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Synthesis, characterization and antimicrobial activity of dextran stabilized silver nanoparticles in aqueous medium

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ABSTRACT

A simple one-step rapid synthetic route is described for the preparation of silver nanoparticles by reduction of silver nitrate ($AgNO_3$) using aqueous dextran solution which acts as both reducing and capping agent. The formation of silver nanoparticles is assured by characterization with UV–vis spectroscopy, atomic force microscopy (AFM), transmission electron microscopy (TEM) and X-ray diffraction (XRD). The absorbance of the silver nanoparticles is observed at 423 nm. The AFM image clearly shows the surface morphology of the well-dispersed silver nanoparticles with size range of 10–60 nm. TEM images show that the nanoparticles are spherical in shape with ~5–10 nm dimensions. The crystallinity of Ag nanoparticles is assured by XRD analysis. The antimicrobial activity of as synthesized silver nanoparticles is tested against the bacteria, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The bacterial growth is inhibited by gradual reduction of the concentration of the silver nanoparticles.

1. Introduction

In the last two decades, the study and preparation of inorganic crystalline particles of the order of nanometer range has attracted considerable attention of scientists from both fundamental and applied research field (Elghanian, Storhoff, Mucic, Letsinger, & Mirkin, 1997). Metal nanoparticles (MNPs), such as silver, gold and copper have received special attraction because of their catalytic (Mallik, Witcomb, & Scurell, 2005), electronic (Kamat, 2002) and unique optical properties (Liz-Marzan, 2006; Malvaney, 1996) making them very attractive in the fields of particularly sensing, bio-conjugation, and surface enhancement Raman spectroscopy (SERS) (Cao, Jin, Nam, Thaxton, & Mirkin, 2003; Shipway, Kartz, & Willner, 2000). Among the noble MNP's, silver has wide recognition for its application in semiconductors (Henglein, 1989; Kamat, 1993; Schimd, 1992), superconductors (Henglein, 1993; Pileni, 1993), super magnets (Lee et al., 1995), micro-electronics (Deheer, 1993; Littau, Szajowski, Muller, Kortan, & Brus, 1993), lithography (Shipway, Lahav, & Willner, 2000; Xia, Rogers, Paul, & Whitesides, 1999), etc. Recently researchers have shown that the silver nanoparticles interact with a human immunodeficiency virus type 1 and prevent the virus from binding to the host cells (Elechiguerra et al., 2005). The antimicrobial activity of silver nanoparticles is comparatively better than the broad-spectrum most prominent antibiotics used worldwide (Roy et al., 2008).

Various methods for the preparation of metallic nanoparticles have been reported. The most common method is chemical reduction of metal salt precursor using chemical reducing agents, such as citrate (Rivas, Sanchez-cartos, Garcia-Ramos, & Marcillo, 2001), polymer substances (Maity et al., 2011; Ohno, Koh, Tsujii, & Fukada, 2003; Zhang et al., 2000), borohydride, or other organic reagents (Gauri et al., 2003; Mayya, Schoeler, & Caruso, 2003; Plynto, Berquier, Jacquiod, & Ricolleau, 1999; Tan, Jiang, Li, & Zhu, 2002; Tanori & Pileni, 1997; Wang et al., 2002). Other methods are laser ablation (Abid, Wark, Brevetm, & Girault, 2002), sono chemical deposition (Pol et al., 2002; Stiger, Gorer, Craft, & Penner, 1999), photochemical reduction (Hunng et al., 1996; Malune, Kohon, Takeda, & Kondow, 2000), electrochemical method (Yu, Chang, Lee, & Wang, 1997; Zhu, Liu, Palchik, Kottypin, & Gedanken, 2000), and heat evaporation (Smetana, Klabundu, & Sorensen, 2005), thermal decomposition in organic solvents (Esumi, Tano, Torigoe, & Meguro, 1990), molecular beam epitaxy (Colli et al., 2005) and green synthesis by plant extract (Bar, Bhui, Sahoo, Sarkar, De, et al., 2009; Bar, Bhui, Sahoo, Sarkar, Pyne, et al., 2009) and fungus (Saha et al., 2010).

