

### Characterizations

**Ultraviolet-Visible (UV-Vis).** UV-vis spectra of PEG stabilized nano silver particles were recorded on an ELICO SL 164 Model UV-vis spectrophotometer (The Elico co., Hyderabad, India) in a wavelength range of between 200 and 700 nm. For this study, 5 mg of PEG stabilized nano silver particles were dispersed in 20 mL of distilled water and allowed to stand for 10 min before recording their UV-vis absorption spectra.

### Fourier Transformed Infrared (FTIR)

The PEG stabilized nano silver particles were studied for functional groups interaction by using the FT-IR. It was performed on PEG and PEG stabilized nano silver particles in the range of  $400\text{--}4000 \text{ cm}^{-1}$ . The FTIR spectroscopy was performed on (Perkin Elmer Spectrum 100 FTIR spectrometer), USA.

### Thermogravimetric Analysis (TGA)

TGA of the samples was carried out using SDT Q 600 DSC instrument (T.A. Instruments-water LLC, Newcastle, DE 19720, USA), at a heating rate of  $1^\circ\text{C}/\text{min}$ , under constant nitrogen flow ( $100 \text{ mL}/\text{min}$ ) for the DSC and a heating rate of  $10^\circ\text{C}/\text{min}$  under a constant nitrogen flow ( $100 \text{ mL}/\text{min}$ ) for the TGA.

### X-ray Diffraction (XRD)

The structural characteristics of the PEG stabilized nano silver particles prepared were performed using an X-ray diffractometer operated at 40 kV and 50 mA (RigakuUltima IV). The samples were recorded using the  $\text{Cu K}_\alpha$  radiation source ( $\lambda = 1.541\text{\AA}$ ) in the  $2\theta$  range of between  $10^\circ$  and  $70^\circ$ .

### Transmission Electron Microscopy (TEM)

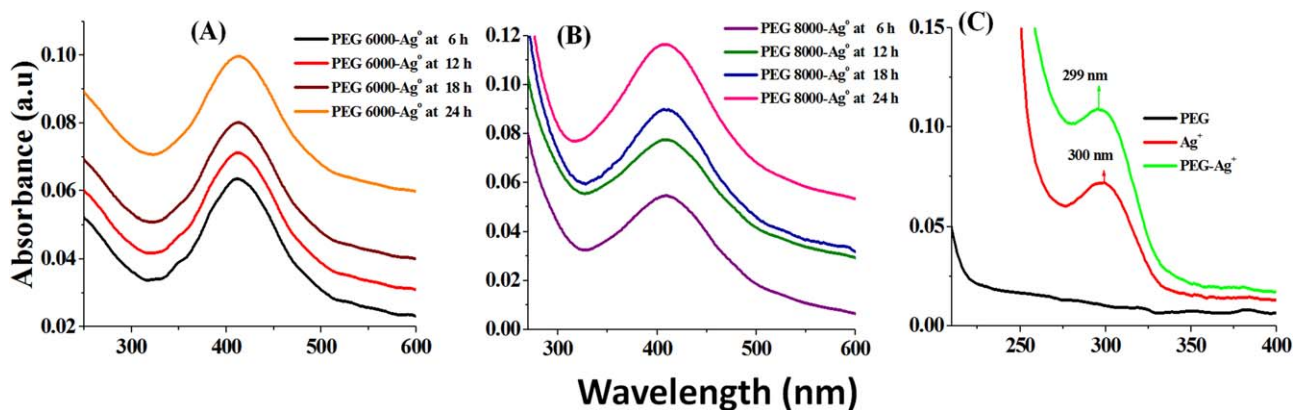
TEM (JEM-1200EX, JEOL, Tokyo, Japan) was used for the morphological observation of the PEG stabilized  $\text{Ag}^0$  nanoparticles. TEM samples were prepared by dispersing 1 mg of PEG stabilized nano silver particles in 1 mL of double distilled water and 1 drop of PEG stabilized nano silver particles solution was placed on a 3 mm copper grid and dried at ambient temperature after removing excess solution using filter paper.

