Eco-friendly, Facile and Rapid Way for Synthesis of Selenium Nanoparticles

Production, structural and morphological characterisation

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A green chemistry approach to synthesize biocompatible selenium nanoparticles is proposed in this work, using hydrogen selenite ($NaHSeO_3$) as selenium precursor and lactose as reducing agent. The formation of nanoparticles was confirmed by dynamic light Scattering, revealing a gaussian size distribution, the maximum percentage being in the range of 20-40 nm. Zeta potential measurement indicates a negative charge -38.2 mV, the stability of selenium colloidal sol being also confirmed by UV-visible spectroscopy. TEM and AFM revealed the homogeneous, spherical shape, confirming the size of nanoparticles in the range of 20-40 nm. Structural investigations of powder selenium nanoparticles by FTIR spectroscopy and XRD patterns emphasise the presence of stretching and bending vibrations of Se-O bonds, respectively the amorphous structure of the synthesized selenium. The proposed method is suitable for biological applications such drug release, functional food or nutritional supplements.

Keywords: selenium nanoparticles, lactose, morphology, TEM, DLS, AFM

Selenium is a major structural component of many enzymes that play important roles in anti-oxidation, reproduction, muscles function and tumour prevention [1]. On the other hand, selenium is used in the electronic industries for manufacturing rectifiers and photoelectric cells, glass and ceramic manufacturing industries and in various aerospace devices [2]. Selenium is also used in the agricultural industry for plant breeding [3]. Among all of these applications, selenium has gained primary importance in medicine for the effective management of different diseases [4].

Nowadays, an important research direction in biotechnology is considered the nanoparticles production by controlling the size and morphology using physical, chemical or biological methods [5]. Although nanoscale materials can be produced using a variety of traditional physical and chemical processes, it is now possible to biologically synthesize materials via environment-friendly green chemistry based techniques [6]. In recent years, the convergence between nanotechnology and biology has created the new field of nanobiotechnology that incorporates the use of biological entities such as actinomycetes algae, bacteria, fungi, viruses, yeasts and plants in a number of biochemical and biophysical processes. The biological synthesis via nanobiotechnology processes have a signficant potential to boost nanoparticles production without the use of harsh, toxic, and expensive chemicals commonly used in conventional physical and chemical processes [7-9].

Selenium in the nano form (nano-Se) has gained importance as a possible supplement in the treatment of several diseases due to a better biocompatibility, efficacy and lower toxicity as compared to various organic and inorganic forms of selenium. The nano form of Se can be used for various medical applications due to its advantages over the bulk form such as low toxicity, better reactivity, and low dosage [10, 11].

The *green* chemistry approach to synthesizing biocompatible selenium nanoparticles has gained attention in recent years. Plant extracts and other natural resources has been found to be an excellent alternative method for green synthesis, since this method does not use any toxic chemicals and also has numerous benefits, including environmental friendliness, cost-effectiveness, and suitability for pharmaceutical and biomedical applications [12, 13].

By chemical route, elemental nano-Se can be synthesized within the reduction of a Se-salt with a reducing agent, usually in the presence of a stabilizing agent to prevent the clusters of Se atoms from growing and to obtain stabilized nanoparticles in colloidal suspension. Selenous acid and sodium selenite are the most frequently used precursors in these reduction methods, and several different reducing agents have been used, such as hydrazine, glutathione, ascorbic acid, sodium citrate and sodium thiosulfate [14, 15].

To avoid the use of toxic reducing agents, availably green reducing agents such as carbohydrates, oleic acid, β-carotene, polyvinyl alcohol were used in dual role as reducers and structural directing agents [16-18]. For example, Wei *et al.* demonstrated that chitosan may act as both reducing and capping agent, and pointed out that reducing sugar must have an aldehyde in open form to be oxidized to carboxylic acid [16]. By extending this finding to other cheap and readily available green reducing agents, we propose in this study a novel eco-friendly method for

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nano-Se production using lactose as reducing agent. Lactose is a reducing disaccharide sugar ($C_{12}H_{22}O_{11}$) composed of galactose and glucose moieties, linked together through a beta-(1,4) glucosidic linkage.

To our knowledge, this is the first paper reporting nano-Se production and characterization by this chemical route. On the other hand, by controlling the synthesis parameters (pressure, temperature, exposure time, *p*H, reagents concentration), the size and shape of nanoparticles can be tailored according to the desired application. Previously, lactose has been used for the reduction of Au and Ag salts and stabilisation of the resultant metallic nanoparticles [19, 22].

In the present work, the structure and morphology of synthesized Se nanoparticles were characterized using transmission electron microscopy (TEM), AFM (atomic force microscopy), UV/Visible and FTIR (Fourier Transform Infrared) spectrophotometry, dynamic light scattering (DLS) and X-ray diffraction method (XRD). Possible applications of the prepared nanoparticles are also discussed.