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Silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents (Krutyakov, Kudrynskiy, Olenin, & Lisichkin, 2008; Sarkar, Saha, Chattopadhyay, Patra, & Acharya, 2011). In small concentrations, silver is safe for human cells, but lethal for bacteria and viruses (Sharma, Yngard, & Lin, 2009). Reduction of the particle size of the materials is an efficient and reliable tool for improving their biocompatibility that can be achieved using nanotechnology.

The antibacterial property of silver nanoparticles has been studied by a number of microbiologists. Sondi and Salopek-Sondi (2004) evaluated the antimicrobial activity of silver nanoparticles against *Escherichia coli*. The results confirmed that the treated *E. coli* cells are damaged, showing pit formation on bacterial cell walls. In another work, Bosetti and co-workers (Bosetti, Masse, Tobin, & Cannas, 2002) tested the antibacterial action of silver nanoparticles-coated polyurethane foam and suggested its use as antibacterial water filter. Recently, Hu and co-workers (Hu, Zhang, Chan, & Szeto, 2006) treated cotton fabrics with suspension of silver oxide in chitosan and studied antibacterial actions against *Staphylococcus aureus*. Now a day, it seems promising to incorporate the specified nano silver drugs as a special class of biocidal agents.

The utility of dextran and its amino derivative as capping agents have been reported in various preparatory methods of silver nanoparticles using silver nitrate and different reducing agents. Silver nanocomposites are synthesized in situ in dextran hydrogel network using N,N methylenebisacrylamide and followed by reduction of silver nitrate (Ma, Yi, & Zhang, 2009). (Ma, Li, Yang, & Yang, 2005) described one-step synthesis of Ag nanoparticles using amino dextran and their application in biosensors. Domingos et al. (Eising, Signori, Fort, & Domingos, 2011) reported synthesis of Ag nanoparticles using sodium borohydride and their catalytic activity. Qi et al. (Yang, Zhang, Ma, Cheng, & Qi, 2004) reported controlled synthesis of silvers microcrystals with novel morphologies using ascorbic acid with dextran.

In this paper, we describe first time the results of the preparation and mechanism of formation of silver nanoparticles using dextran as both reducing cum stabilizing agent. This work has been conducted to develop one pot, cost-effective preparation of spherical silver nanoparticles at ambient temperature and in short reaction time. We have shown by AFM that silver nanoparticles are trapped between dextran molecules and we have also described in detail the mechanism of formation of silver nanoparticles capped with dextran molecules. Also, we have investigated the antimicrobial activity of the synthesized Ag nanoparticles against five bacteria. Our result signifies that silver nanoparticles synthesized by dextran are suitable for formulation of new types of bactericidal materials.

2. Experimental

2.1. Materials

Silver nitrate ($AgNO_3$) and sodium hydroxide (NaOH) were purchased from Merck (Mumbai, India) and used without further purification. Dextran T40 was purchased from Pharmacia (Sweden). All aqueous solutions were made using ultra high purity water purified using a Mill-Q Plus system (Millipore Corporation Co., USA).

2.2. Preparation of silver nanoparticles using dextran

All glassware was cleaned in a bath of freshly prepared aqua regia solution (HCl–HNO_3, 3:1), and rinsed first thoroughly with double distilled water and then acetone for prior to use. A stock solution of 5% dextran was prepared by dissolving the dextran in Millipore water. Silver nitrate solution (0.01 M) was prepared by adding $AgNO_3$ in Millipore water. Then, the silver nitrate solution was mixed with dextran solution followed by the addition of 0.4 ml of very dilute solution of sodium hydroxide (0.001 M) at room temperature. The transparent colorless solution was converted to the characteristics pale yellow color, indicating the formation of silver nanoparticles. It was observed that the aqueous solution of silver nanoparticles was stable more than one month at room temperature.

