International Journal of Science, Environment and Technology, Vol. 5, No 2, 2016, 827 – 838

ISSN 2278-3687 (O)
2277-663X (P)

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING DEXTRAN FROM WEISSELLA CONFUSA

B. Srinivas[1] and P. Naga Padma[*2]
[1]SRK Degree and PG College, Siddipet
[2]BVB Vivekananda College, Secunderabad 500094, India
E-mail: [1]bandari.srinu@gmail.com, [2]naga_padmathota@yahoo.com

Abstract: Dextran a polymer of glucose is produced by different microorganisms like *Leuconostoc mesenteroides, Lactobacillus sps, Streptococcus mutants, Weissella confusa* etc. It has a wide range of applications in the food, pharmaceuticals and other industries. It also has significant role in green synthesis of silver nanoparticles. In the present study an efficient low molecular dextran produced by an indigenous isolate *Weissella confusa* isolated from idli batter was used. The dextran produced was a low molecular weight one as indicated by HPLC analysis. Both the sample dextran and commercial dextran silver nanoparticles were prepared by simple one step method. In this method dextran acted as both reducing and stabilizing agent that reduced silver nitrate ($AgNO_3$) and also acted as protecting agent for nanoparticles. The formation of nanoparticles is assured by characterization with UV-vis spectroscopy, scanning electron microscopy (SEM). The absorbance of silver nanoparticles is observed at 430nm. Scanning electron microscopy images shows that the nanoparticles are spherical in shape with 48-53nm dimensions. The antimicrobial activity of these synthesized silver nanoparticles was tested against bacteria like *Staphylococcus aureus* and *Escherichia coli*. The results confirmed that the tested bacteria were inhibited as indicated by higher zones of inhibition. These non-toxic nanomaterials which can be prepared in a simple and cost-effective manner by green synthesis may be suitable for the formulation of new types of bactericidal agents.

Keywords: Antimicrobial activity, dextran, green synthesis, reducing agent, silver nanoparticles, *Weissella confusa*.

1.0 INTRODUCTION

Dextran is structurally an exopolysaccharide [1], biochemically a branched glucan made up of glucose molecules joined into chains of varying length [2]. It is produced as low molecular weight and high molecular weight dextrans (From 10 to 150 Kilo Daltons) [3]. It is produced by certain lactic acid bacteria like *Leuconostoc mesenteroides* [4], Lactobacillus brevis, *Streptococcus mutants* and *Weissella confusa* [5]. Dextran is of particular interest because of its use as blood-plasma volume expander [6]. It finds various other industrial applications in pharmaceutical, food and chemical industries as emulsifier, adjuvant, carrier and stabilizer [7]. Dextran can also play significant role in field of nanotechnology for preparation of silver

*Received Mar 25, 2016 * Published April 2, 2016 * www.ijset.net*

nanoparticles [8]. In the last twenty years, the study and synthesis of inorganic crystalline particles of nanometer range has attracted considerable attention of scientists from both fundamental and applied research field [9]. Metal nanoparticles (MNPs), such as gold, silver and copper have received special attraction due to their electronic, catalytic and unique optical properties making them very attractive in the areas of particularly bio-conjugation, sensing and surface enhancement Raman spectroscopy (SERS) [8]. Among the noble nanoparticles silver has wide significance for its applications in super magnet, semiconductor, super conductor [10] and antimicrobial activity [11]. Recent studies show that the silver nanoparticles prevent the binding of human immunodeficiency virus type 1 with the host cells [12]. Silver nanoparticles show good antimicrobial activity when compared to broad spectrum antibiotics. Silver is safe for human cell but toxic for virus and bacteria when used in low concentration [8]. By using nanotechnology reduction of particle size of material can be achieved for improving their biocompatibility. In present study, we synthesized silver nanoparticles using dextran as both reducing and stabilizing agent. The antimicrobial activity of as synthesized silver nanoparticles was also studied against bacteria.

2.0 MATERIALS AND METHODS

2.1 Inoculum Preparation

An efficient dextran producer *Weissella confusa* identified by 16s rRNA gene sequencing analysis was inoculated into cortezi medium [13] containing sucrose as main carbon source.

2.2 Fermentation and Recovery

Dextran production was done in 250ml Erlenmeyer flasks containing 50 ml cortezi medium. The inoculum size was 5% and it contained 10^6 cells/ml. The flasks were incubated at 35^0C for 48 hours. Dextran was recovered from broth by alcohol precipitation, dried under vacuum over $CaCl_2$ at 30^0C [14]. Molecular weight of dextran was analysed by HPLC using Agilent Zorbax GF-250, and it indicates presence of low molecular weight dextran [15].

2.3 Preparation of silver nanoparticles using dextran

Silver nitrate solutions (0.005M, 0.01M and 0.02M) were prepared by adding silver nitrate in distilled water separately. Then the silver nitrate solutions were mixed with 2.5%, 5% and 7.5% sample dextran and commercial dextran solution followed by the addition of 0.4ml of very dilute solution of sodium hydroxide (0.001M) at room temperature. The transparent colorless solution was converted to the pale yellow color, indicating the formation of silver nanoparticles.

