Research Article

Chemical Synthesis and Characterization of Selenium Nanoparticles

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Abstract

The present study was carried out to synthesize SeNP by chemical reduction method at room temperature, using glutathione as reducing agent. This method is capable of producing SeNP in a size range about 80-100 nm at room temperature. The synthesized nanoparticles separated easily from the reduced glutathione and aqueous solution by a continuous magnetic stirring and centrifugation followed by lyophilization. The synthesized SeNP have been characterised by sophisticated analytical techniques such as zeta potential and Transmission electron microscopy. Nano red Se is fine powder form, stable for several years. It was concluded from zeta size analysis and TEM that production of 80-100 nm SeNP is possible by simple chemical reduction method. The bovine serum albumin stabilized SeNP are uniform and can be used as therapeutic material for several years.

Keywords: Nanoparticles, Selenium, glutathione, synthesis, characterization

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Introduction

Nanotechnology is an emerging discipline fundamentally changing the way in which materials are synthesized at nanoscale level. Present study focused on Se mainly because of its unique properties and great potential applications. Selenium is an essential trace element with important antioxidative, pro-oxidative effect, physiological functions, nourishment and extensive pharmacological actions [1]. Nano selenium has unique properties over the bulk form such as low toxicity, better reactivity, low dosage and high biological activities. It is the important micronutrient required at low concentration for most living organisms but it is toxic at high concentration. Nanoparticle formulations have been developed to provide more effective selenium dosing regimens, as SeNP provide slow release of Se ions having very high surface to volume ratio and are known to bind proteins and reactions may be catalysed by the nanoparticle surface [2].

Se in nano form shows excellent biological activities like down regulation of m-RNA, expression of pro-inflammatory cytokines including inducible NO synthase, IL-1, TNF-alpha, reducing inflammation [3]. Thus SeNP caused the great interest of researchers and a few of synthesis methods have been developed [4]. Zhang *et al*. (2001) [5] reported that chemically synthesized SeNP were proven to be effective antioxidants without notable cytotoxicity which is a typical side effect of other chemical forms of Se. Thus SeNP have attracted much attention to be used as potential therapeutics [6] as well as nutritional supplement [7].

The main synthetic approach for preparing SeNP is chemical reduction required reducing agent and stabilizer.

Materials and Methods

All the reagents used were of AR grade, sodium selenite, bovine serum albumin (BSA) and glutathione (GSH) were from Sigma Aldrich, USA. MilliQ water and double distilled water is used throughout the experiment to prepare the solution and dialyze respectively. Equipments used are magnetic stirrer (Remi 2 MLH), Lyophilizer (Scanvac), Sonicator (Citizen), Digital pH meter (Toshniwal), Zeta sizer nano ZS-90 analyser (Malvern Instr. Inc. UK), Ultracentrifuge (Optima TLX-120), Transmission Electron Microscope (TEM) (Hitachi H-7500, Japan).

Synthesis of Se-NP

5 ml of 25 mM sodium selenite was mixed with 20 ml 25 mM of glutathione containing 200 mg bovine serum albumin. The mixture pH was adjusted to 7.2 with 1.0 M sodium hydroxide to generate red elemental selenium and oxidized glutathione. The red solution was dialyzed for 96 hours against double distilled water which was replaced every 24 hours to separate oxidized glutathione from the SeNP under magnetic stirring (**Figure 1**). The final solution containing Nano-Se and BSA was subjected to centrifugation at 13000 rpm for 10 min. The pellet thus recovered was subjected to washing by its resuspension in milliQ water followed by centrifugation at 13000 rpm for 10 min, to remove possible contamination present in nanoparticles. Finally, pellet was freeze dried using a lyophilizer and obtained fine powder was stored at 4^{o}C in aluminium foil. Whenever required sonicated for 10 min after required dilution in PBS and used immediately for treatment as long time after sonication it loses its stability (**Figure 2**). Selenium nanoparticles prepared using the high concentration of bovine serum albumin are uniform and stable for several years [8, 5].

Figure 1 Dialysis of red solution against double distilled water to separate SeNP from oxidized glutathione under magnetic stirring.

Figure 2 Sonicated SeNP in PBS.

Figure 3 Conformation of SeNP formed by observing colour change through naked eye

Characterization of Selenium nanoparticles

SeNP formed were characterized by Zeta potential and transmission electron microscopy imaging method. The surface charge (Zeta potential) and particle size of SeNP was determined by electrophoretic light scattering (ELS), using a Zeta sizer Nano ZS 90 analyzer. A He-Ne laser with fixed wavelength of 633 nm used as a light source and the intensity of scattered light was measured by a detector at 90^{o}C. This instrument passes laser which provides 90^{0} scattering angle. This instrument read the sample with appropriate medium i.e. water. The temperature was set at 25^{0}C, the sample were put into the cuvette (clean disposable zeta cells). The charges applied on the sample solution and results were read by zeta sizer after 12 consecutive recording (Zeta run).

