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Synthesis and characterization of nano-selenium and its antibacterial response on some important human pathogens

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Synthesis of nano-selenium was achieved from sodium selenite by a simple precipitation method using the reducing power of ascorbic acid. The high-speed centrifuge was used to separate selenium nanoparticles from aqueous solution. The selenium nanoparticles were characterized by UV–Vis spectroscopy, X-ray diffraction, Fourier transform-infrared spectroscopy (FT-IR), scanning electron microscopy, energy dispersive X-ray analysis and transmission electron microscope. Presence of various functional groups responsible for the production and stability of the nanoparticles was confirmed by FT-IR. Some of the important human pathogens like *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were used for examining the antibacterial response of selinium nanoparticles. Results of this study demonstrate that synthesized selenium nanoparticles exhibit a spherical shape with average diameter range between 15 and 18 nm. They can be used as an antibacterial agent and also in medicinal applications for the treatment of humans with certain bacterial diseases.

Keywords: Antibacterial activity, characterization, human pathogens, synthesis, selenium nanoparticles.

NANOTECHNOLOGY is able to observe, measure, manipulate and manufacture things at the nanometre scale[1]. Currently, many nanosubstances are produced with the help of this emanating technology which occupies an important place in scientific research. Chemical composition, size, shape and morphology of nanoparticles are dealt within the synthesis process which is considered as a vital step in nanotechnological research[2]. Due to the size of the nanoparticles, their properties accustomed to giving a larger surface area compared to the bulk material. Thus, materials made up in such a way will have atoms that have more contact with the external environment; whereas those which are considered as bulk hold the atoms closer to the centre[3]. Application of nanoparticles can be associated with many fields like medical, food industries, environmental studies, electronics production, energy generation and agriculture[4].

The most commonly used nanoparticles which have wide applications are silver, gold, zinc, copper and iron.

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For this study, an essential mineral selenium (Se) was used. This is a metalloid and an essential micronutrient. It plays vital functions in the human body by improving the action of enzymes such as glutathione peroxidase and seleno-enzymes which defend the body against immunity-related diseases[5]. As a semiconductor selenium possesses optical, photoconductor and catalytic properties. It also has some uncommon properties like efficient chemical and biological functions compared to bulk materials[6].

There arises complexity with the frequent use of antibiotics in the medical field; among them bacterial resistance is a major problem faced by the microbiologists. So there is a crucial need to develop a substitute operator to control pathogenic bacterial growth. It has been reported that metalloid nanoparticles can be used as disinfectants which are applied in the food industries to preserve food materials[7]. Though necessary, very less support has been extended to the pharmaceutical industry to design new antibacterial operators. To overcome the resistance of microorganisms to antibiotics, it is indispensable to design a non-antibiotic treatment[8].

Silver nanoparticles are generally used for their antimicrobial function. Hence their functions are unsettled and the latest choice is Se nanoparticles which have many medical applications. Due to their unique morphological and chemical nature, Se nanoparticles are more reactive and lethal to bacteria[9]. Se nanoparticles have also been utilized in food preservation methods like packing of foods items and antiseptic coating over food materials[8]. Studies have been carried out to highlight the disinfectant properties of Se nanoparticles against *Trichophyton rubrum*[10], *Candida albicans*[11], *Pseudomonas aeruginosa* and *Proteus mirabilis*[12]. The present study focuses on a solution to the problem of drug resistance.

Sodium selenite was used for producing Se nanoparticles (Na_2SeO_3) where ascorbic acid ($C_6H_8O_6$) acted as a reducing operator[13]. A stock of aqueous solution of 100 mM Na_2SeO_3 and 50 mM $C_6H_8O_6$ was prepared in 1 : 4 ratio. The solution was kept under magnetic stirring condition at different rpm (rotation per minute) and at ambient temperature for 30 min. The mixtures were allowed to react with each other in the concentrated form until a change was observed from colourless to red colour. Then the solution was centrifuged at 3000 rpm, pellets were collected and nano-selenium was obtained.