2.4 Characterization

UV-vis spectroscopy

The reduction of pure Ag^+ ions was monitored by measuring the UV-vis spectrum of the reaction. UV-vis absorption spectrum of the sample (silver nanoparticles prepared from 5% sample dextran and commercial dextran) was done in an Electronics India-371 UV-vis spectrophotometer in the wavelength range from 300 to 600nm to determine absorption maxima. The measurements of silver nano particle synthesis under different conditions like variation of concentration of sample dextran and standard dextran, molarity of silver nitrate solution and incubation time were taken at the particular wave length that gave absorption maxima.

Scanning electron microscopy (SEM)

Scanning electron microscope (SEM) was also used to observe the size, shape of the synthesized nanoparticles (silver nanoparticles prepared from 0.01M, 0.02M silver nitrate solution with 5% sample dextran and commercial dextran. SEM study was observed on a Hitachi-S-3700N at an accelerating voltage of 30Kv.

2.5 Assay for antibacterial activity of silver nanoparticles

Two bacterial strains, *Staphylococcus aureus* (Gram positive) and *E.coli* (Gram negative) were tested for antimicrobial activity of the green synthesized silver nanoparticles by agar well assay method. The zones of inhibition were measured after 24 hours at 37^0C.

3.0 RESULTS

Dextran was used for green synthesis of silver nanoparticles as both a reducing and a stabilizing agent. The reduction of silver ions into silver nanoparticles during exposure to the dextran is indicated by color change that could be read spectrophotometrically using UV.Vis spectrophotometer. Silver nanoparticles exhibit pale yellow color in aqueous solution due to excitation of surface plasma vibrations in silver nanoparticles [8]. Silver nanoparticles prepared from sample dextran and commercial dextran (5% dextran with 0.01M silver nitrate) exhibit a sharp emission peak at 430nm and this remained stable even after 30 days indicating stability of the green synthesized silver nanoparticles (Figure-1a and 1b). This also indicated that there was almost no agglomeration of silver nanoparticles.

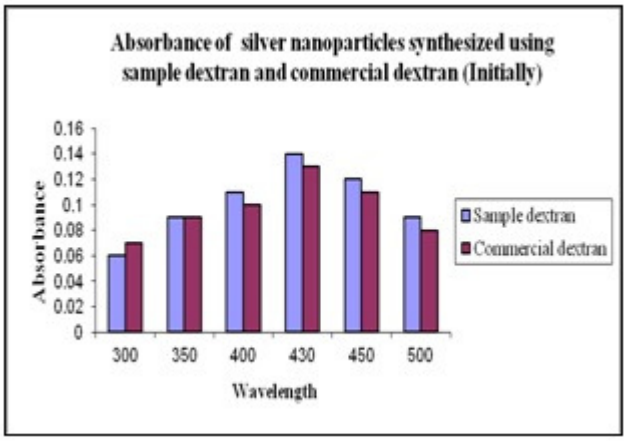

Figure-1a

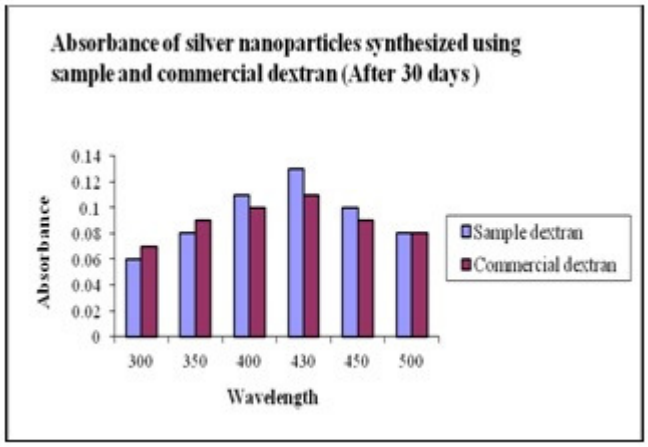

Figure-1b

Figure-1a-1b: UV-vis spectroscopy of silver nanoparticles synthesized from 0.01M silver nitrate solution with 5% sample dextran and commercial dextran. Figure-1a shows absorbance of nanoparticles initially and Figure-1b indicates absorbance of silver nanoparticles after 30 days.

Further absorbance of silver nanoparticles synthesized from different concentration of silver nitrate and dextran (sample dextran and commercial dextran) was observed at 430nm (Figures 2a-2f). The results indicated that as concentration of silver nitrate and reducing agent (dextran) increase optical density also simultaneously increased.

