Academia Journal of Biotechnology 6(1): 021-028, January 2018
DOI: 10.15413/ajb.2018.0100
ISSN 2315-7747

Research Paper

Preparation of sliver and selenium nanoparticles and its characterization by dynamic light scattering and scanning electron microscopy

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Accepted 13th February, 2018

ABSTRACT

Nanotechnology dealing with metal and metalloid nanoparticles has been usually applied in nearly each field of science, engineering and technology including biology and medicine etc due to the presence of size and shape dependent unusual physical and chemical properties and in the most recent decade, numerous groups including appreciably developed metal and metalloid nanoparticles based theranostic approaches for the treatment of almost human diseases. Amongst many nanoparticles, recently silver and selenium nanoparticles have been broadly used in the antimicrobial coatings, textiles, paints, keyboards, engineering, food industry, electronics, cosmetics, bio-sensing, wound dressings and even in biomedical devices. In our study, two different methods were used to determine the size and size distribution of the sliver and selenium nanoparticles through Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Due to characterization by DLS technique, nanoparticles size was found in the range of 79.22 and 178 nm for sliver and selenium nanoparticles, respectively. Sliver nanoparticles shown morphological average size and shape with scanning electron microscopy (SEM) reveals spherical shape particles with the size of 80.32 nm whereas selenium nanoparticles shown rod shape particles with the size of 74.29 nm.

Keywords: Dynamic light scattering, scanning electron microscopy, selenium nanoparticles, biomedical devices, nanotechnology, microbial infections.

INTRODUCTION

Nano-sized materials possess desirable novel and superior properties to macro-sized materials and homogeneous nanoparticle suspensions play an important role in many scientific and industrial applications, for example, in colloidal science (Brambilla et al., 2009), nanotoxicological studies (Iker et al., 2010), high thermal conductivity fluids (Madrid et al., 2009) and nanofluidics (Schoch et al., 2008). In these applications, recent experience has identified the need for a well-defined and accurate method of characterizing nanoparticles in suspension. Among the available experimental methods (for example, photocentrifuges, x-ray disc centrifuges, scanning electron microscopy and scanning mobility particle sizer), dynamic light scattering (DLS) can provide information about particles with sizes in the range from a few nanometers to several micrometres. Even though the application of DLS to particle sizing in dilute solutions has become increasingly common, because the diffusion of particles in a solution is influenced by the kinetic and hydrodynamic conditions, the repeatability and the uncertainty of DLS measurements are often insufficient to accurately evaluate the real size of nanoparticles.

Silver and its compounds are known to have antimicrobial properties. Early in the 19th century, 0.5% $AgNO_3$ was used for the treatment and prevention of microbial infections such as Ophthalmia neonatorum (by

German obstetrician Carl Crede). When the era of antibiotics began with the discovery of penicillin, the use of silver slowly diminished (Klasen, 2000), but currently, due to the lack of effectiveness of conventional drugs, the use of silver for treating infections has regained importance. However, the use of ionic silver has one major flaw: it is easily inactivated by complexation and precipitation. As a result, the use of silver ions has been limited (Atiyeh et al., 2007). Silver nanoparticles, which are zerovalent, can be a valuable alternative to ionic silver (Sintubin et al., 2009). Silver nanoparticles are a non-toxic and safe antibacterial agent (Verma, 2015; Verma and Maheshwari, 2017) for the human body. In addition, nano-Ags are also reported to possess antifungal activity (Kim et al., 2009), anti-inflammatory properties (Nadworny et al., 2008), antiviral activity (Rogers et al., 2008) and anti-angiogenic activity (Gurunathan et al., 2009). Nano-Ags can be applied safely in therapy when the effective concentrations against various types of organisms have been determined.

Selenium (Se) is an essential element in human and animal body in low concentration. It is a necessary dietary constituent of at least 25 human selenoproteins and enzymes containing selenocysteine (Zhang and Spallholz, 2011). In the environment, it exists in many oxidation states (−2, 0, +4 and +6) and forms, such as ionic selenite (Na_2SeO_3) and selenate (Na_2SeO_4), solid state Se (0) and selenomethionine (SeMet)/ selenocysteine, etc (Biswas et al., 2011; Bo et al., 2014). SeNPs can be synthesized through physical methods, such as laser ablation, UV radiation and hydrothermal techniques (Iranifam et al., 2013; Overschelde et al., 2013). Chemical synthesis is mediated by precipitation, acid decomposition and catalytic reduction using ascorbic acid, glucose, sulfur dioxide, sodium dodecyl and sulfate, etc (Zhang et al., 2010; Dwivedi et al., 2011).