Experimental part

Preparation of selenium nano colloidal sol, UV-visible and DLS analysis

25 mL of sodium hydrogen selenite ($NaHSeO_3$) in concentration of 10000 ppm was selected as starting selenium precursor and mixed with 25 mL lactose solution in a ratio 1:8 (w/w), by vigorous stirring using magnetic stirrer. The resulted mixture was heated for 3 min at 120° C in a vertical autoclave (Sanyo), resulting a characteristic dark red coloured nano-colloidal sol. All the reagents were purchased from Sigma Aldrich. The selected ratio 1:8 emerged as a result of optimisation study, upon testing different ratio sodium hydrogen selenite/lactose (1:1; 1:6; 1:8 and 1:12 w/w). After an appropriate dilution, UV-Vis spectrum was recorded in the range 200-800 nm using Shimadzu UV-VIS 1700 Pharma Spec (Shimadzu Corp. Kyoto) spectrophotometer. DLS (Dynamic Light Scattering) was applied to selenium nano-colloidal sol using ZEN 3690 (Malvern Instruments) in order to determine the average particle size, size distribution and Zeta potential. After cooling, the red mixture was centrifuged at 6000 rpm for 10 min. The supernatant was removed and the red nano-Se particles were washed with distilled water, followed by repeated centrifugation (4 times), filtration and drying.

Morphological and structural characterization of selenium nanoparticles

X-rays Diffraction (Rigaku Miniflex, with Cu-Ká radiation) was performed on powder nano-Se after filtration and freeze-drying procedure (using Martin Christ Alpha 1-2 LD equipment). The same powder was investigated by FTIR (Fourier Transform Infrared Spectroscopy) in the range 400-4000 cm^{-1}, using Spectrum BXII spectrophotometer (Perkin Elmer), equipped with MIRacle ATR accessory (ZnSe crystal), at scanning speed of 32cm^{-1} and spectral width 2.0 cm^{-1}. AFM microscopy (SPM/AFM 5500 Keysight Technologies) was applied in order to observe the surface topography of the drop-coated film of nano-Se, using tapping mode with RTESP tip. The shape and morphology of selenium nanoparticles was emphasized by TEM (Tecnai G2 F30 S-TWIN, FEI) equipped with Energy Dispersive X-ray Spectroscopy (EDAX) for qualitative and quantitative microanalysis.

Results and discussions

Preparation of selenium nano-colloidal sol, UV-visible and DLS analysis

The reaction of selenium ions with lactose occurred rapidly and the solution displayed a time-dependent colour change during the heating procedure. During the heating, in alkaline solution, lactose undergoes an isomerisation reaction to lactulose, where the glucose moiety of lactose is converted to fructose. Both lactose and lactulose are reducing disaccharides having anomeric carbon that may convert to an open-chain form with an aldehyde group [23].

The schematic mechanism of the red nano-Se particles production by this route is proposed in figure 1. After cooling, the red colour of the mixture remained stable. Figure 2a displays the UV/Visible spectrum of the red coloured colloidal mixture, while figures 2b and 2c shows the histogram of particle size distribution and apparent zeta potential determined by dynamic light scattering (DLS) measurement.

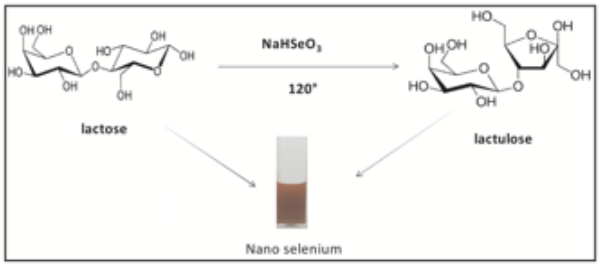

Fig.1 The proposed mechanism for selenium nanoparticles synthesis using $NaHSeO_3$ as selenium precursor and lactose as reducing agent