For TEM imaging dip preparation for SeNP by floatation method [9] was done. A drop of sample was placed on a piece of parafilm and the carbon coated copper grid was placed. After 5-10 minutes the excess was drained with the help of filter paper. The sample was washed with distilled water and then stained with 2% Uranyl acetate, air dried and observed under Transmission electron microscope (Model Hitachi, H-7500) at various magnifications as per the standard protocol at Ruska Lab., College of Veterinary Sciences, Hyderabad.

Results and Discussion

Synthesis,Coating and Characterization of SeNP

Synthesis of SeNP was confirmed by color change into red which was very well visible to naked eye (**Figure 3**) as reported by Qureshi *et al*. (2015) [10], Khil *et al*. (2016) [11], Bhattacharjee *et al*. (2015) [8] who employed glutathione as reducing agent to prepare spherical SeNP by reducing sodium selenite.

Maintaining stability of nanoparticles is a fundamental requirement for any further application of these nanoparticles as they tend to agglomerate or precipitate. As the size of the particle increases its reactivity increases, so to protect these particles from any reaction, coating of the particle is essential. The simplest method of stabilizing the particles is by a single layer coating of any polymer or surfactant which is a dense matrix. This will result in increase in viscosity and therefore less interaction between the nanoparticles hindering the further growth of these particles. The coating does not only help in protecting the particle but also help in the functionalization with specific compounds such as drugs, functional groups, available binding site etc. also giving solubility, specificity and process ability to the system [12].

Tran and Webster (2011) [13] used BSA for the stabilization of the particles where they observed an increased size of the particles of about 100 nm while these particles without the coating were of 40-60 nm, which is in accordance with the present study. Similar studies are conducted by Allah and Khalid (2015) [14] and Mohapatra *et al*., (2014) [15].

The nanoparticles are characterized to enhance the quality of development and also to improve the method of synthesis. The size distribution of SeNP determined by Zeta sizer. The major fraction of the SeNP had a diameter in the range of 85 to 200 nm with a maximum of the size distribution (mean $\pm$ one s.d.) at 186.5 $\pm$ 53.78 nm. (**Figure 4**). The zeta potential analysis of SeNP revealed that the average potential (mV) of SeNP was observed to be -17.6 with the maximum of apparent zeta potential (mV) -15.0 $\pm$ 12.3 mV (mean $\pm$ one s.d.) (**Figure 5**). The size distribution as determined immediately before and after the animal study to ensure that the SeNP with the same size characteristics were administered to the rabbits throughout the study. The synthesized SeNP showed very low negative zeta potential of -17.6 mV which generally have electrostatic repulsion with one another, thus preventing cluster formation and causes low toxicity. The sample observed to be stable for a period of six months without any cluster formation as opined by Karnik *et al*., (2008)[16] who produced SeNP with a zeta potential of -28 mV.

Transmission electron microscopy (**Figure 6**) confirmed the diameter on an average 100 nm and showed that the particles had a spherical shape. The nanoselenium produced was fine soft powder in consistency, transmission electron microscope image of nano particle revealed the spherical shaped selenium nanoparticle which were well dispersed and the diameter measured on an average 100 nm. Similar finding were reported by Ahmed *et al*., (2015)[17], Hu *et al*., (2012)[18], Chen *et al*., (2008)[19] and Sasidharan and Balakrishnaraja (2014)[20] who produced spherical shaped nano selenium.

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	167.0	Peak 1:	186.5	100.0	53.78
PdI:	0.232	Peak 2:	0.000	0.0	0.000
Intercept:	0.904	Peak 3:	0.000	0.0	0.000
Result quality	Good				