Metal and metalloid nanoparticles has been frequently useful in almost every field of science, and technology including biomedical etc due to presence of small size and shape dependent curious physical and chemical properties. In last decades applications of Ag and Se based nanoparticles were discussed in field of infectious diseases treatment like Biofilms on medical devices (Verma *et al.,* 2013; Verma and Singh, 2015), and as an antimicrobial (Verma, 2015; Verma and Maheshwari, 2017). In other aspect, other researchers (Haris and Khan, 2017) studied that Novel selenium nanoparticle-enhanced photodynamic therapy of toluidine blue O against biofilm forming *Streptococcus mutans* bacterial isolates. (Haris and Ahmad, 2017) were studied the Impact of Metal Oxide (ZnO and TiO_2) nanoparticles on beneficial soil microorganisms and their Secondary Metabolites. The antibacterial potential of nanoparticles were determined by growth kinetics of *P. aeruginosa*, *P. fluorescens* and *B. amyloliquefaciens.* Soni, 2017 was discussed the Biodegradable Nanoparticles for Delivering Drugs and Silencing Multiple Genes or Gene activation in Diabetic Nephropathy. Chauhan *et al.,* 2017 was also discussed the applications of Nanotechnology in Forensic investigation that reveals the hidden evidences, which can prove to be helpful for the forensic scientists to give an outcome to their investigation.

MATERIALS AND METHODS

Chemicals

All chemicals of silver and selenium nanoparticles used in this experiment were highest, purified and purchased from CDH (Central Drug House) (P) Ltd, Delhi, India. Distilled water was also used for the preparation of reagents. Further study was done in the School of Biotechnology, IFTM University Moradabad, India.

Preparation of nanoparticles by chemical reduction method

Preparation of silver nanoparticles

In our study, silver colloid was prepared using the chemical reduction method. All solutions of reacting materials were prepared in distilled water.

Solution 1: 0.0849 g of Silver nitrate ($AgNO_3$) was dissolved in 500 ml distilled water and the solution heated to boiling.

Solution 2: Thereafter, 1 g of tri-sodium citrate ($C_6H_5O_7Na_3$) was dissolved in 100 ml distilled water.

Working solution: 5 ml of tri-sodium citrate were added to 500 ml of $AgNO_3$ after boiling (drop by drop). During the process, the solution was forcefully mixed. The solution was heated and left on hot plate for 2 h at 90°C (un-continuous manner) thereafter, it was allowed to cool at room temperature and a reddish green color (Sileikaite et al., 2009) appeared. Prepared AgNPs were collected in 1.5 ml Eppendorf tube and kept at room temperature for further experiment.

Preparation of selenium nanoparticles

Selenium nanoparticles (SeNPs) were synthesized by the chemical reduction of sodium selenite by glutathione (reduced form) and stabilized by bovine serum albumin (BSA). Specifically, 3 ml of 25 mM Na_2SeO_3, 3 ml of 100 mM GSH and 0.15 g BSA were added to 9 ml of double distilled water (dH_2O) in a sterile cabinet. All solutions were made in a sterile environment using a sterile cabinet and double distilled water. After mixing the reactant solution, 1 M NaOH was added to bring the pH of the solution to the alkaline media. Selenium nanoparticles were formed

immediately following the addition of NaOH as visualized by a color change of the reactant solution from clear white to clear red color. Thereafter, selenium nanoparticles were collected by centrifuging (Cooling centrifuge C-24 BL) the solution at 13,000 rpm at -4°C temperature with 30 min duration sterilized by ultra-violet light exposure, before use in bacteria experiment (Tran and Webster, 2011). Prepared SeNPs were collected in 1.5 ml Eppendorf tube and kept at room temperature for further experiment.

Characterization of silver and selenium nanoparticles

Particle size analyzer (Dynamic light scattering)

Dynamic light scattering (DLS) was used to measure the hydrodynamic diameter. The particle size of the silver nanoparticle sample was analyzed by dynamic light scattering using the Litesizer™ 500 under the following conditions (at wavelength 658 nm, particle absorption coefficient 0.01, diffusion coefficient 6.2 $\mu m^2/s$, water refractive index 1.33, and intensity 79.67 nm). To measured the particle size, a volume of 0.1 ml of the Ag-NPs solution in 2 ml deionized water (DI H_2O) water was placed in a polystyrene cuvette and measured at 25°C (Bootz et al., 2004).