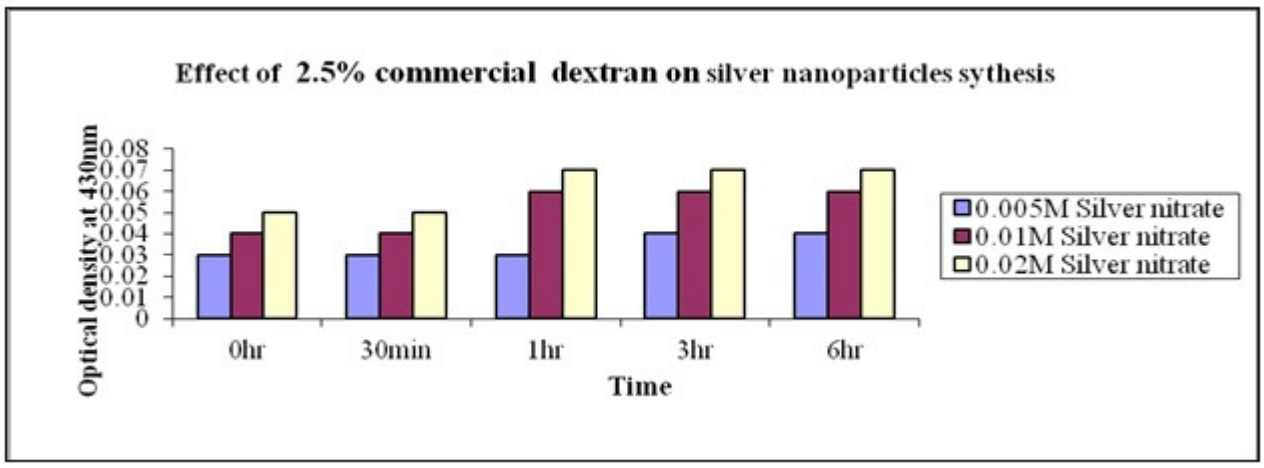

Figure-2a: Effect of 2.5% commercial dextran on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution

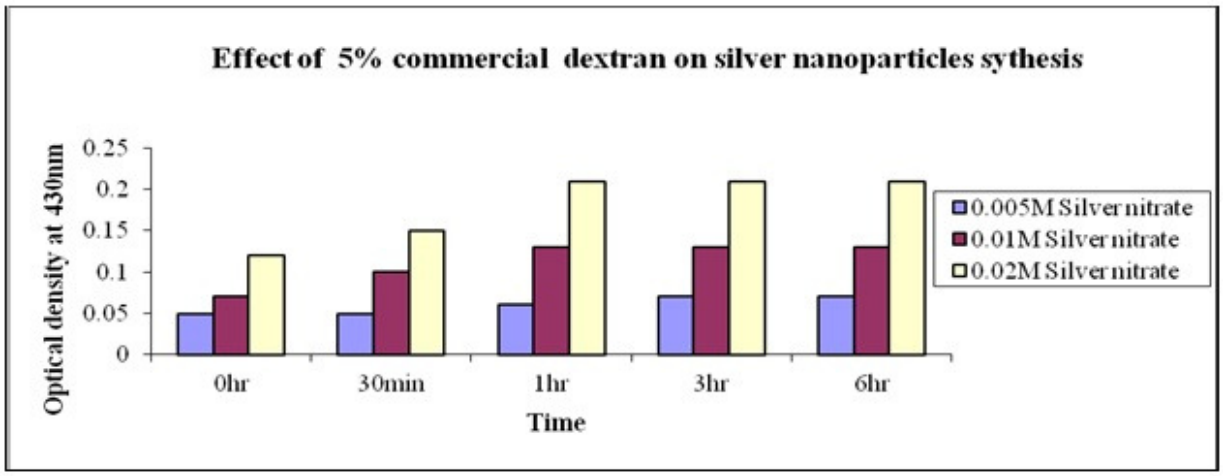

Figure-2b: Effect of 5% commercial dextran on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution

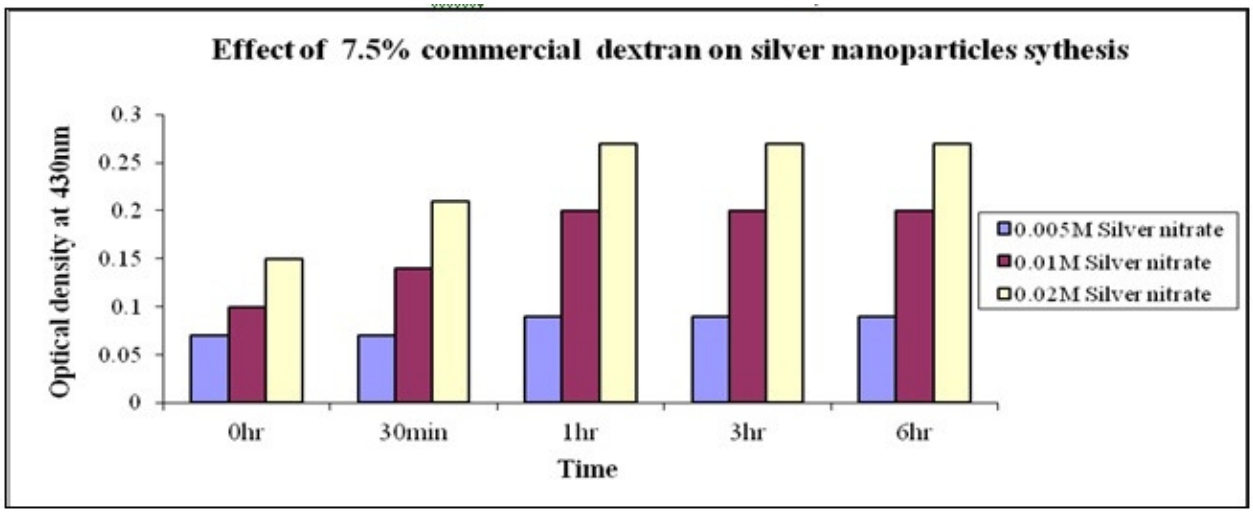

Figure-2c: Effect of 7.5% commercial dextran on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution

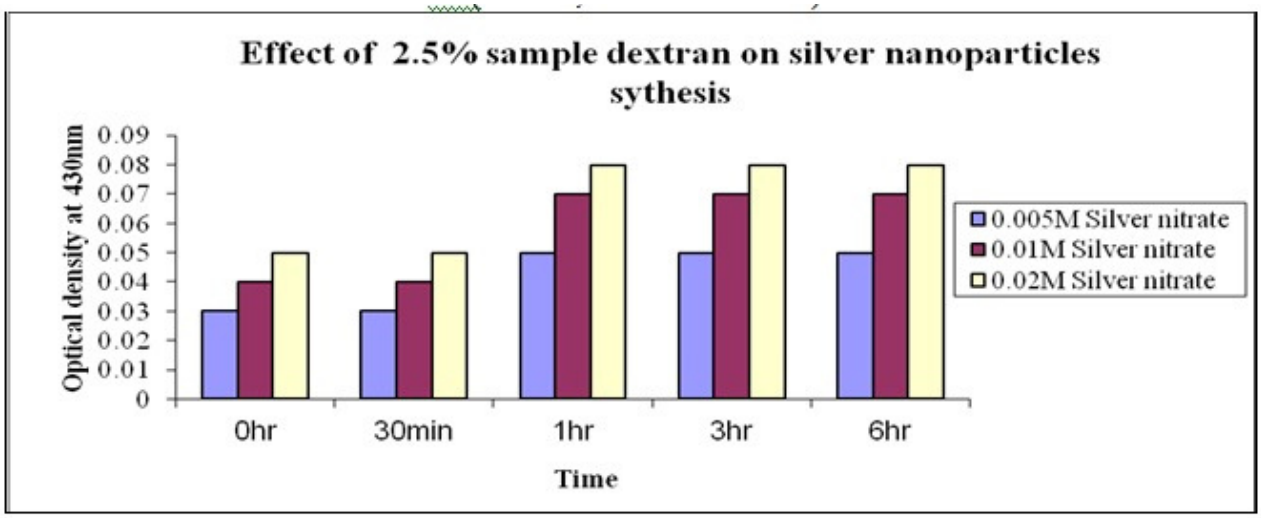

Figure-2d: Effect of 2.5% sample dextran on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution.

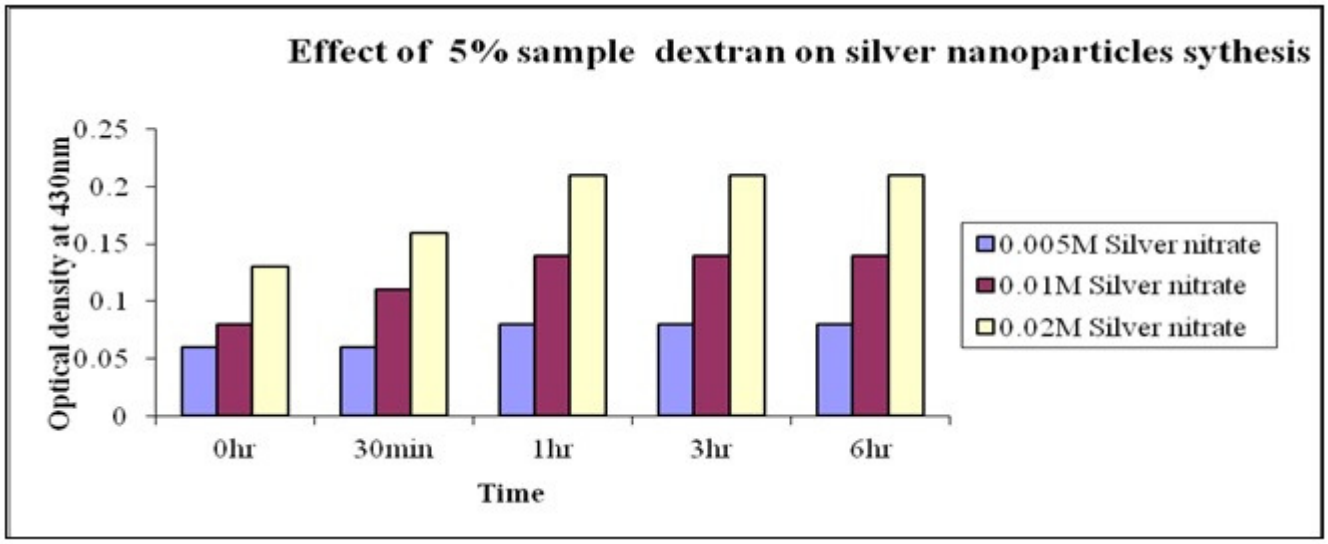

Figure-2e: Effect of 5% sample dextran on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution

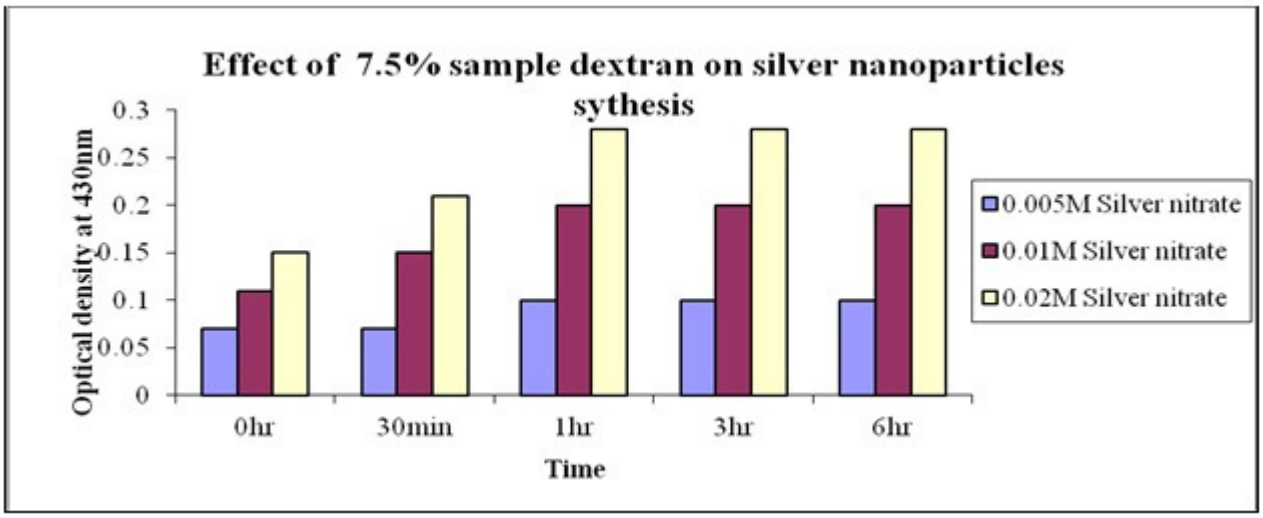

Figure-2f: Effect of 7.5% sample dextran on synthesis of silver nanoparticles using different concentrations of (0.005M, 0.01M and 0.02M) silver nitrate solution.

The size and morphology of synthesized nanoparticles (prepared from 0.01M, 0.02M with 5% commercial dextran and sample dextran) was determined by scanning electron microscope (SEM) (Figures-3a-3d). SEM images indicate that the nanoparticles prepared from 0.01M silver nitrate with 5% commercial dextran and sample dextran were spherical in shape with 52nm-70nm and 48nm-53nm diameter respectively where as silver nanoparticles synthesized from 0.02M with 5% commercial dextran were spherical in shape with 72nm-133nm and 98-119nm diameter respectively. The formation of large size particles may be due to increase in concentration of silver nitrate solution [16].

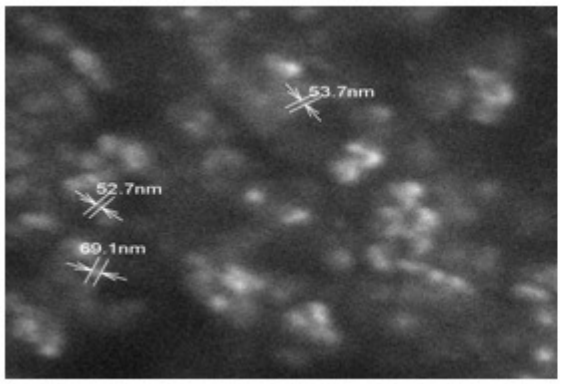

Fig-3a

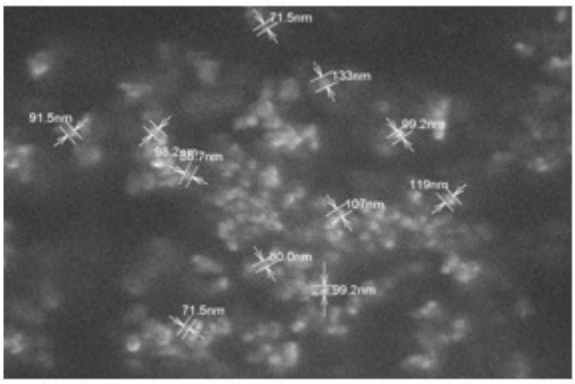

Fig-3b

Figure-3a-3b: SEM images of silver nanoparticles synthesized from 0.01M (Fig-3a) and 0.02M (Fig-3b) silver nitrate solution with 5% commercial dextran