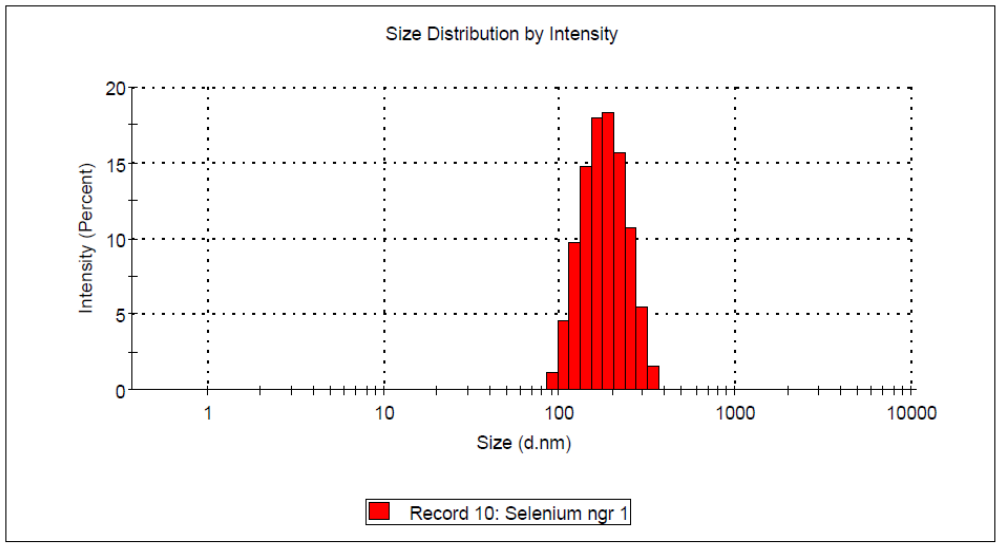

Figure 4 Zeta size of SeNP

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV):	-17.6	Peak 1: -15.0	100.0	12.3
Zeta Deviation (mV):	24.6	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm):	0.0342	Peak 3: 0.00	0.0	0.00
Result quality	See result quality report			

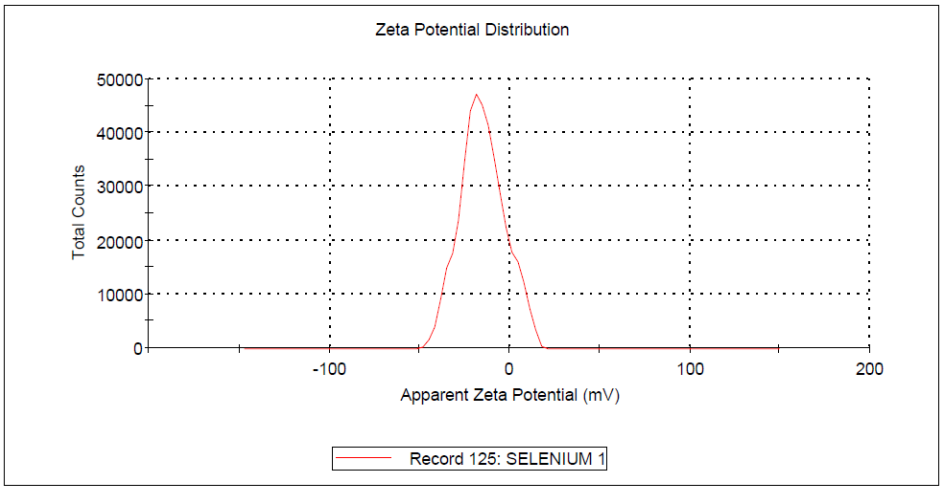

Figure 5 Zeta potential of SeNP

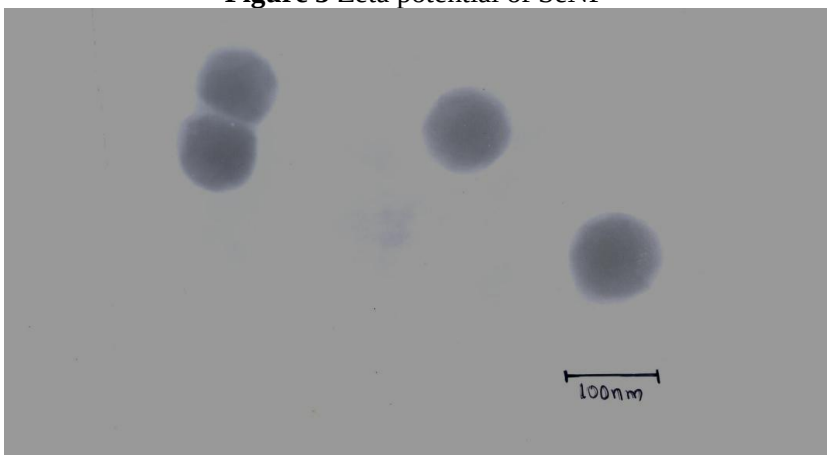

Figure 6 High resolution TEM image of Se-NP, the bar indicates 100 nm. The sizes of elemental SeNP distribute from 80-200 nm with the average size of 100 nm magnification X193000.

Conclusion

The present study was carried out to synthesize SeNP by chemical reduction method at room temperature, using glutathione as reducing agent and characterize SeNP by sophisticated analytical techniques.

The reduction of sodium selenite by glutathione was found to be simple chemical method for the production of SeNP at room temperature. Further the glutathione not only used as a reducing agent but also acts as a stabilizer to avoid the aggregation of particles. It was concluded from zeta size analysis and TEM that the formation of 80-100 nm SeNP is possible by chemical reduction method. The BSA stabilized SeNP are uniform, can be stored for several years and used as therapeutic material.

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Publication History
Received 04.11.2019
Revised 26.11.2019
Accepted 27.11.2019
Online 30.11.2019