Selenium nanoparticle sample was analyzed by dynamic light scattering using the Microtrac, Bluewave model, Germany under the following conditions (at water refractive index 1.33 and wavelength 780 nm). To measure the particle size, a volume of 0.1 ml of the Se-NPs solution in 2 ml deionized water (DI H_2O) was placed in a polystyrene cuvette and calculated at 25°C temperature (Bootz et al., 2004).

Scanning electron microscopy (SEM)

Scanning electron microscopy is a type of electron microscope that takes image to the sample by scanning with a high-energy beam of electrons in a raster scan pattern. The electrons interact with atoms that can make up the sample producing signals that hold information about the sample's surface topography, composition and other properties such as electrical conductivity. Nanoparticles of the silver colloid were prepared using chemical reduction method while selenium nanoparticles were synthesized by the reduction of sodium selenite by glutathione (reduced form) and stabilized by bovine serum albumin (BSA). Nanoparticles (Ag and Se) were sterilized by ultra-violet light in laminar air flow. The sterilized nanoparticles were carefully mounted on SEM stubs using adhesive tape and uniformly coated with gold. Both nanoparticles samples were placed in a sample chamber of SEM (JEOL JSM-6490LV, Japan) and scanning performed under different magnifications ranging from 15,000x to 35,000x and voltage 20 to 30 kV (Sileikaite et al., 2009; Razi et al., 2011).

RESULTS

Preparation of silver and selenium nanoparticles

Silver nanoparticles synthesis adopting chemical reduction process was primarily confirmed by the color change in the reaction mixture from pale yellow to reddish green clearly indicating the synthesis of silver nanoparticles (Sileikaite et al., 2009). The reddish green color formation was observed within 10 min after the drop wise addition of tri-sodium citrate to the silver nitrate solution. Antibacterial activity of silver nanoparticles (consisting of silver nitrate) and nanoparticle-coated face masks serve as protection against biofilm forming infectious agents *Staphylococcus aureus*. Silver nanoparticles are now extensively used as anti-microbial agents and utilized in the development of anti-microbial dressings and anti-microbial medical devices etc.

Selenium nanoparticles (SeNPs) were synthesized by the reduction of sodium selenite by glutathione (reduced form) and formed immediately following the addition of NaOH (make alkaline medium) as visualized by a color change of the reactant solution from clear white to clear red (Tran and Webster, 2011). Selenium nanoparticles were then collected by centrifuging (Cooling centrifuge C-24) 13,000 rpm at -4°C for 30 min sterilized by ultra-violet light exposure.

Characterization of silver and selenium nanoparticles

Particle size analyzer (Dynamic light scattering)

Particle size was determined by dynamic light scattering (DLS) measurement. The hydrodynamic diameters of silver nanoparticles and selenium nanoparticles in aqueous solution were determined using dynamic light scattering.

The particle size of the silver nanoparticle sample was analyzed by dynamic light scattering using the Litesizer™ 500 found 79.22 nm size under the following conditions: diffusion coefficient 6.2 $\mu m^2/s$, water refractive index 1.33, intensity 79.67 nm and temperature 25°C (Sarkar et al., 2011) under the 658 nm Laser wavelength with an angle detection of 175° (Back scatter). DLS method has several pitfalls, in particular with respect to the influence of dust particles or small amounts of large aggregates in addition to a main component of distinctly smaller size (Berne and Pecora, 2000). In many chemical companies, sedimentation velocity analysis in the analytical ultracentrifuge has been a standard technique for studying the size of the colloidal particles, frequently using non-commercial apparatus (Machtle, 1999). Figure 1 shows the size distribution by intensity of Ag-NPs in solution and correlation function of silver nanoparticles as shown in Figure 2.