Chemically synthesized nano-selenium was determined by UV–Vis spectroscopy using an automated spectrometer (Spectro UV–Vis double beam DUV 3500). The morphology and elements percentage of nano-selenium were measured using transmission electron microscopy (TEM) and scanning electron microscopy (SEM; LEO 1455 VP) provided with energy dispersive X-ray (EDX) analytical instrument. Fourier transform infrared spectroscopy (FT-IR; JASCO (FTIR-6200)) was employed to explore the functional groups present in the sample. X-ray diffraction (XRD) analysis was done using an X-ray diffractometer (Shimadzu XRD-6000, Japan) to analyse the crystalline nature of the sample.

After characterization, the antibacterial activity of nano-selenium against *S. aureus* which is Gram-positive, and *E. coli* and *P. aeruginosa* which are Gram-negative strains was assessed by well diffusion technique. An active bacterial culture was obtained by shifting the culture to nutrient broth containing test tubes from stock, and it was incubated for 24 h at 37°C to obtain fresh and active bacterial culture. For the assay, approximately 25 ml of nutrient agar plates was prepared, and 100 µl of bacterial culture was swabbed evenly over this with the aid of disinfected cotton bud. Then 50 µl of Se nanoparticles (1 mg of Se nanoparticles diluted in 1 ml of distilled water) was poured in one well, while an ampicillin antibiotic disc (50 µg) and distilled water in another well served as control. Zone of inhibition on these plates was observed after incubation at 37°C for about a day.

Se nanoparticles of various shapes and sizes were synthesized by chemical precipitation method. Chemical reduction method helps in maintaining better uniformity of the particles which can be used for various applications, especially in medicine. Nguyen *et al.*[8] fabricated nano-selenium from Na_2SeO_3 using glutathione and bovine serum albumin as reducing agents, with an average diameter of 79 nm. Similarly, Li *et al.*[14] synthesized Se nanoparticles using L-cysteine as a reducing agent. The change from colourless to red colour indicates the formation of nano-selenium

$$Na_2SeO_3 \rightarrow 2Na^+ + SeO_3^{2-},$$

$$C_6H_8O_6 \rightarrow C_6H_7O_6^- + H^+,$$

$$2Na^+ + SeO_3^{2-} + C_6H_7O_6^- + H^+ + H_2O \rightarrow Se + 2Na^+ + C_6H_7O_6^- + H_2O.$$

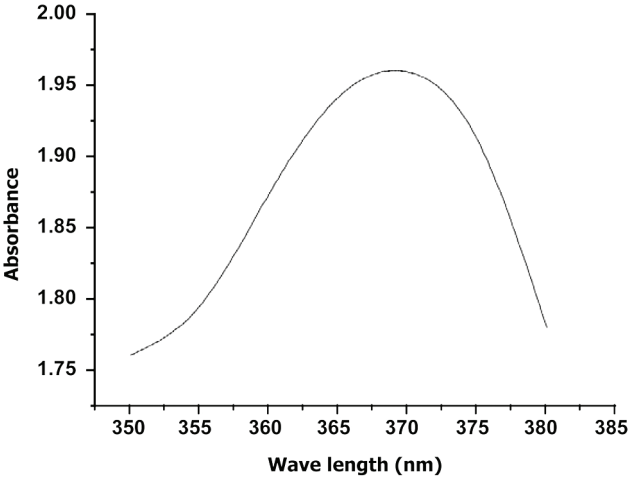

Figure 1. UV–Vis analysis of nano-selenium.