### Antibacterial Activity

The antibacterial activity of the PEG stabilized nano silver particles under study, was investigated by employing a similar standard procedure described elsewhere in the literature but with a little modification by making wells with the wide end of a  $1000 \mu\text{L}$  pipet tip into the agar medium and removing the agar plug.<sup>4,23,39,40</sup> Nutrient agar medium was prepared by mixing peptone (5.0 g), beef extract (3.0 g) and sodium chloride (NaCl) (5.0 g) in a  $1000 \text{ mL}$  double distilled water and the pH was adjusted to 7.0. Finally, agar (15.0 g) was added to the solution. The agar medium was sterilized in an autoclave at a pressure of  $15 \text{ lbs in}^{-2}$  ( $\sim 103.4214 \text{ kPa}$ ) for 30 min. This medium was transferred into sterilized petri dishes in a laminar air flow chamber (Microfilit Laminar Flow Ultra Clean Air Unit, Mumbai India). Following the solidification of the media, bacteria (*Bacillus* and *Pseudomonas aeruginosa*) ( $50 \mu\text{L}$ ) culture was spread on the solid surface of the media and wells were formed. In to these wells, one drop ( $1 \text{ mg}/\text{mL}$ ) of PEG stabilized nano silver particles, prepared at different time intervals (6, 12, 18, and 24 h) was poured and the plates were incubated for 2 days at  $37^\circ\text{C}$  in an incubation chamber.

### Results and Discussions

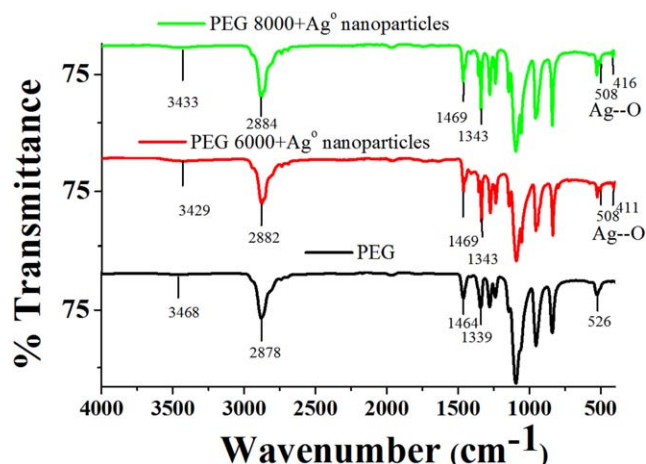
The formation of PEG stabilized nano silver particles were analyzed by using UV-Visible spectrum. Figure 1 shows the UV-Visible spectrum of the nano silver particles synthesized by ball mill process with stabilizing agent, PEG. Figure 1(A) shows PEG 6000-nano silver particles surface plasmon resonance at 411 nm and Figure 1(B) shows PEG 8000-nano silver particles surface plasmon resonance at 409 nm with time intervals of



**Figure 1.** UV-visible spectra of: (A) PEG 6000 stabilized nano silver particles, (B) PEG 8000 stabilized nano silver particles, obtained at different time intervals and (C) PEG, silver ions and PEG-silver ions combined [i.e., initial samples before stabilization]. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

between 6 h and 24 h, respectively. PEG 8000-nano silver particles are highly stabilized than the PEG 6000-nano silver particles, as a consequence of its superior molecular weight of PEG 8000. Recently, Shkilnyy *et al.*,<sup>35</sup> and Vimala *et al.*,<sup>41</sup> observed that nano silver particles stabilization increases with increasing molecular weight of PEG. Moreover, PEG, silver ions and PEG-silver ions particles at 0 h (initial) of milling do not show any absorption peaks around 411 and 409 nm [Figure 1(C)]. This result confirms that nano silver particles were successfully synthesized *via* ball mill process.