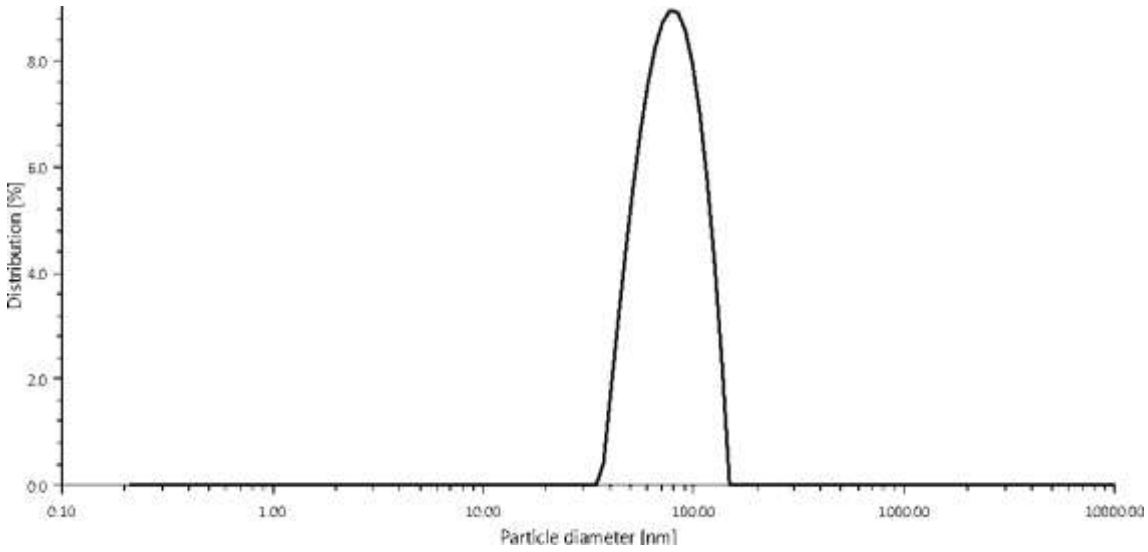

Figure 1: DLS image of Silver nanoparticles size distribution by Intensity in solution.

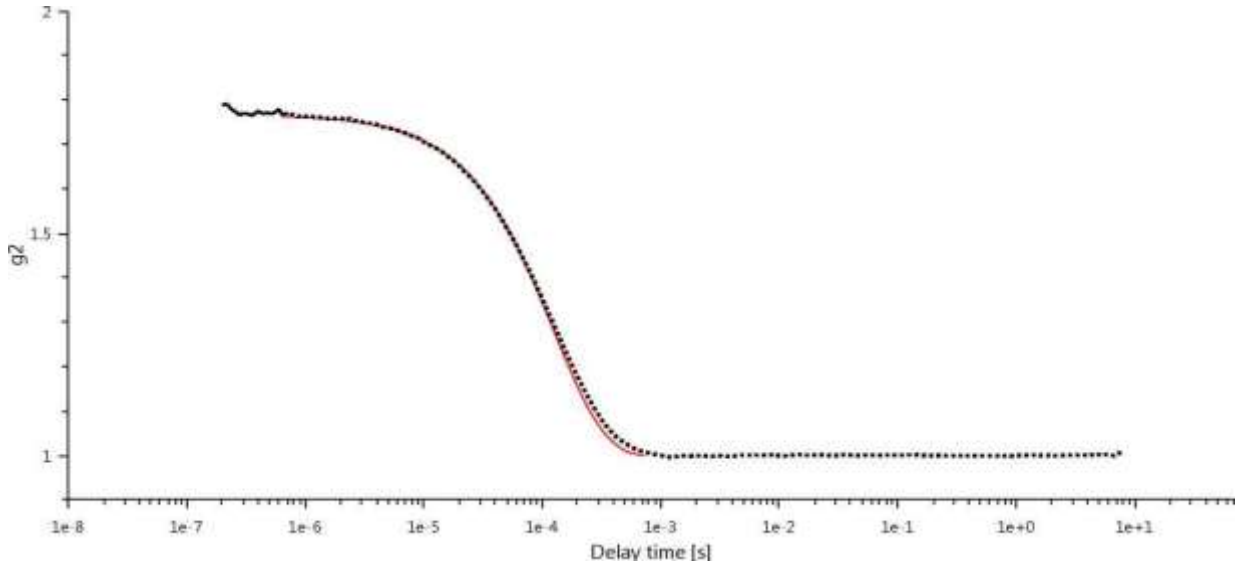

Figure 2: DLS image of silver nanoparticles of correlation function.