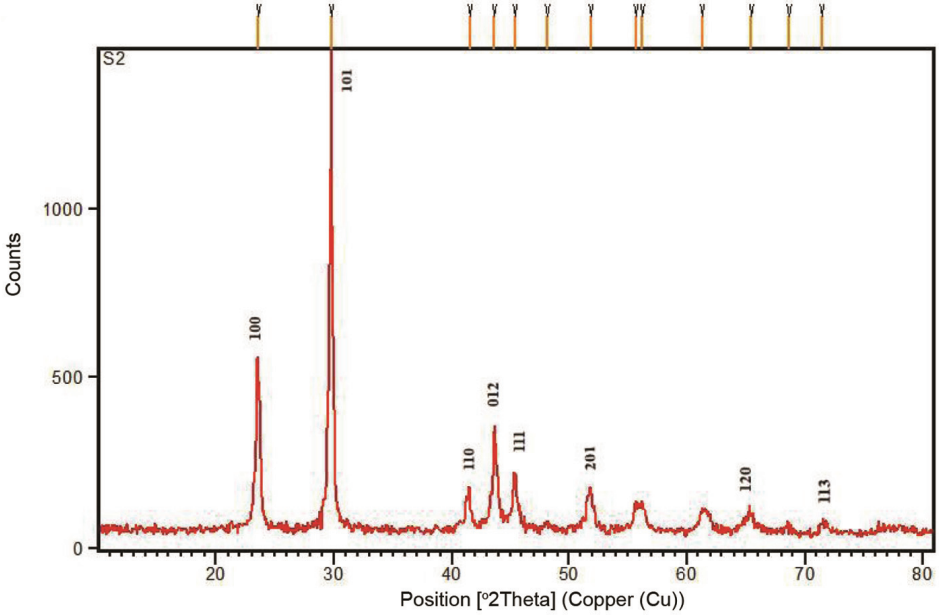

Figure 2. X-ray diffractometer analysis of nano-selenium.

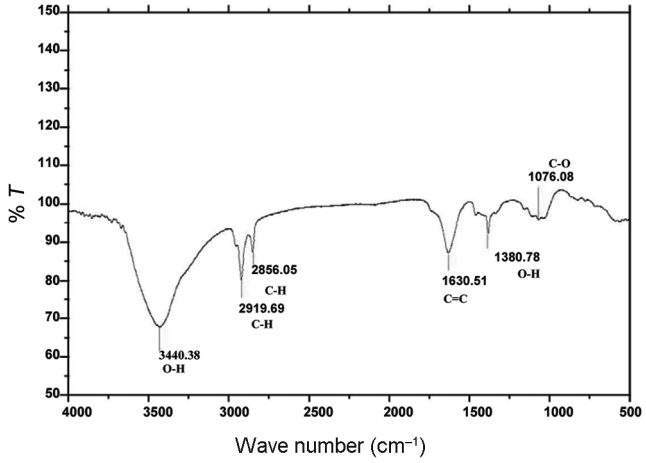

Figure 3. Fourier-transform infrared spectroscopy analysis of nano-selenium.

Conversion of selenium ions into nano-selenium during reaction with ascorbic acid was observed as a result of the colour change. The most easily observed property of nanoparticles is their change in colour at different sizes. So as the size changes, the colour of the particles formed will also change; absorbing in the visible region of the spectrum. Thus, UV–Vis spectrum is the most basic and important technique for the identification and characterization of nanoparticles[15]. In UV analysis, chemically synthesized Se nanoparticles were scanned under a spectrophotometer between 350 and 400 nm; the highest peak was visible at 370 nm (Figure 1). Malhotra *et al.*[13] observed similar strong absorbance peak between 320 and 550 nm, with a maximum at 390 nm. Nano-selenium obtained using *Klebsiella pneumonia* also showed an absorption band between 200 and 300 nm (ref. 16). Zhang *et al.*[17] and Harikrishnan *et al.*[18] have fabricated nano-selenium using *Pseudomonas alcaliphila* and *S. cerevisiae*, which showed absorption peaks at 200 and 300 nm respectively.