In order to ascertain the formation of the nano silver particles in PEG stabilized nano silver particles and their interaction with PEG, the samples were analyzed by FTIR spectroscopy. Figure 2 shows the FT-IR spectra of PEG and PEG stabilized silver nanoparticles. PEG absorption bands at  $3468\text{ cm}^{-1}$  was due to the O-H stretching vibrations,  $2878\text{ cm}^{-1}$  was due to the aliphatic C-H stretching,  $1464$  and  $1339\text{ cm}^{-1}$  were due to C-H bending vibrations,  $1278$  and  $1095\text{ cm}^{-1}$  were due to O-H and C-O-H stretching vibrations, respectively, which are consistent with data in the previous report.<sup>45</sup> All these peaks are observed in PEG-stabilized



**Figure 2.** FT-IR spectra of PEG, PEG 6000 stabilized nano silver particles and PEG 8000 stabilized nano silver particles. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

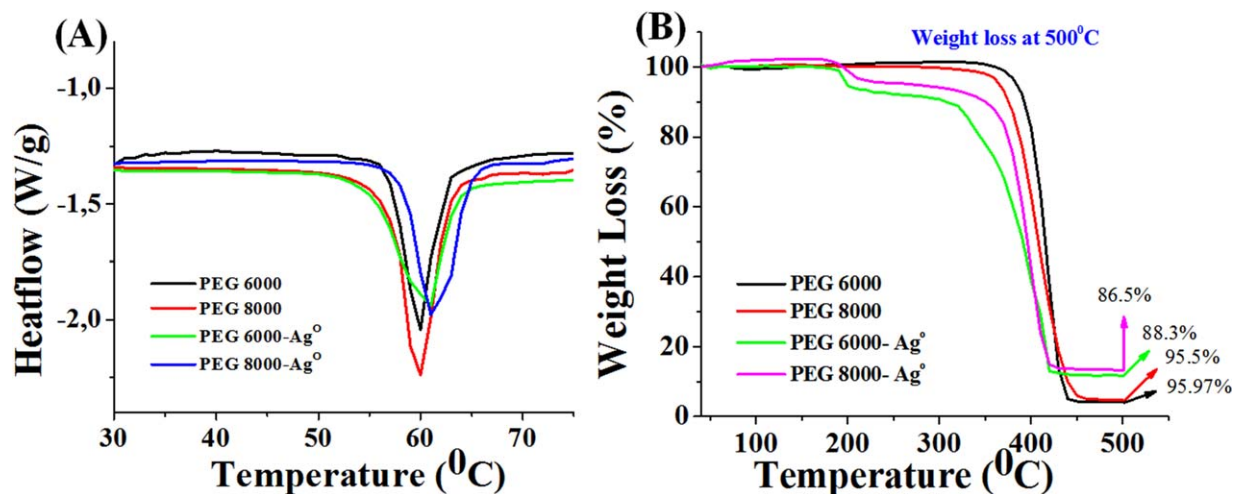
nano silver particles with their wavelengths slightly shifted; in addition, new peaks were also observed at around 509 and  $411\text{ cm}^{-1}$ . These new peaks are due to the coordination bond formed between nano silver particles and the hydroxyl groups of PEG.<sup>45</sup> Based on the FTIR data, it is evident that strong interaction of PEG and nano silver particles occurred.

The thermal stability of the PEG stabilized nano silver particles synthesized were characterized using the TGA. Figure 3 shows the DSC thermograms of the PEG stabilized nano silver particles. Figure 3(A) shows the DSC curves of PEG 6000 and PEG 8000, displaying melting endothermic peaks<sup>42–44</sup> at  $\sim 59.86^\circ\text{C}$  and  $60.15^\circ\text{C}$ , whereas these melting endothermic peaks were shifted to  $\sim 60.91^\circ\text{C}$  and  $61.07^\circ\text{C}$  in PEG stabilized nano silver particles, as a consequence of nano silver particles formation.