Selenium nanoparticle size sample was analyzed by dynamic light scattering using the Microtrac, Bluewave model, Germany in the range of 178 nm, under the following conditions: laser wavelength 780 nm, water refractive index 1.33 at room temperature (25°C) (Sarkar et al., 2011). Figure 3 shows the size distribution by particle number of Se-NPs in solution and size distribution by volume particle of selenium nanoparticles (Figure 4).

Scanning electron microscopy (SEM)

The shape and size of the silver and selenium nanoparticles was measured by Scanning electron microscopy (SEM). Particle morphology shape and size with scanning electron microscopy reveals spherical shape with the size of particle in the range 80.32 nm for AgNPs (Figure 5) (Table 1) whereas, rods shape particles with the size of particle was in the range 74.29 nm for SeNPs (Figure 6) (Table 1). Smaller sized Ag and Se nanoparticles have many positive attributes, such as good conductivity, chemical stability and catalytic and antibacterial activity, which would make them suitable for many practical applications.

A scanning electron microscope (SEM) is a type of electron microscope that produces sample images due to scanning of the surface of object with the focused beam of electrons and widely considered gold standard for nanoparticle characterization.

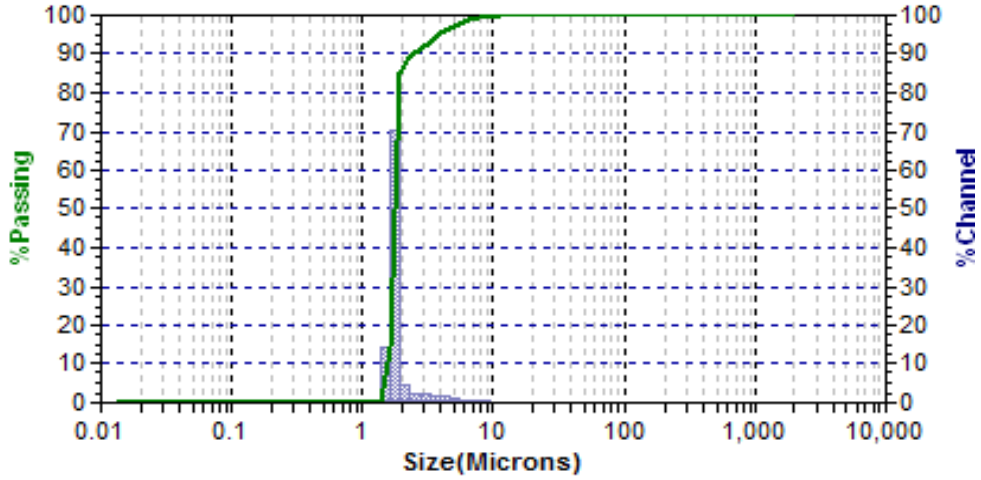

Figure 3: DLS image of selenium nanoparticles size distribution by number of particles.

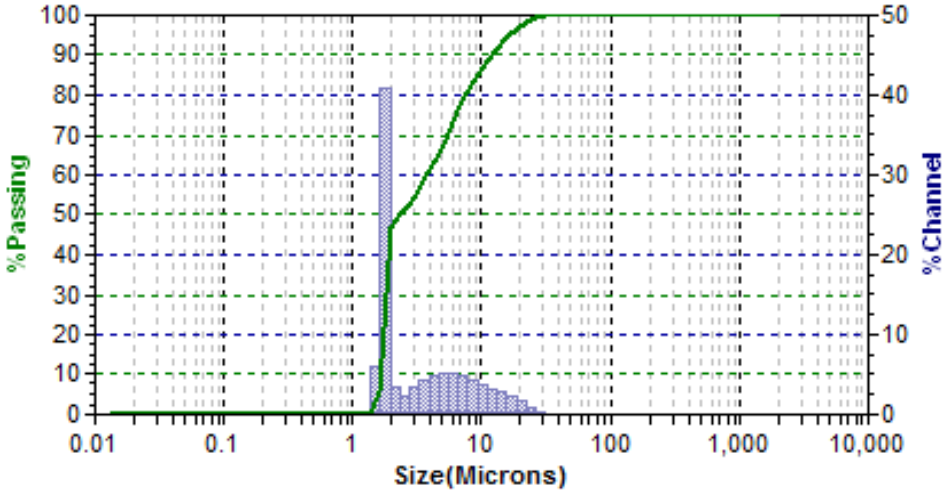

Figure 4: DLS image of selenium nanoparticles size distribution by volume of particles.