The crystallite nature of chemically prepared nano-selenium was examined by XRD. This analytical method aids in the determination of crystallite materials and also provides details of unit cell dimensions. Dorofeey *et al.*[19] reported that shape and breadth of reflection help detect substructures in the nanosubstances. The obtained nano-selenium was highly crystalline and all diffraction peaks have been well indexed as 23.5616°, 29.7572°, 41.4821°, 43.6615°, 45.4223°, 48.1882°, 51.8509°, 55.6733°, 56.2526°, 61.3117°, 65.4128°, 68.6359° and 71.4573°, which correspond to 100, 101, 110, 012, 111, 200, 201, 003, 112, 013, 120, 211 and 113 crystal planes respectively, in accordance with JCPDS 86-2246 (Figure 2). Using Scherrer's equation the crystalline nature of nano-selenium was confirmed.

$$D = 0.94\lambda/\beta\cos\theta.$$

The crystallite size of nano-selenium was found to be 28 nm. A similar study was also carried out by Ghada and Bahig[20], who identified sharp peaks numbers from 5 to 80 in the 2θ values.

FT-IR spectra were captured in the frequency range from 400 to 4000 cm^{-1}. This frequency absorption helps identify elements involved in the fabrication of nano-selenium. Figure 3 shows the FT–IR spectra of chemically synthesized nano-selenium. The spectrum of nano-selenium has vibrational and stretching functions at wave

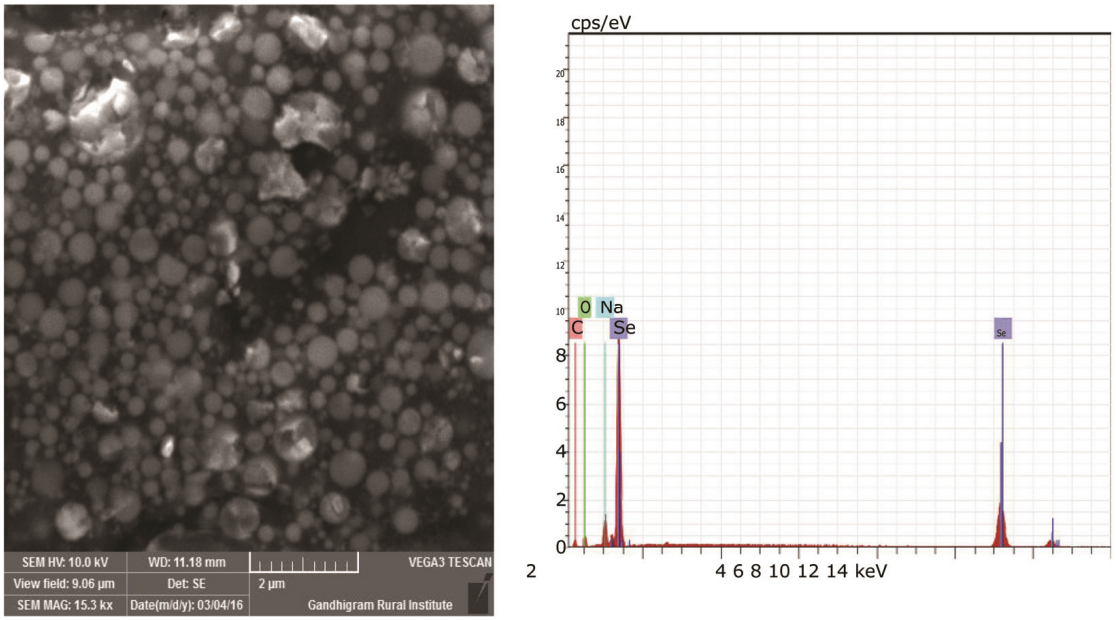

Figure 4. Scanning electron microscopy-energy dispersive image of nano-selenium.

Table 1. Fourier transform-infrared spectroscopy analysis of nano-selenium

Frequency (cm^{-1})	Functional group	Types of vibration	Intensity
3440.38	O–H	Stretch bonded	Strong, broad
2919.69	C–H	Stretch bonded	Strong
2856.05	C–H	Stretch bonded	Strong
1630.51	C=C	Stretch bonded	Variable
1380.78	O–H	Bending bonded	Medium
1076.08	C–O	Stretch bonded	Strong

numbers 2919.69, 1630.51, 1380.78 and 1076.08 cm^{-1} corresponding to C–H, C=C, O–H and C–O respectively, suggesting the presence of reducing groups aiding in nano-selenium fabrication (Table 1). The band at 2361 cm^{-1} is the C–H stretch of aryl acid. The strong band found at 1654 cm^{-1} is characteristic of C=C stretch of an aromatic ring, N–H bending of amine and a C=O stretch of polyphenols. The C–O group can be indicated by peaks observed between 1100 and 1000 (ref. 21). Mallikarjuna *et al.*[22] reported that bands at 3250 cm^{-1} indicate O–H groups in water and alcohol.