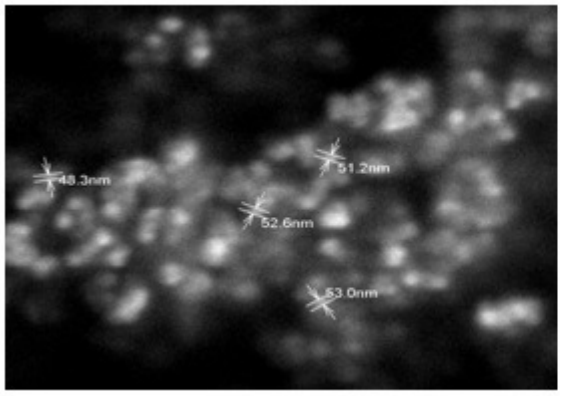

Fig-3c

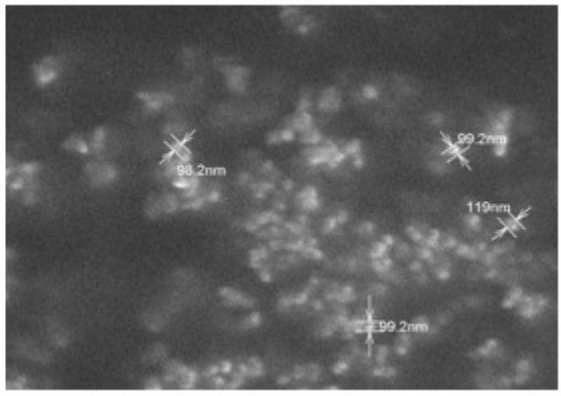

Fig-3d

Figure-3c-3d: SEM images of silver nanoparticles synthesized from 0.01M (Fig-3c) and 0.02M (Fig-3d) silver nitrate solution with 5% sample dextran

The silver nanoparticles solution (prepared from 0.01M silver nitrate with 5% sample dextran) showed excellent antibacterial activity against *Staphylococcus aureus* (Gram positive) and *E.coli* (Gram negative) by showing zone of inhibition around the cavities with bacteria growth plate. The radial diameter of the inhibitory zones of *Staphylococcus aureus* and *E.coli* were 25mm and 20mm respectively (Figure-4 and Figure 5).

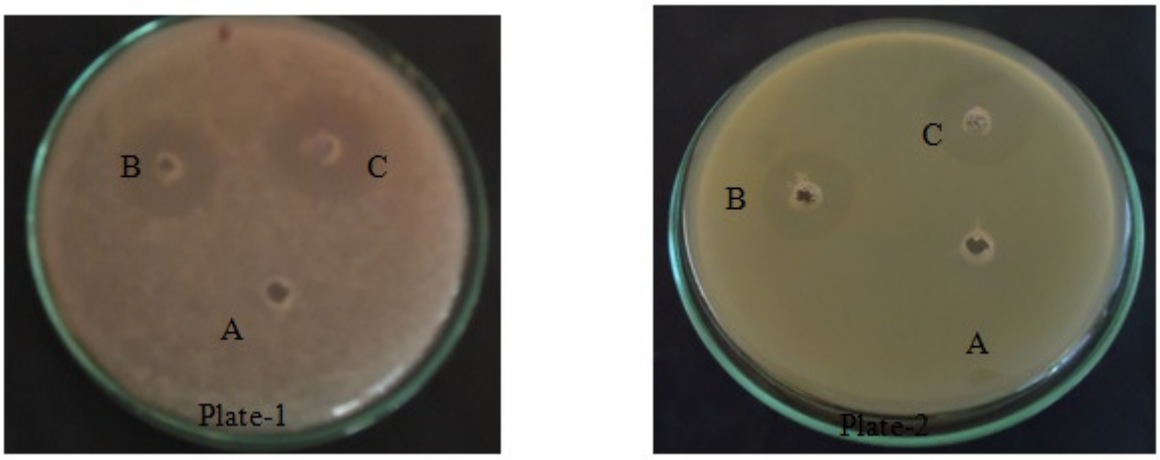

Figure-4: Antibacterial activity of silver nanoparticles assayed by the agar well method. In the plate-1 and plate-2, the lower cavity-contained sterilized water, cavity-B contained silver nitrate solution and cavity-C contained silver nanoparticles. The clear zone indicating growth restriction by diffused nanoparticles and silver nitrate solution against *Staphylococcus aureus* (plate-1) and *E.coli* (plate-2)