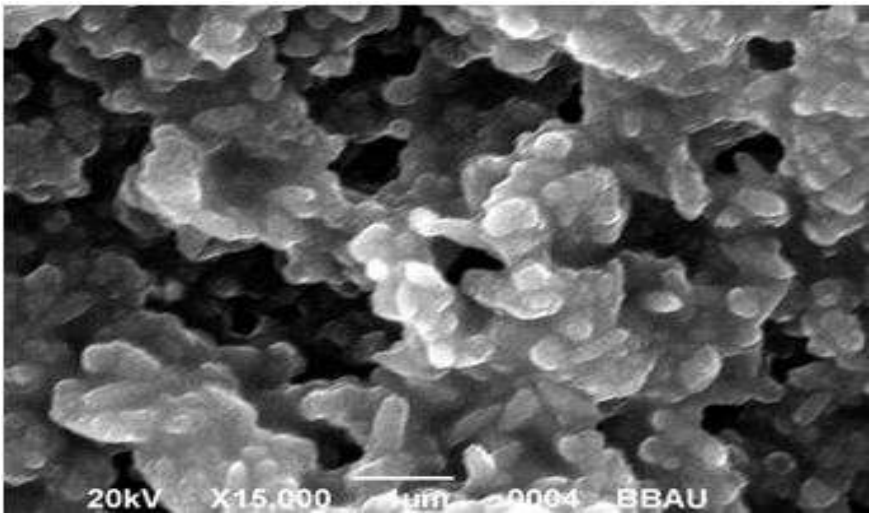

Figure 5: SEM image of silver nanoparticles.

Table 1: Measurement of the nanoparticles (Ag and Se) shape and size under different microscopy.

S/No	Characteristics	Dynamic light scattering (DLS)		Scanning electron microscope (SEM)	
		AgNPs	SeNPs	AgNPs	SeNPs
1	Size	178 nm	780 nm	80.32 nm	74.29 nm
2	Shape	-	-	Spherical	Rods

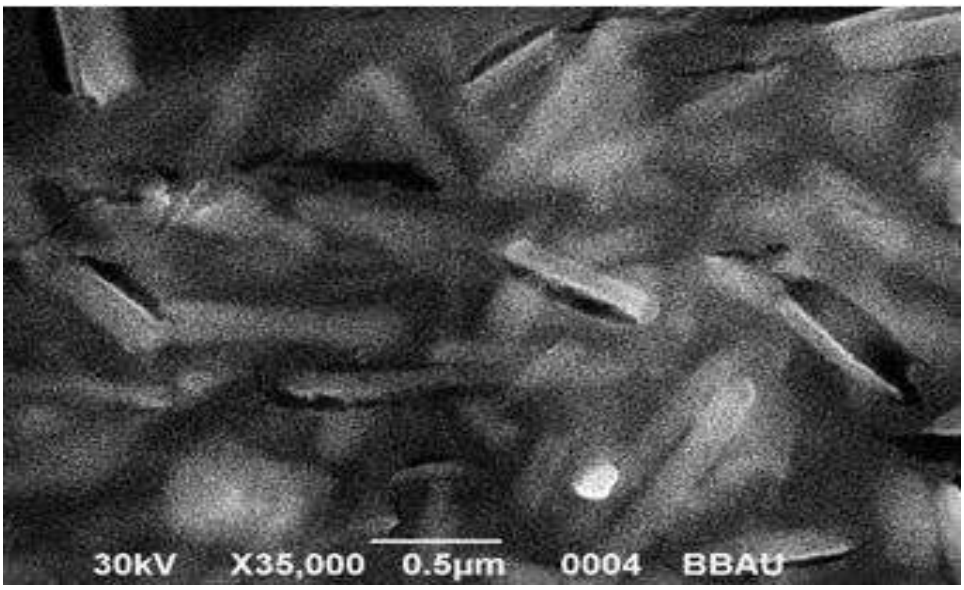

Figure 6: SEM image of selenium nanoparticles.