TGA is a powerful technique, usually employed for the analysis of the decomposition and thermal stability of materials. TGA measures the weight change as a function of temperature. As the temperature increases, the weight of the sample decreases, indicating the continuous decomposition of the sample. Figure 3(B) shows the TGA thermogram of: PEG 6000, PEG 8000 and their PEG stabilized nano silver particles. From Figure 3(B), a significant weight loss<sup>45</sup> is observed in PEG stabilized nano silver particles between  $180^\circ\text{C}$  and  $220^\circ\text{C}$  and there is no weight loss below  $180^\circ\text{C}$ . This can be attributed to the evaporation of water and organic components. Both PEG and PEG stabilized nano silver particles undergo a substantial weight loss at  $\sim 420^\circ\text{C}$ , corresponding to the pyrolysis of PEG functional groups. In nitrogen atmosphere and at  $500^\circ\text{C}$ , the weight loss observed<sup>46</sup> in PEG 6000 and PEG 8000 are 95.97% and 95.5%, respectively, whereas the weight loss in PEG 6000 and PEG 8000 stabilized nano silver particles are 88.3% and 86.5% at this particular temperature ( $500^\circ\text{C}$ ). Therefore, the weight loss ratios between PEG and PEG stabilized nano silver particles were determined to be 7.67% and 9%.

The XRD of PEG and PEG stabilized nano silver particles are shown in Figure 4. PEG is observed to show major XRD peaks at  $2\theta = 19.31^\circ$ ,  $23.32^\circ$  and a few minor peaks at:  $2\theta = 26.40^\circ$ ,  $36.28^\circ$ ,  $39.74^\circ$  and  $45.22^\circ$ , respectively, which is consistent with data in previous report.<sup>44</sup> Similar peaks were observed in PEG stabilized nano silver particles and also a few other peaks were





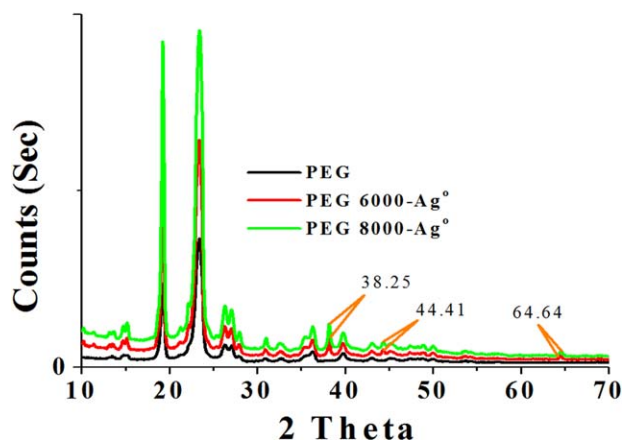
**Figure 3.** (A) DSC curves of: PEG 6000, PEG 8000, PEG 6000 stabilized nano silver particles and PEG 8000 stabilized nano silver particles and (B) TGA curve of: PEG 6000, PEG 8000, PEG 6000 stabilized nano silver particles and PEG 8000 stabilized nano silver particles. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

observed at:  $2\theta = 38.25^\circ$ ,  $44.41^\circ$  and  $64.64^\circ$ . These peaks correspond to lattice planes of silver (111), (200) and (311), respectively, as presented in Figure 4. XRD patterns also represent the face-centered cubic (FCC) structure of nano silver particles<sup>26,47</sup> and the data was found to correspond with the database in the JCPDS File No. 04-0783 of WinXpov software. The XRD patterns, thus clearly show that the nano silver particles were formed through the ball mill process. The average grain size of the nano silver particles was determined using the Scherer's equation:

$$D = \frac{K\lambda}{\beta} \cdot \cos \theta \quad (1)$$

where  $D$  is the particle diameter in nm,  $K$  is a constant,  $\lambda$  is the wavelength of X-ray source (0.15406 nm),  $\beta$  is the full width half maximum (FWHM) of the X-ray diffraction peak and  $\theta$  is the diffraction angle in degree ( $^\circ$ ). By using the above relation, eq. (1), the average sizes of the nano silver particles, estimated from the two main diffraction peaks are 9 and 12 nm.