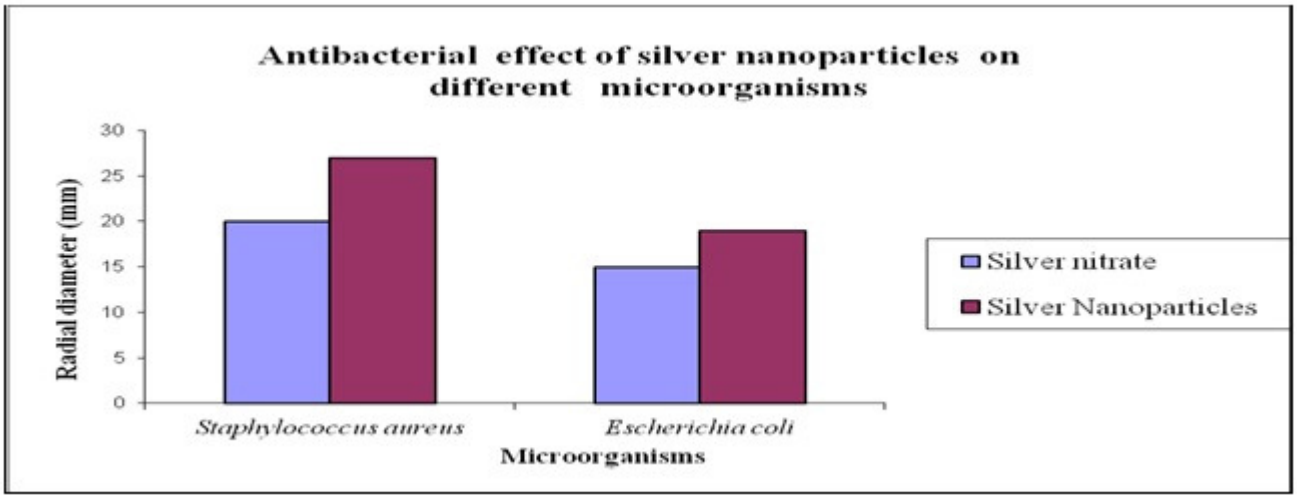

Figure 5: Antibacterial effect indicated by Zone of inhibition (mm) of silver nitrate (0.01M) and silver nanoparticles synthesized from silver nitrate (0.01M) with 5% sample dextran

At the same time the cavities containing the sterile distilled water did not show any inhibitory zone. Cavity filled silver nitrate solution (0.01M) showed a good antimicrobial activity against *Staphylococcus aureus* and *E.coli*. Higher dose of silver was toxic to human that is why the smaller concentration (nanorange) is much more applicable.

4.0 Discussion

Dextran is a water soluble polysaccharide used as both reducing and stabilizing agent in preparation of metal nanoparticles. Due to surface plasmon resonance silver nanoparticles absorb radiation maximally at 430nm [17]. The surface plasmon resonance transition is responsible for the striking yellowish coloration of silver nanoparticles [8]. Synthesized

nanoparticles were stable even after 30 days, this reveals that the silver nanoparticles are well capped with dextran molecule.

SEM images indicate the synthesized silver nanoparticles (prepared from 0.01M with 5% sample dextran) were spherical in shape with 48nm-53nm diameter. Particle-particle aggregation of nanoparticles takes place due to their small size and large surface, making physical handling of nanoparticles difficult in liquid and dry forms [17]. A part from agglomerations, small size silver nanoparticles (3mm) is more cytotoxic [18]. Not only the size but shape of the nanoparticles is also important [19]. Low concentration of silver nanoparticles is attractive due to its non-toxicity to the human body, its broad spectrum antibacterial action [11], and also due to its bactericidal action against multiresistant bacteria like Methicillin-resistant *Staphylococcus aureus* (MRSA), as well as multi drug resistant *Pseudomonas aeruginosa* [20]. Silver nanoparticles interact with a wide range of metabolic process with in microorganisms, resulting in inhibition of growth, loss of infectivity leading to cell death, but this mainly depends on size, shape and concentration of silver nanoparticles (AgNPs) [21]. Positive charge on the Ag^+ ion is crucial for its antimicrobial activity through the electrostatic attraction between the negatively charged cell membrane of the microorganisms and the positive charged nanoparticles. Irregularly shaped pits formation take place in the outer membrane that changes membrane permeability, which may lead to release of lipopolysaccharides and membrane proteins due to metal deposition [22]. The mode of action of AgNPs was also found to be similar to that of Ag^+ ion, however, the effective concentrations of AgNPs and Ag^+ ion were at nanomolar and micro molar levels. Synthesized silver nanoparticles show excellent antibacterial activity against *Staphylococcus aureus* (Gram positive) and *E.coli* (Gram negative), as indicated by observing inhibitory zone that is 25mm and 23mm respectively around growth plate of bacteria. The synthesized AgNPs has great potential due to its antimicrobial activity.

5.0 Conclusion

A critical need in the area of nanotechnology is the development of reliable and eco-friendly process for preparation of metallic nanoparticles. Here, we developed a simple, one-step, cost effective and eco-friendly method for the synthesis of silver nanoparticles using dextran solution at room temperature. Dextran acts as both reducing and stabilizing agent in the preparation of silver nanoparticles. The synthesized nanoparticles are remained stable for 30 days with out agglomeration. The synthesized silver nanoparticles shows significant antibacterial activity against Gram positive bacteria (*Staphylococcus aureus*) and Gram

negative bacteria (*E.coli*). These non-toxic nanomaterials which can be prepared by green synthesis may have in future valuable application as antimicrobial agents to combat drug resistant bacteria.

Acknowledgments

The authors (Srinivas and Naga Padma) are grateful to JNTU Hyderabad and the management of BVB Bhavan's Vivekananda College for encouraging to carry out this work.

References

[1] Tallgren, A.H. Airaksinen, R. Von Weissenberg, U. Ojamo, H. Kuusisto, J. and Leisola, M. 1999. Exopolysaccharide producing Bacteria from Sugar Beets, Applied Environmental Microbiology. 65, 862-864.