2.3. Assay for antimicrobial activity of silver nanoparticles against microorganisms

The silver nanoparticles in sterilized distilled water were tested for their antibacterial activity by the agar diffusion method. Five bacterial strains, *Bacillus subtilis* [MTCC 736], *Bacillus cereus* [MTCC 306], *E. coli* [MTCC 68], *S. aureus* [MTCC 96] and *Pseudomonas aeruginosa* [MTCC 8158] were used for this analysis. These bacteria were grown on liquid nutrient agar media (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for 24 h prior to the experiment, were seeded in agar plates by the pour plate technique. For every bacterial strain two different plates were prepared. In one plate, two cavities were made using a cork borer (10 mm diameter) at an equal distance. In one petri plate, the cavities were filled with the silver nano particle solution (0.2 mg/ml) and only sterile distilled water, respectively. Simultaneously, in another plate, two cavities were made using the same method described above and filled with only 5% dextran solution and only 0.01 M silver nitrate solution, respectively. All the plates were incubated at 37 °C for 24 h. Every experiment was repeated for three times.

3. Characterization

3.1. UV–vis spectroscopy

The reduction of pure Ag^+ ions was monitored by measuring the UV–vis spectrum of the reaction medium. UV–vis absorption spectrum of the sample was done in a UV–vis spectrophotometer HITACHI U-4100 (Japan) in the wavelength range from 200 to 700 nm. The absorption in the visible range directly reflects the perceived color of the chemical involved.

3.2. Atomic force microscopy (AFM)

Atomic force microscopy was done to observe the surface morphology and size of the resultant silver nanoparticles. Sample was dropped onto new cleaved mica slices and dried overnight. AFM study of the silver nanoparticles deposited on mica slices was performed in a microscope VEECO, Multimode Nanoscope IIIa (USA).

3.3. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) was also used to observe the size, shape and morphology of the resultant nanoparticles. A specimen for TEM sample was made by casting a drop of suspension on a carbon coated copper grid and the excess solution was removed by tissue paper and allowed to air dry at room temperature for over night. TEM study was observed on a TEM, JEOL JEM 2010 (Japan) at an accelerating voltage of 200 kV and fitted with a CCD camera.

3.4. X-ray diffraction (XRD)

The XRD technique was used for determination and confirmation of the crystal structure of silver nanoparticles. The sample for XRD analysis was prepared by depositing the centrifuged sample on a microscopic glass slide and air-dried overnight. The diffractogram was recorded from PANalytical, XPERT-PRO diffractrometer (Netherlands) using Cu Kα (λ = 1.54060) as X-ray source.

4. Results and discussion

Dextran is a water-soluble polysaccharide. It is produced industrially in a large scale and has wide range of applications in food and medical related areas. Dextran has been used as protecting agent for the synthesis of metal nanoparticles but no reports have found describing the use of this polysaccharide both as reducing and as stabilizing agent in synthesis of metal nanoparticles in aqueous solution at room temperature. The method for the preparation of silver nanoparticles using dextran as both reducing and protecting agent in water is quite simple. The mixture of silver nitrate and sodium hydroxide forms silver hydroxide instead of silver nanoparticles. Silver nanoparticles cannot be synthesized by using dextran solution in Millipore water. Because Millipore water is faintly acidic (p^H-6) which can prevents the reduction of Ag^+ to Ag^0 in nanometer range by forming $R{-}O^+H_2$ (step-I). Fig. 1 shows the schematic diagram of formation and stabilization of silver nanoparticle. The presence of very low concentration of sodium hydroxide can free the hydroxyl groups of dextran by removing the protons (step-II) and assist the formation of silver nanoparticles immediately by changing the color of solution (step-III).