Formation of nano silver particles size was confirmed by TEM analysis. Figure 5 shows the TEM images of PEG 6000 and PEG

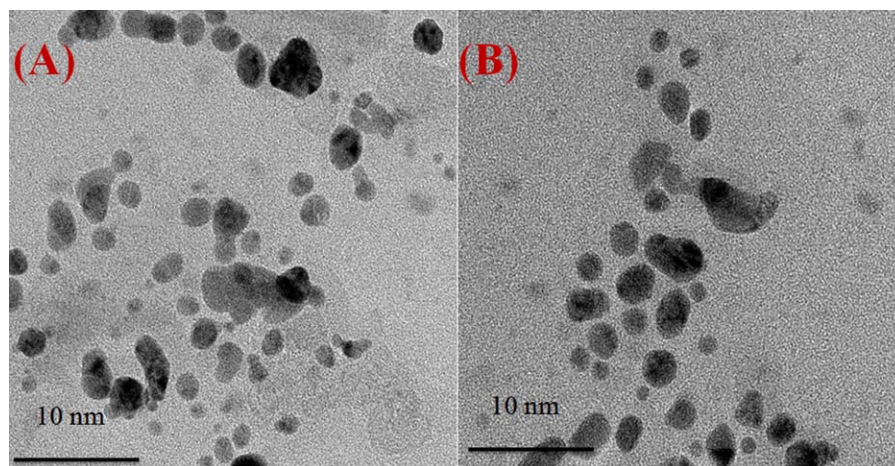


**Figure 4.** XRD patterns of: PEG, PEG 6000 stabilized nano silver particles and PEG 8000 stabilized nano silver particles. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

8000 stabilized nano silver particles. The particles observed were spherical without aggregation. Incorporation of PEG to the nano silver particles efficiently protect them from aggregation by steric repulsion.<sup>12</sup> This is similar to the effective protection of aggregation of nano particles by surface coating of nano particles with layers of neutral organic molecules.<sup>12</sup> Further, according to Luo *et al.*,<sup>38</sup> increasing the molecular weight of the polymer facilitates the formation of stable nano particles without aggregates. From the TEM images, it can be clearly seen that the smaller size nano silver particles were formed in PEG 8000 ( $\sim 10$  nm) [Figure 5(B)], rather than the PEG 6000 ( $\sim 12$  nm) [Figure 5(A)]. This is an indicative of comparatively more effective stabilization of higher molecular weight PEG 8000 than PEG 6000. The extra stabilization was believed to be due to more steric repulsion generated among higher molecular weight PEG 8000, which allows the associated nano silver particles to lesser nano dimension. The sample milled for 24 h (Figure 5) shows a spherical shape with uniform size distribution of particles, with sizes in the range of  $\sim 10$  nm (PEG 8000) and 12 nm (PEG 6000). These sizes are also in agreement with those obtained using the Debye-Scherrer equation ( $\sim 9$  nm for PEG 8000 and 12 nm for PEG 6000). This is a remarkable result, achieved in the present study, in the quest to produce ultrafine spherical nanoparticles of silver on a large scale, from the bulk, which might be useful for antibacterial applications. Furthermore, in order to determine the amount of nano silver particles in the PEG 6000 and PEG 8000, EDS analyses were carried out and a strong signal for nano silver particles at the spectrum of 3 keV was characteristic of nano silver particles. Figure 6 shows the EDS spectra of nano silver particles synthesized using the ball mill process. From these results, it can be concluded that marginally, higher percentage of nano silver particles was formed in PEG 8000 (6B-90.24%) than in PEG 6000 (6A-89.31%).