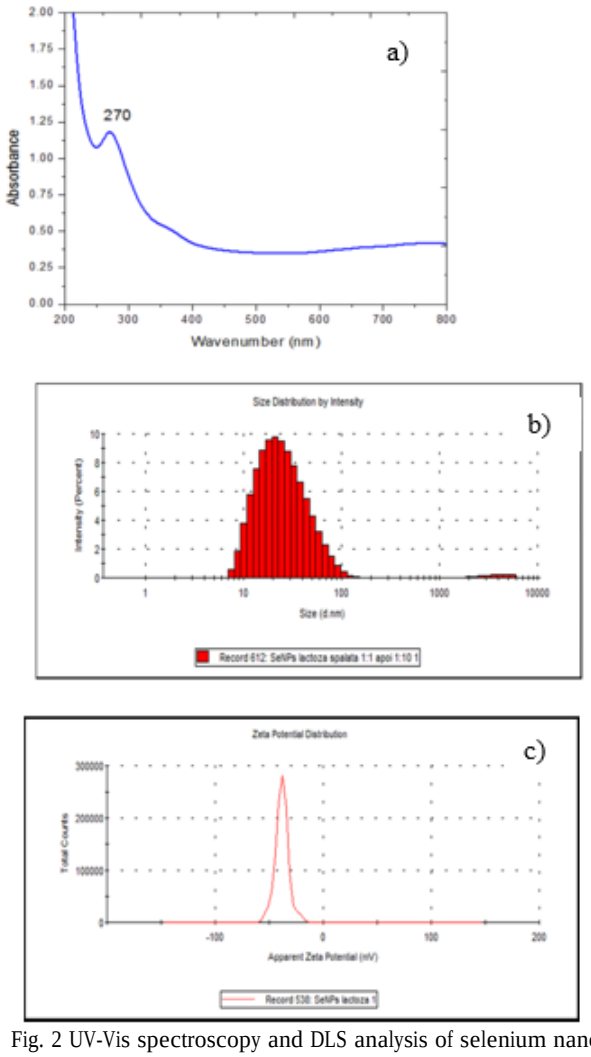

Fig. 2 UV-Vis spectroscopy and DLS analysis of selenium nano-colloidal sol: a) UV-Vis spectrum of nano-colloidal selenium; b) Particle size distribution; c) Zeta potential measurement

The maximum absorbance at 270 nm is characteristic for colloidal nano-selenium, as previously reported by Shah et al. [24]. This results is in agreement with other studies in literature [24-25], demonstrating that the reducing agent (lactose) is strong enough to ensure complete conversion of the precursor molecules into nano sized selenium particles. Selenium nanoparticles are known to exhibit a regular absorption maximum in the wavelength region of about 300 cm^{-1} when spherical particles of size 50-100 nm are formed, depending on the experimental conditions. However, it was previously demonstrated that heat treatment causes selenium nanoparticles to aggregate into larger size spheres or nanorods [26]. The formation of nanoparticles was further confirmed by laser diffraction, revealing that particle size obtained from highly dispersed mixture was in the range of 8-100 nm, with gaussian distribution, the maximum percentage being in the range of 20-40 nm. The zeta potential measurement indicates negative charge -38.2 mV on the selenium nanoparticles. The high negative value charge indicates a stability of the selenium nanoparticles without forming aggregates; these particles do not transform to black amorphous during the storage time [7].

Morphological and structural characterization of selenium nanoparticles

The morphology of selenium nanoparticles was revealed by TEM (fig. 3) indicating homogeneous, spherical shape; this fact is very important for the Zeta Sizer measurements, since the method presumes round shape of the measured object and therefore we consider the measurements accurate [12]. However, a slightly aggregation observed in figure 3b occurred as a consequence of storage time (about 30 days).

According to TEM images, majority of the particles are of size between 30-50 nm. On the other hand, bi- and three-dimensional AFM images presented in figure 4 (a, b) also confirm the morphology and distribution of selenium nanoparticles, attaching with each other, showing the tendance to form a layered structure. Moreover, the size of the selenium nanoparticles was found to be similar with the data collected from the DLS study, while the variation in size of nanoparticles was commonly found during chemical and biological synthesis [9, 14].

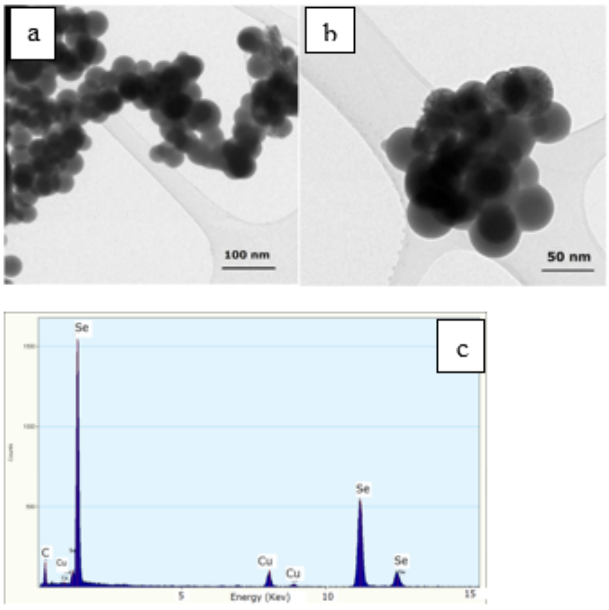

Fig. 3 Morphological evidence of selenium nanoparticles: (a, b) TEM images of selenium nanoparticles recorded with different details and magnification; c) Corresponding EDAX spectrum