4.1. UV–vis spectroscopic analysis

Silver nanoparticles absorb radiation in the visible region of the electromagnetic spectrum (380–450 nm) due to the surface plasmon resonance (SPR) transition. This SPR transition is responsible for the striking yellowish brown coloration of silver nanoparticles. The UV–vis absorption spectrum of the silver nanoparticles is shown in Fig. 2(a) and (b). The absorbance of the silver nanoparticles is observed at 423 nm. The related result was also reported in silver nanoparticles prepared by chemical reduction method (Wang, Qiao, Chen, & Ding, 2005). The UV–vis absorption spectrum of the same sample is taken after 30 days and it has been found that the absorption maxima is shifted to 425 nm which is very close to the initial peak position indicating almost no agglomeration of silver nanoparticles. This phenomenon reveals that the silver nanoparticles are well capped with dextran macromolecules.

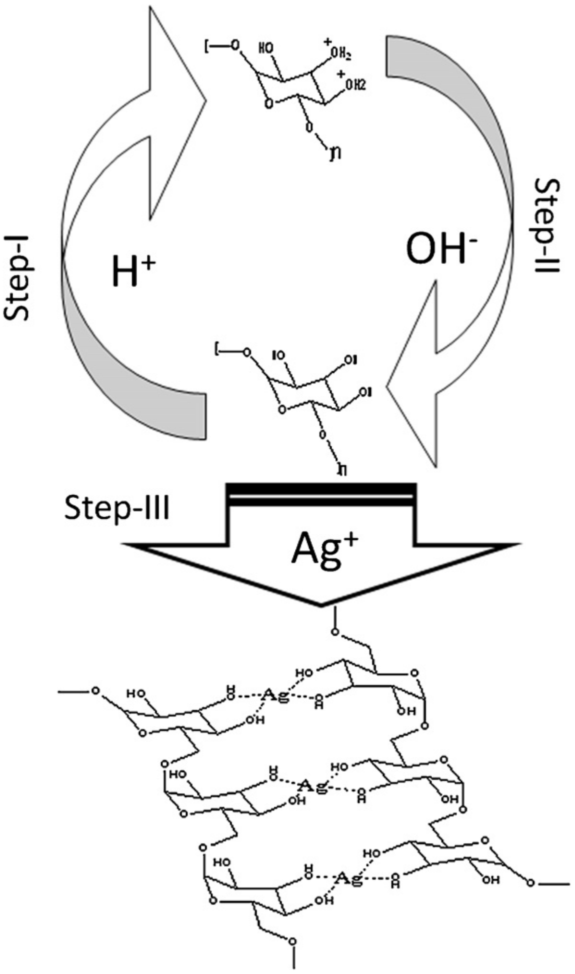

Fig. 1. Schematic presentation of mechanism of protonation (step-I), deprotonation (step-II) of hydroxyl group of dextran and coordination bond between dextran molecules and silver nanoparticles (step-III).

4.2. AFM analysis

The formation of silver nanoparticles is confirmed by AFM analysis .The AFM image clearly shows the surface morphology of the well-dispersed silver nanoparticles. The particle size is in the range of 10–60 nm. Fig. 3a shows the 3D AFM photograph of synthesized silver nanoparticles. The synthesized silver nanoparticles are