#### Antibacterial Studies

In recent years, noble metals have been actively studied for biomedical applications.<sup>48</sup> Among them, silver has gained the most

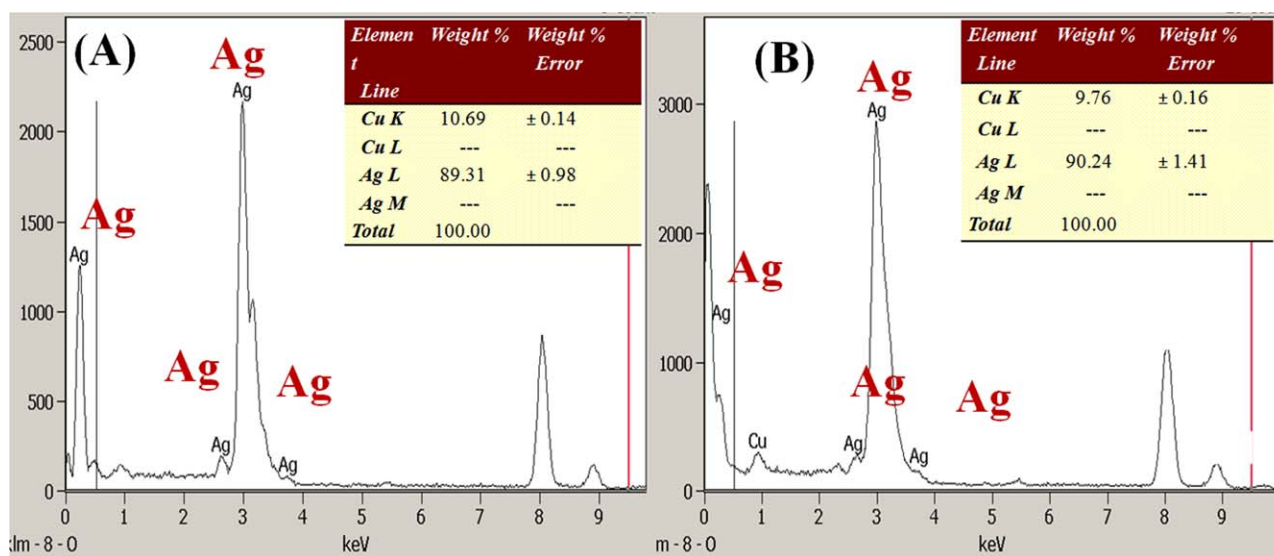


**Figure 5.** TEM images of (A) PEG 6000 stabilized nano silver particles and (B) PEG 8000 stabilized nano silver particles. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

important acceptance as universal antibacterial substance. In general, silver containing salts having antimicrobial activity has gained a lot of popularity. However, bulk silver cannot work effectively on microbes and it's more harmful to living system when compare to nano silver. Nano silver is very useful in order to avoid the harmful effect of silver on the consumers and it works efficiently on bacteria growth arrest.<sup>13,49</sup>