DISCUSSION

Characterization of silver and selenium nanoparticles

AgNPs and SeNPs characterization by particle size analyzer (Dynamic Light Scattering)

Two main types of particle size analyzers, that is, dynamic light scattering and laser particle analyzers. Dynamic light scattering (DLS), one of the most popular light scattering modalities can probe the size distribution of small particles at the scale from sub-micron down to one nanometer in solution or suspension using a monochromatic light source (Sapsford et al., 2011). Dynamic light scattering was used to measure the hydrodynamic diameter. Dynamic light scattering analyzers measure particles in solutions in the 0.6 to 6000 nm range of any nanoparticle. The main advantage of DLS is the short time required to perform the measurement and the relatively low cost of the apparatus, therefore, DLS becomes the preferred method for nanoparticle sizing (Berne and Pecora, 2000).

The DLS technique measures the diffusion coefficient of suspended nanoparticles undergoing Brownian motion in a solution by analyzing the fluctuating scattered intensity from the nanoparticles in the solution. The scattered light from the suspension of nanoparticles fluctuates in a characteristic time, that is, inversely proportional to the particle diffusion coefficient. This can provide information about particles with sizes in the range from a few nanometers to several micrometers. Even though the application of DLS to particle sizing in dilute solutions has become increasingly common, because the diffusion of particles in a solution is influenced by the kinetic and hydrodynamic conditions, the repeatability and the uncertainty of DLS measurements are often insufficient to accurately evaluate the real size of nanoparticles. In the case of a concentrated solution, these effects become more significant and therefore, a process, extrapolating the concentration and scattering angle to extremely low values, is used to obtain the real size of nanoparticles in solutions (Johnson and Gabriel, 1981; Takahashi et al., 2008).

Figures 1 and 3 shows the DLS pictures of Silver nanoparticle size distribution by intensity in solution and selenium nanoparticle size distribution by particle number respectively as well as, Figures 2 and 4 shows the DLS pictures of silver nanoparticle of correlation function and DLS image of selenium nanoparticles size distribution by particle volume respectively. The particle size determined by DLS detector was slightly larger than the nominal size, probably because both methods measure a hydrodynamic

size, rather than a physical size.

In our result, it was noticed that the synthesized AgNPs were polydispersed in nature and Polydispersity index was found to be 18.9%, while silver nanoparticle size 79.22 nm was analyzed. With respect to the observations made in the present study most literature report a similar particle size ranging of silver nanoparticle from 55 to 85 nm by dynamic light scattering (Mukherjee et al., 2014), whereas for selenium nanoparticle size of 178 nm was analyzed. Certain reports, have however documented a lesser particle size range of 3 to 18 nm (Sharma et al., 2014) and closely similar size range (30 to 150 nm**)** as observed by Sarkar et al. (2011).

AgNPs and SeNPs characterization by scanning electron microscopy (SEM)

Presently, numerous microscopic techniques are commercially available, whenever Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) are the most popular microscopes for the analysis of the nanoparticles.

The shape and size of the silver and selenium nanoparticles was measured by Scanning electron Microscopy (SEM). Particle morphology shape and size with scanning electron microscopy reveals spherical shape with the size of particles in the range 80.32 nm for AgNPs. Figure 5 showed close similarity with our result ranging from 60 to 80 nm (Namasivayam et al., 2012; El-Kheshen and El-Rab, 2012; Dimitrijevic et al., 2013; Devaraj et al., 2013) whereas, rod shape particles with size of particles in the range of 74.29 nm for SeNPs (Figure 6), showed similarity with our result ranging from 50 to 150 nm, respectively (Ramamurthy et al., 2013).

Conclusion

Silver and selenium nanoparticles were successfully prepared using the chemical reduction method. Both nanoparticles preparation confirmation was done by qualitative analysis, that is, silver nanoparticles appeared as a reddish green color while selenium nanoparticles appeared as a clear white to clear red color and characterization of NPs were shown by particle size analyzer [Dynamic Light Scattering (DLS)] as well as, Scanning electron microscope (SEM). By DLS, nanoparticles size were found in the range of 79.22 and 178 nm for AgNPs and SeNPs, respectively and through SEM, nanoparticles size were found in the range of 80.32 nm and spherical as well as, 74.29 nm with rods shape of AgNPs and SeNPs, respectively. These nanoparticles can be used as therapeutic drug for various diseases like antibacterial, antifungal, antiviral, biofilm eradication and cancer treatment. Smaller sizes of nanoparticles have great capacity to eradicate any disease due to high surface area.

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Cite this article as:

Verma P and Maheshwari SK (2018). Preparation of sliver and selenium nanoparticles and its characterization by dynamic light scattering and scanning electron microscopy. Acad. J. Biotechnol. 6(1): 021-028.

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