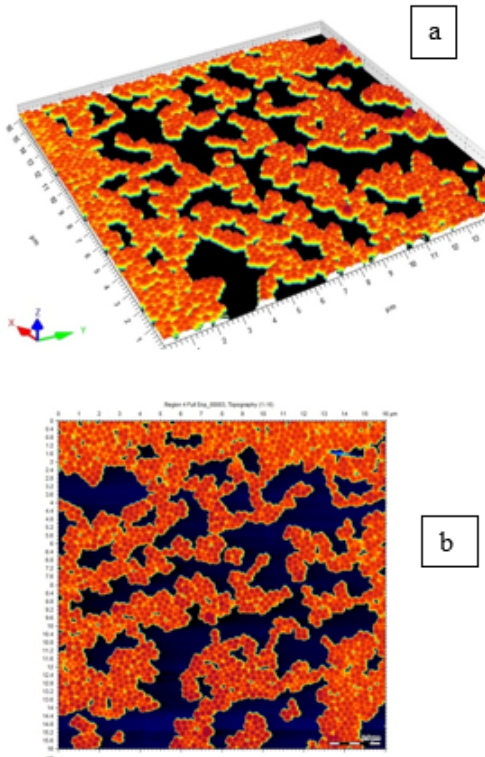

Fig. 4 AFM images (tapping mode) of selenium nanoparticle: a) 3D topographic image of 16μm/16μm scanned area; b) 2D image of the same area.

Structural characterization of the selenium powder was performed by FTIR and XRD analysis. In figure 5a, a medium intensity band is shown at 465 cm^{-1} and a sharp peak at 668 cm^{-1}, which are due to the bending vibrations of Se-O bonds. A sharp and strong intensity peak is shown at 1051 cm^{-1} due to stretching vibration of Se-O bonds [27-29]. From this point of view, the result is similar with previous work reporting the synthesis of selenium nanorods with assistance of biomolecules (amino acids) and demonstrates that fingerprints of

FTIR spectrum of synthesized Se nanoparticles is almost identical with that of commercial powder [30-31]. So, the formation of different types of nanostructures (nanospheres, nanorods, nanowires) mainly depends on the nature of the stabilizers.

The XRD pattern of the Se nanoparticles shows a broad and intense peak at about $2\theta = 23°$ (fig. 5b) which suggests that the nanoparticles are not crystalline. Some previous studies related to crystalline phase investigation of selenium nanoparticles suggested that stable amorphous forms (or even low crystallinity) are advantageous for biological applications, as they exhibit better solubility and

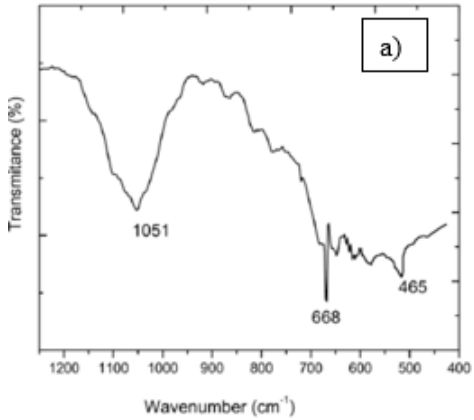

Fig. 5 Structural characterization of powder selenium nanoparticles: a) FTIR ATR spectrum of powder selenium nanoparticles

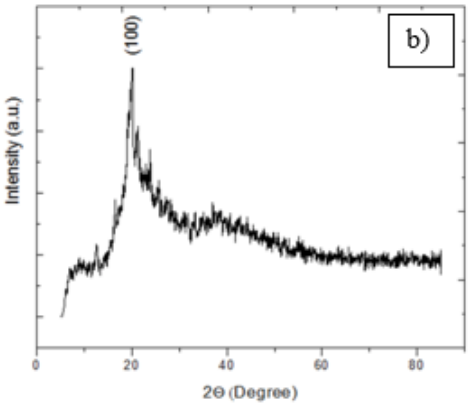

Fig. 5 Structural characterization of powder selenium nanoparticles: b) XRD pattern of powder selenium nanoparticles

subsequent adsorption and bioavailability [32, 33].We consider that this method is suitable for different biological applications such drug release, functional food or nutritional supplements, being not only facile and rapid, but also very safe for the environment and low cost.

Conclusions

Synthesis of selenium nano spheres was performed by *green chemistry* route, using hydrogen selenite ($NaHSeO_3$) as selenium precursor and lactose as reducing agent. The size and morphology of selenium particles were investigated by DLS, TEM and AFM, demonstrating that majority of the particles were in the range of 20-50 nm. The stability of selenium colloidal sol was confirmed by UV-visible spectroscopy. Structural investigations of powder selenium nanoparticles by FTIR spectroscopy and XRD patterns confirmed the presence of stretching and bending vibrations of Se-O bonds, respectively the amorphous structure of the synthesized selenium. The proposed technique is eco-friendly, facile and rapid way for production of selenium nanoparticles suitable for biological applications.

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