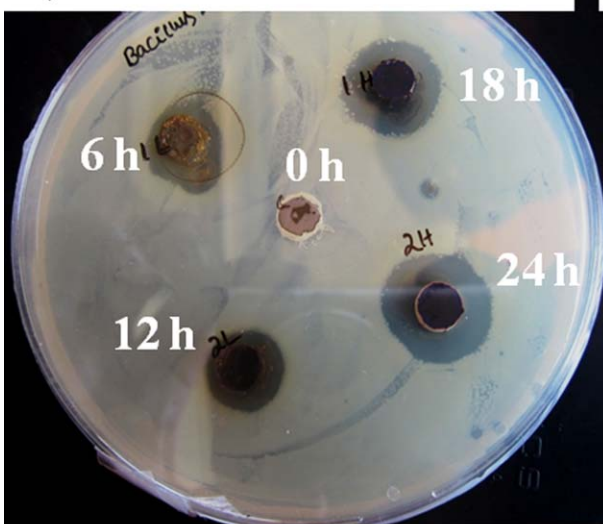
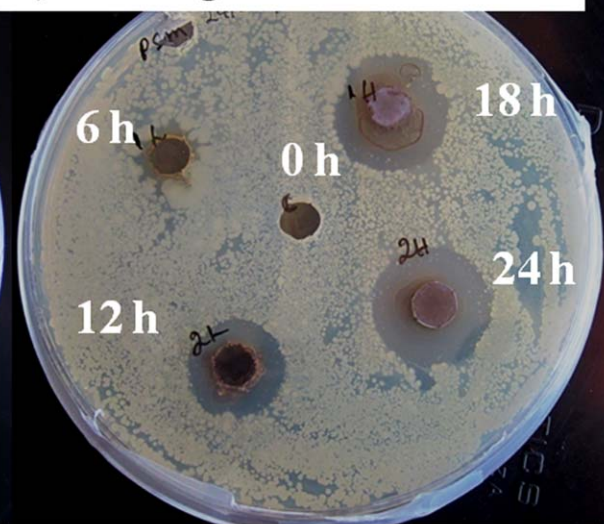
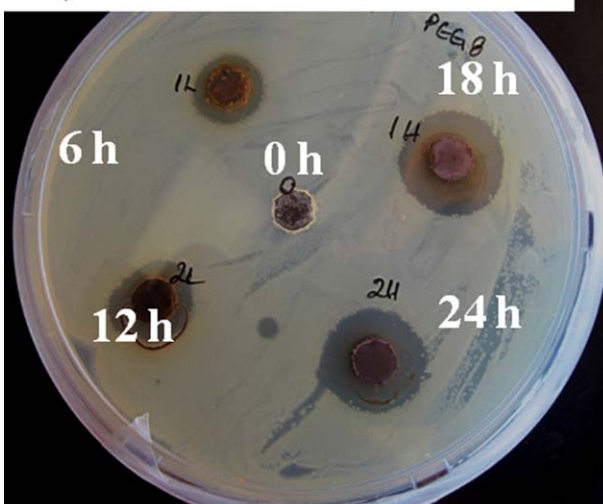
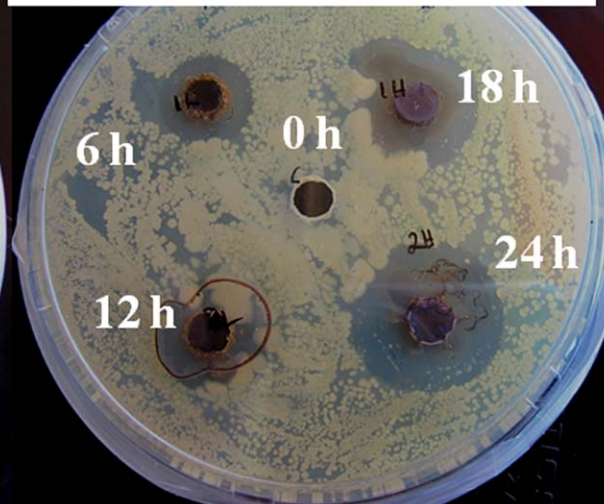
In the current investigation, PEG stabilized nano silver particles synthesized were shown antibacterial activity against gram-positive bacteria (*Bacillus*) and gram-negative bacteria (*Pseudomonas aeruginosa*). Figure 7 shows the different time intervals of the synthesized nano silver particles from 0 h to 24 h. The PEG 6000 stabilized nano silver particles exhibited inhibition zones were followed on both bacteria: *Bacillus* [Figure 7(A): 0 h (0 mm), 6 h (6.5 mm), 12 h (6.8 mm), 18 h (7.2 mm) and 24 h (7.5 mm)] and *Pseudomonas aeruginosa* [Figure 7(B): 0 h (0 mm), 6 h (5.0 mm), 12 h (7.0 mm), 18 h