SEM is used for studying morphological characteristics of nanoparticles. It is capable of imaging photographs with high resolution. EDX diffraction is commonly associated with SEM, which is used to analyse the chemical-level elemental composition of samples[23]. SEM image of chemically synthesized Se nanoparticles revealed that it is spherical in shape and uniformly distributed (Figure 4 *a*). Hariharan *et al.*[24] used this technique for analysing the morphology and structure of Se nanoparticles, and produced spherical and smaller-sized particles ranging between 30 and 100 nm. EDX analysis proves that the obtained nanoparticles are pure in nature (Figure 4 *b*). The chemical constituents of the sample were analysed by Razi *et al.*[25], which yielded about 99% pure selenium. Keerthika *et al.*[26] have synthesized iron oxide nanoparticles that are almost globular in shape and range in size between 30 and 110 nm.

Size and morphology of nano-selenium were identified by this technique. Nano-selenium exhibits spherical shape with an average diameter between 15 and 18 nm. Figure 5 *a* and *b* shows TEM image and its histogram of chemically synthesized nano-selenium respectively. These images depict that the shape of nano-selenium is spherical, which is in conflict with the SEM analysis result. Hu *et al.*[27] have also reported a comparable result (nano-selenium of size 20–80 nm). Chen *et al.*[28] synthesized spherically shaped and 44–92 nm sized nano-selenium.

Figure 6 shows the antibacterial response of nano-selenium against *S. aureus*, *E. coli* and *P. aeruginosa*. Hamouda *et al.*[29] found that nanoparticles can play a vital role in designing efficient bactericidal substances. Klabunde *et al.*[30] and Stoimenov *et al.*[31] reported that metal nanoparticles display excellent antibacterial activity against disease-causing bacteria. The zone of inhibition of nano-selenium against the three above-mentioned strains was measured (Table 2), which revealed that nano-selenium possesses antibacterial efficiency. The antibacterial effect may be due to the fact that at this particular concentration nano-selenium interact with the bacterial cell surface and penetrates into the cell, thus causing damage. As concentration of the nanoparticles