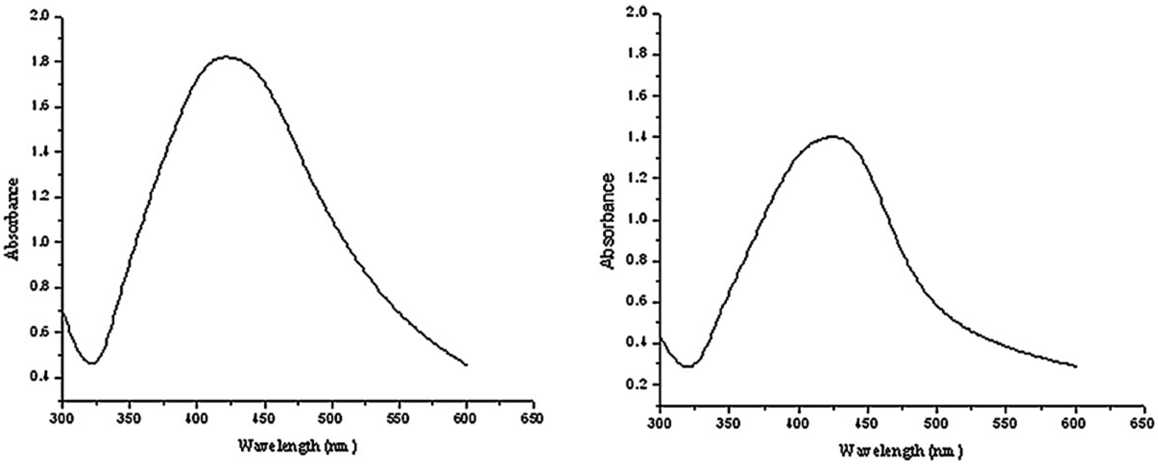

Fig. 2. UV–vis spectroscopy of silver nanoparticle. (a) Initially and (b) after 30 days.

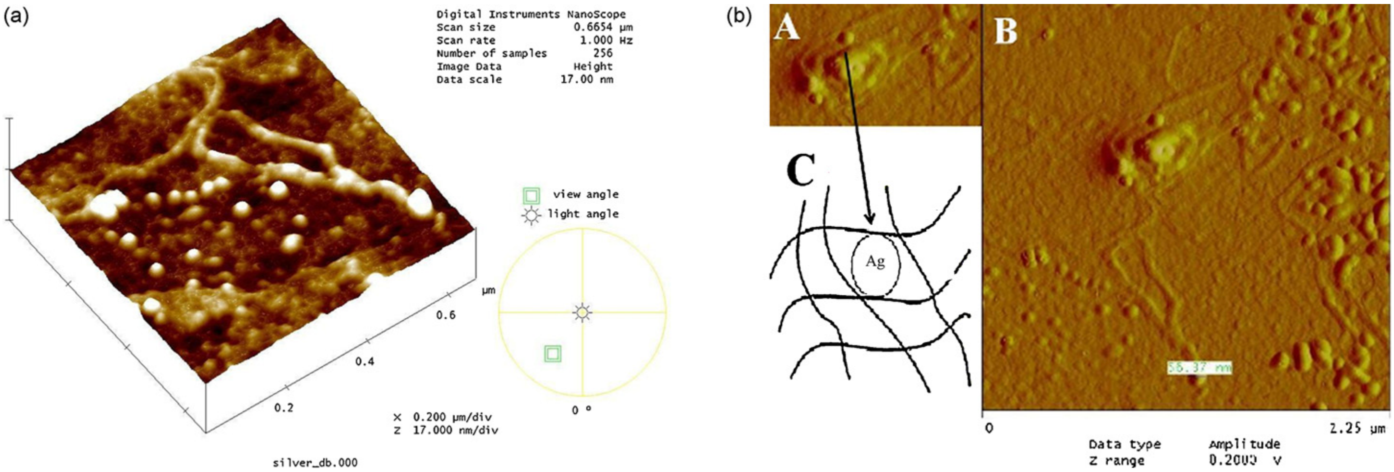

Fig. 3. (a) 3D AFM image of silver nanoparticles. (b) A: AFM image of silver nanoparticles, B: single silver nanoparticle entrapped by dextran molecules, and C: schematic representation of entrapped silver nanoparticle.