(7.5 mm) and 24 h (9.5 mm)] and PEG 8000 stabilized nano silver particles exhibited inhibition zones were followed on both bacteria: *Bacillus* [Fig. 7(A<sup>1</sup>): 0 h (0 mm), 6 h (7.0 mm), 12 h (7.2 mm), 18 h (9.9 mm) and 24 h (10.2 mm)] and *Pseudomonas aeruginosa* [Figure 7(B<sup>1</sup>): 0 h (0 mm), 6 h (7.5 mm), 12 h (7.0 mm), 18 h (8.5 mm) and 24 h (10.0 mm)], respectively. From the above results (Figure 7) the order of the bacterial inhibition zones as followed in this manner: 0 h < 6 h < 12 h < 18 h < 24 h and also PEG 8000 stabilized nano silver particles had shown good antibacterial activity against both positive and negative bacteria when compared to PEG 6000 stabilized nano silver particles. These results are quite expected and the trend seemed to have manifested according to the nanoparticles content in the PEG stabilized nano silver particles. It was evident from the TGA analysis that the percentage nano silver particles content<sup>23</sup> is higher for PEG 8000 than PEG 6000, respectively.



**Figure 6.** EDS peaks of: (A) PEG 6000 stabilized nano silver particles and (B) PEG 8000 stabilized nano silver particles. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]



**A) *Bacillus*-PEG 6000****B) *P. Aeruginosa*-PEG 6000****A<sup>1</sup>) *Bacillus*-PEG 8000****B<sup>1</sup>) *P. Aeruginosa*-PEG 8000**

**Figure 7.** Antibacterial activity of PEG (6000, 8000) and PEG (6000, 8000) stabilized nano silver particles: 0 h pure PEG, PEG stabilized nano silver particles at different time intervals 6 h, 12 h, 18 h, 24 h on *Bacillus* (A, A<sup>1</sup>) and *Pseudomonas aeruginosa* (B, B<sup>1</sup>). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

These results can be understood on the basis of the source of bacterial action of nano silver particles, as per their sizes and quantities. Therefore, decreasing the nano silver particle size leads to an increase in the precise surface of a bacterial specimen, suggesting an increase in their ability to enter the cell membrane and improving the antibacterial activity. The nano silver particles showed efficient antimicrobial property when compared to bulk silver. This is due to their extremely large surface area, which provides better contact with microorganisms<sup>49</sup> and also the possibility of the nano silver particles not only interacting with the surface of membrane, but can also penetrate inside the bacteria.<sup>50</sup> Furthermore, according to various literatures and the standard antibacterial test “SNV 195920–1992”,<sup>51,52</sup> specimens showing more than 1 mm microbial zone inhibition can be considered as good antibacterial agents.<sup>4,23,51</sup>

Hence, the PEG stabilized nano silver particles synthesized from ball mill process can be considered as excellent antibacterial agents and has been proved to, effectively, work on the inactivation of the two bacteria tested.

### CONCLUSION

Nano silver particles were successfully synthesized *via* the ball mill process and using PEG as a stabilizing agent. PEG hydroxyl groups were involved in the stabilization of ball milling of the silver ions into nano silver particles. The nano silver particles formed were characterized using UV–Visible, FTIR, TGA, DSC, EDS and TEM. The nano silver particles synthesized exhibited very good antibacterial properties and their (nano silver particles) size depended on the molecular weight of PEG. The

present process has more advantages for the production of bulk, cost-effective, formation of highly stable nanoparticles, straightforward and scalable. In addition, as the nano silver particles synthesized in the present approach are in solid form rather in liquid. Hence, the volume occupied is significantly less, hence it can be utilized even by proportionate dilution and applied for more valuable applications in the antimicrobial field in designing surgical wound dressing, antimicrobial finishing, antimicrobial functional coating materials etc. This demonstrates the practicability of the current process.

## ACKNOWLEDGMENTS

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