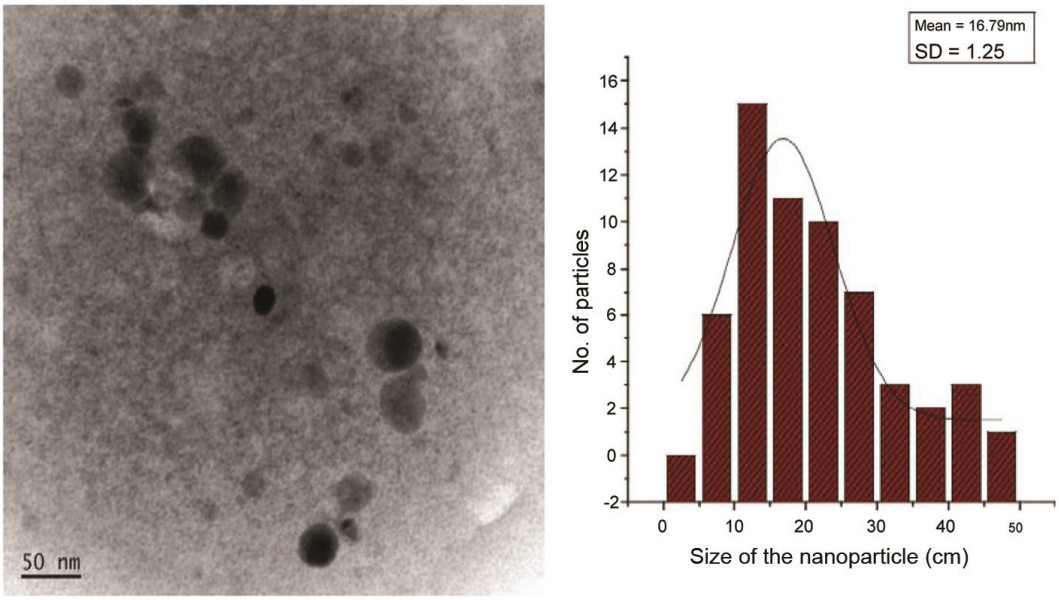

Figure 5. Transmission electron microscopy analysis of nano-selenium.

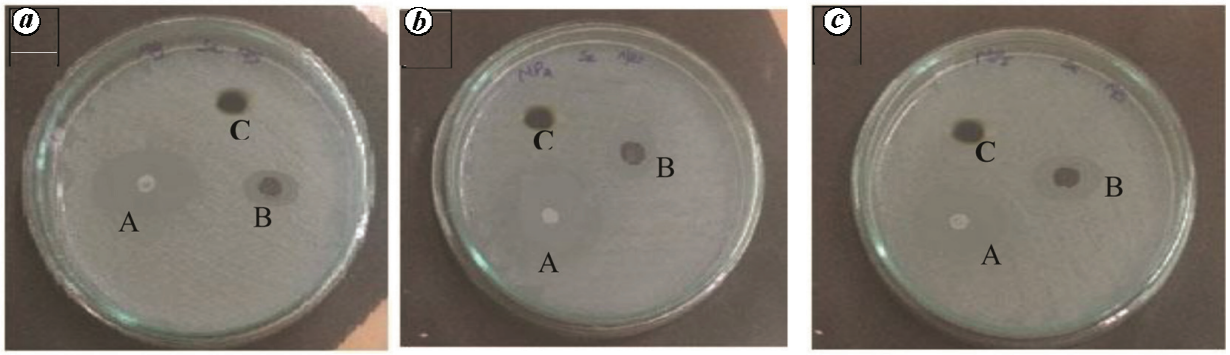

Figure 6. Antibacterial response of nano-selenium. ***a***, *Pseudomonas aeruginosa*; ***b***, *Staphylococcus aureus*; ***c***, *Escherichia coli*. A, Antibiotic (ampicillin); B, Selenium nanoparticles; C, Control.

Table 2. Zone of inhibition of nano-selenium against pathogens

Pathogens	Zone of inhibition (mm)	
	Nano-selenium	Antibiotic (ampicillin)
Pseudomonas aeruginosa	18	20
Staphylococcus aureus	21	22
Escherichia coli	18	21

increases, their bactericidal response also increases[32]. Chudobova *et al.*[33] found that *S. aureus* is inhibited by nano-selenium at 60 times better than the control. More research needs to be carried out to deduce the antimicrobial response of the disease causing microorganisms. This study is focused on fabrication of highly stable nano-selenium (15–18 nm size) using precipitation method with simple ingredients which can be used as an effective antibacterial agent similar to that of ampicillin. This antibiotic is used to prevent and treat a number of bacterial diseases or infections in humans, like respiratory tract infections, urinary tract infections, meningitis, whooping cough, salmonellosis and endocarditis. Hence, nano-selenium can be an alternative to antibiotics like ampicillin.

Thus, in this study Se nanoparticles were synthesized through chemical precipitation method using ascorbic acid as the reducing agent. The fabricated nano-selenium was characterized using UV–Vis, XRD, FT-IR, TEM and SEM-EDX. It exhibits a spherical shape with an average diameter between 15 and 18 nm, which was confirmed by TEM analysis. Chemically synthesized Se nanoparticles could be a potential antibacterial agent to treat humans affected with bacterial diseases caused by important pathogenic bacteria.

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