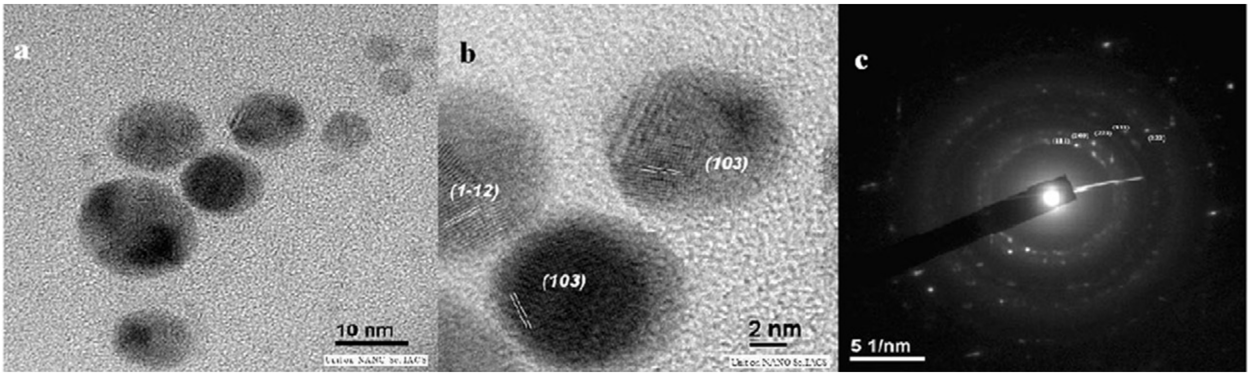

Fig. 4. (a) TEM images of silver nanoparticles, (b) crystal analysis and (c) SAED picture.

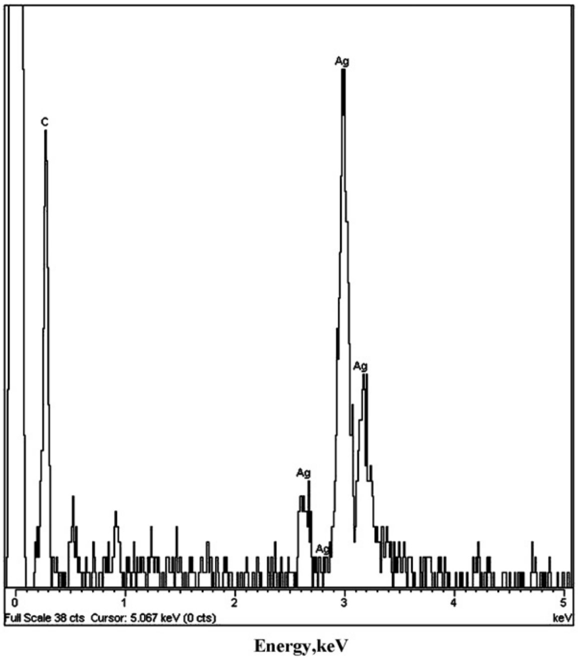

Fig. 5. EDX spectrum of silver nanoparticles.

coordinated with hydroxyl group of dextran molecule preventing their large-scale aggregation. Thus, the silver nanoparticles are well capped with dextran molecules. Fig. 3b depicts the photographs of silver nanoparticles, single silver nanoparticle entrapped by dextran molecules and schematic representation of entrapped silver nanoparticle (Pelillo et al., 2009).

4.3. *TEM studies*

The size and morphology of the synthesized silver nanoparticles are further determined by TEM images. The typical TEM images are shown in Fig. 4a. It is clear from TEM images that the nanoparticles are spherical in shape and size is ~5–10 nm. The AFM image shows that the particle size of silver nanoparticles is in the range 10–60 nm. This inconsistency/increase of particle size of silver nanoparticles observed by AFM with respect to TEM may be due to the geometric effects of a symmetrical tip apex (Ramirez-Aguilar & Rowlen, 1998). The image obtained with the atomic force microscope is a convolution of the tip and sample geometry (Markiewicz & Goh, 1994). Fig. 4b and c shows the TEM images with clear lattice fringes with a spacing of 0.2 nm which reveals that the growth of Ag nanoparticles occur preferentially on the (1 1 1) plane. The TEM images have been analyzed with anticlockwise sense. The d spacing of the leftmost particle has been calculated and the value shows 0.22 nm. The d spacing of rest of the two particles is at 0.207 nm and 0.196 nm, respectively, which is close to the JCPDS value 0.20 nm [PDF # 411402]. Nearly, same d values have been obtained as described above. The clear lattice fringes in TEM images and the typical selected area electron diffraction (SAED) pattern