[2] Naessens, M. Cerdobbel, A. Soetaert, W. and Vandamme, E.J. 2005. Leuconostoc dextranase and dextran production properties and application. J.Chem.Technol.Biotechnol. 80, 845-860.

[3] Shah Ali UL Qader, Lubna Iqbal, Afsheen Aman, Erum Shireen, and Abid Azhar. 2005. Production of dextran by newly isolated strains of *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9, Turkish Journal of Biochemistry. 31, 21-26.

[4] Onilude, A.A. Olaoye, O. Fadahunsi, I.F. Owoseni, A. Garuba, E.O. and Atoyabi, T. 2013. Effects of cultural conditions on dextran production by *Leuconostoc spp*. International Food Research Journal. 20(4), 1645-1651.

[5] Maina, N.H. Tenkanen, M. Maaheimo, H. Juvonen, R. and Virkki, L. 2008. NMR spectroscopic analysis of exopolysaccharides produced by *Leuconostoc citreum* and *Weissella confuse*. Carbohydr. Res. 343, 1446-1455.

[6] Anthony, J. and Leonsins, M.B. 1952. Valuable plasma volume expander. S.A. Medical Journal. 546-549.

[7] Lakshmi Bhavani, A. and Nisha, J. 2010. Dextran-The polysaccharide with versatile uses. International Journal of Pharma and Biosciences. Vol-1, 569-573.

[8] Bankura, K.P. Maity, D. Mollick, M.M.R. Mondal, D. Bhowmick, B. Bain, M.H. Chakraborty, A. Sarkar, J. Acharya, K. and Chattopadhyay, D. 2012. Synthesis, characterization and antimicrobial activity of dextran stabilized silver nanoparticles in aqueous medium. Carbohydrate Polymers. 89, 1159-1165.

[9] Elghanian, R. Storhoff, J.J. Mucic, R.C. Letsinger, R.L. and Mirkin, C.A. 1997. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. Science. 277, 1078-1081.

[10] Pileni, M.P. 1993. Reverse micelles as microreactors. Journal of Physical Chemistry. 97, 6961-6973.

[11] Baker, C. Pradhan, A. Pakstis, L. Pochan, D.J. and Shah, S.I. 2005. Synthesis and Antibacterial Properties of Silver Nanoparticles. J.Nanotechnol. 5, 224-249.

[12] Elechiguerra, J.L. Burt, J.L. Morons, J.R. Camacho-bragado, A. Gao, X. Lara, H.H. 2005. Interaction of nanoparticles with HIV-1. Nanobiotechnology. 3, 6-16.

[13] Cortezi, M. Monti, R. and Contiero, J. 2005. Temperatures effects on dextransucrase production by *Leuconostoc mesenteroides* FT045 B isolated from alcohol and sugar mill plant. Afr J Biotechnol. 4, 279-285.

[14] Farwa Sarwat, Shah Ali UL Qader, Afsheen Aman, and Nuzhat Ahmed. 2008. Production and Characterization of a unique dextran from an indigenous *Leuconostoc mesenteroides* CMG713. International Journal of Biological Sciences. 4, 379-386.

[15] Vijayabaskar, P. Babinastarlin, S. and Shankar, T. 2011. Quantification and Characterization of Exopolysaccharides from *Bacillus subtilis* MTCC 121. Advances in Biological Research. 5(2), 71-76.

[16] Kondow, T. and Mafune, F. 2000. Structures and dynamics of molecules on liquid beam surfaces. Annu Rev Phys Chem. 51, 731-761.

[17] Amany, A. El-Kheshen, Gad El-Rab, and Sanaa, F. 2012. Effect of reducing and protecting agents on size of silver nanoparticles and their anti-bacterial activity. Der Pharma Chemica. 4(1), 53-65.

[18] Yen, H.J. Hsu, S.H. and Tsai, C.L. 2009. Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes. Small. 5, 1553-1561.

[19] Fukuoka, A. Sakamoto, Y. Guan, S. Inagaki, S. Sugimoto, N. Fukushima, Y. Hirahara, K. Iijima, S. and Ichikawam, M. 2001. Novel templating synthesis of necklace shaped mono and bimetallic nanowires in hybrid organic-inorganic mesoporous material. J Am Chem Soc. 123, 3373-3374.

[20] Lara, H.H. Ayala-Nunez, N.V. Ixtepan-Turrent, L. Rodriguez-Padilla, C. 2010. Bactericidal effect of silver nanoparticles against multi-resistant bacteria. World Journal of Microbiology and Biotechnology. 26, 615-621.

[21] Asharani, P.V. Hande, M.P. and Valiyaveettil, S. 2009. Anti-proliferate activity of silver nanoparticles. BMC Cell Biol. 10, 65.

[22] Amro, N.A. Kotra, L.P. Wadu-Mesthrige, K. Bulychev, A. Mobashery, S. and Liu, G. 2000. High-resolution atomic force microscopy studies of the *Escherichia coli* outer membrane: structural basis for permeability. Langmuir. 16, 2789-2796.