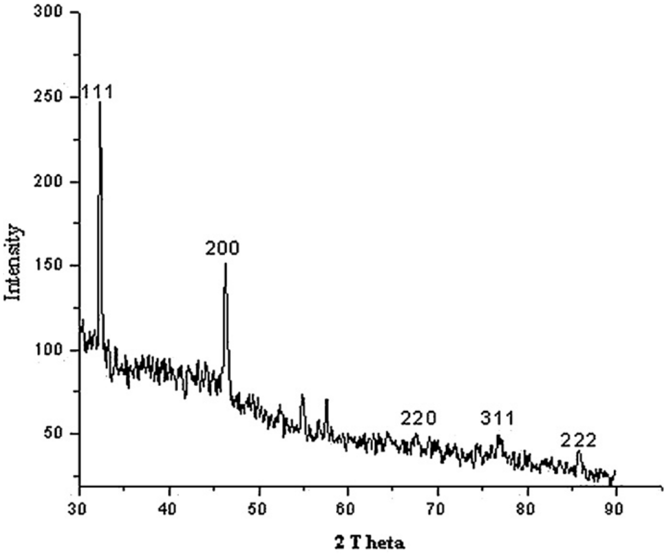

Fig. 6. XRD spectrum of silver nanoparticles.

with circular rings corresponding to the (1 1 1), (2 0 0), (2 2 0), (2 1 1) and (2 2 2) planes show that the nanoparticles obtained are highly crystalline. Similar results were reported in silver nanoparticles synthesized using *Cochlospermum gossypium* gum (Kora, Sasidhar, & Arunachalam, 2010).

4.4. EDX measurement

Fig. 5 shows the energy dispersive X-ray analysis (EDX) of silver nanoparticles, has revealed strong signal in the silver region, and thus confirms the formation of silver nanoparticles. Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance (Magudapatty, Gangopaghyayrans, Panigrahi, Nair, & Dhara, 2001).

4.5. XRD studies

The crystalline nature of silver nanoparticles is further confirmed from X-ray diffraction (XRD) analysis. The XRD pattern of as-synthesized silver nanoparticles (Fig. 6) matches the face centered cubic (fcc) structure of the bulk silver with the broad peaks at 32.27°, 46.27°, 64.49°, 76.83° and 85.67° corresponding to (1 1 1), (2 0 0), (2 2 0), (3 1 1) and (2 2 2) planes, respectively. In addition to the Bragg peaks representative of fcc silver nanoparticles, additional and unassigned peaks are also observed suggesting that the crystallization of reducing and capping agent occurs on the surface of the silver nanoparticles. Similar results are reported in case of silver nanoparticles synthesized using geranium leaf extract (Shankar, Ahmad, & Sastry, 2003) and mushroom extract (Philip, 2009).

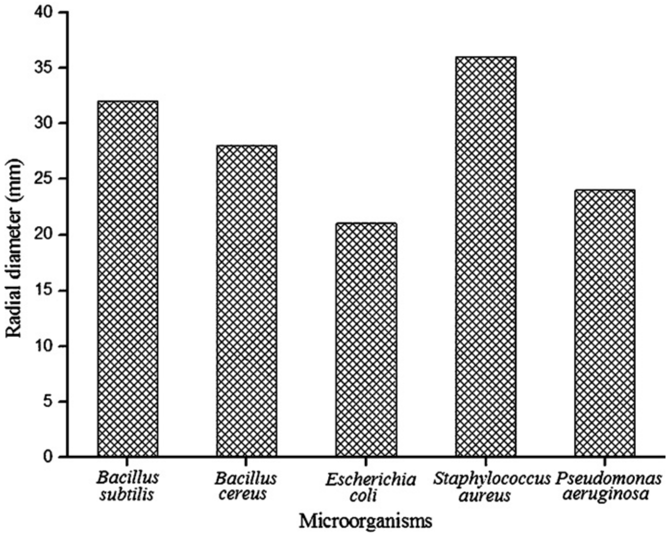

Fig. 7. Radial diameter of inhibitory zone by silver nanoparticles (0.2 mg/ml) against different microorganisms along with the control set (only 10^{-2} M silver nitrate solution).

4.6. Antimicrobial activity against microorganism

The silver nanoparticle solution exhibited excellent antibacterial activity against the bacteria, *B. subtilis*, *B. cereus*, *E. coli*, *S. aureus* and *P. aeruginosa* by showing the clearing zones around the holes with bacteria growth on Petri plates by cup plate method. The radial diameter of the inhibiting zones of *B. subtilis*, *B. cereus*, *E. coli*, *S. aureus* and *P. aeruginosa* are 32, 28, 21, 36, 24 mm, respectively. Silver nanoparticles at the concentration of 0.2 mg/ml showed a range of specificity towards its antimicrobial activity (Fig. 7). At the same time the cavities containing the dextran solution and

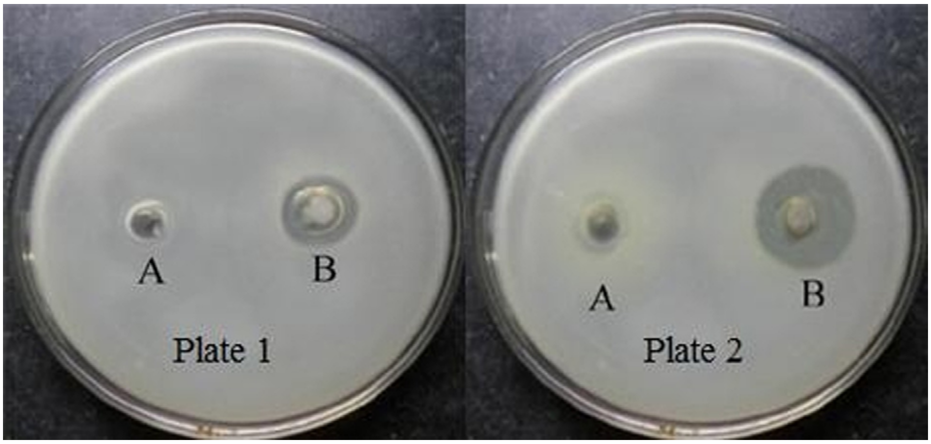

Fig. 8. Antibacterial activity of silver nanoparticles assayed by the agar diffusion method in petri plates. Silver nanoparticles poured in the circular wells show the zone of inhibition against *Bacillus subtilis*. In the Plate 1, the left side cavity contained deionized water (A) while the right side cavity contained silver nanoparticles (B). But in the Plate 2, the left side cavity contained dextran solution (A) while the right side cavity contained silver nitrate solution (10^{-3} M) (B). The clearing zone indicated bacterial growth restriction by diffused silver nanoparticles as well as silver nitrate solution.

sterile distilled water respectively did not show any inhibition zone (Fig. 8). But the plate having the cavity filled by the silver nitrate solution (0.01 M) showed a good antimicrobial activity against the strain of *B. subtilis* (Fig. 8). Results are mean of three separate experiments, each in triplicate. We have already described earlier in the introductory section that several silver containing salts showed a good antimicrobial activity. But the higher concentration of silver was harmful to both consumer and the microbes. That is why the smaller concentration (nano range) is much more applicable for that purpose. This design of silver nanoparticle synthesis has great potential due to its antibacterial activity.

5. Conclusions

A simple, safe, one-step, cost effective and eco-friendly approach is developed for the preparation of silver nanoparticles in aqueous dextran solution at room temperature. Dextran acts as a reducing and stabilizing agent for the synthesis of silver nanoparticles. The synthesized silver nanoparticles are remaining stable without agglomeration for more than one month. The prepared silver nanoparticles have significant antibacterial activity against different types of bacteria. Application of silver nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial agents.

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