Biosynthesis of selenium nanoparticles and their effect on changes in urinary nanocrystallites in calcium oxalate stone formation

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Abstract

Plant bio constituents have the ability to prepare nanoparticles, and usually, plant polyphenols are tested to reduce sodium selenite to selenium nanoparticles (SeNPs). In this work, we showed the biosynthesis of SeNPs using *Ocimum tenuiflorum* leaf extract. The as obtained SeNPs were in the size range of 15–20 nm and spherical in shape. Also, TEM microscopic images represented the aggregation of crystal structures as extracellular deposits. Moreover, scanning electron microscopy was performed to examine the chemical transition of calcium oxalate (CaC_2O_4) crystal's shape and structure due to the influence of SeNPs. SeNPs inhibited the aggregation and growth of CaC_2O_4 monohydrate crystals and hence the prepared SeNPs could have important prospects in medical and pharmaceutical applications as a potential inhibitor of CaC_2O_4 urinary stones.

Keywords Se NPs · Calcium oxalate · Aggregation · Sub phases

Introduction

Selenium (Se) is recognized as one among the essential trace minerals, vital for the maintenance of proper health and good maintenance of the human body. Selenium combines with proteins to form essential antioxidant enzymes, seleno proteins (SPs). These SPs were utilized for proper immune system functioning, controlling the thyroid functioning and to avoid cellular injury from free radicals.

Moreover, various studies reported anti-carcinogenic action of Se opposite to various kinds of cancers. The Se intervention is helpful in decreasing the threat of cancer occurrences in all the cancer forms, particularly in prostate, liver, lung cancers, and colorectal (Rayman 2005; Knekt et al. 1998; Clement 1998). Recently, it was also discovered that Se cannot only exhibit its effect on cancer threat

but can also help to prevent its metastasis and progression (Rayman 2005). Selenium can also be utilized to reduce the possibility of miscarriage, prevention of HIV evolution to AIDS through contravene the growth of virulence and sperm motility (Rayman 2000). The precise method of Se activities are not totally understood, though it is projected that improved antioxidant protection, carcinogen detoxification, prevention of angiogenesis, prevention of tumor cell attack, change of cell proliferation (apoptosis and cell cycle) and improved immune surveillance may be accredited to Se (a key selenoproteins constituent) (Knekt et al. 1998; Clement 1998; Rayman 2000; Zeng and Combs 2008). The low toxicity and great biological activity (Xia 2007) of SeNPs were presented with its exceptional medicinal diagnostic applications (Li et al. 2010). SeNPs also owed exceptional semiconductor and photoelectric properties. Se is broadly utilized in photographic exposure meters, photocells, photocopy machines and also in semiconductor rectifiers (Zhang et al. 2011). Moreover, Se can also be utilized as a catalyst in oxidation–reduction reactions and chemical sciences (Zhang et al. 2011). The chemical method of reduction is described as one among the feasible approaches of SeNPs preparation (Shin et al. 2007). The key disadvantages of these approaches are involvement of toxic chemicals, high cost, high pressure and high temperature (Wang et al. 2010). The ability of biological systems (fungi, bacteria, actinomycetes, and plants) to recycle, utilize, survive and grow

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in high concentration of metal- and toxic environment was clearly established in literature (Dhanjal and Cameotra 2010). Metal ions can be reduced to metal NPs using these biological systems. Hence, there is an increase in demand for biosynthetic approaches for the production of nanomaterials for their use in biological applications, it is well known that the green synthesized nanomaterials are biocompatible and can be easily produced by simple, low-cost ways without using any toxic chemicals. There are several biomolecules or green plant extracts that have already been reported for the production of different kinds of nanomaterials such as metal nanoparticles, graphene (Sireesh Babu 2017; Sireesh Babu et al. 2015, 2017a, b, 2018).

In this work, we have showed the green synthesis of SeNPs using *Ocimum tenuiflorum* plant extract as reducing agent. Also, we studied the effect of SeNPs on the chemical transition of CaC_2O_4 crystal's (calcium oxalate) morphology using scanning electron microscopy (SEM). SeNPs inhibited and prevented the aggregation and growth of CaC_2O_4 monohydrate (COM) crystals and induced the spherical shaped CaC_2O_4 dihydrate (COD) crystals.

Materials and methods

Materials

Using these stock solutions of sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$, 10 mmol L^{-1}), sodium chloride (NaCl , 10 mmol L^{-1}) and calcium chloride (CaCl_2 , 10 mmol L^{-1}), the sub phases of supersaturated CaC_2O_4 were prepared. The absolute concentration of $\text{C}_2\text{O}_4^{2-}$ and Ca^{2+} was maintained as 30 mmol L^{-1} . This method of preparation may induce the nature of COM crystals formation. Selenious dioxide (SeO_2) was reduced with the help of plant extract and then the solution of SeNPs was prepared by reducing SeO_2 .

Fabrication of selenium nanoparticles (SeNPs)

The precursor and extract concentrations were maintained in between 10 and 50 mM and 1 to 5%, respectively for the SeNPs synthesis. When the *O. tenuiflorum* leaf extract of 1% was mixed with 10 mM solution of Sodium Selenite, optimized results were observed for the synthesis by maintaining a 9:1 ratio at room temperature. When considering variable temperature, no change in color of the reaction mixture was observed indicating the incomplete reaction. We also observed similar results when the reaction pH was recorded throughout the process of the reaction, alkaline at 9 remained constant. Under stirring conditions of 130 rpm, the reaction sustained for about 75 h (Ganesan 2015).

Crystal growth and transfer

The sub phase of supersaturated CaC_2O_4 was considered as sub phase (I). In sub phase (I) the SeNPs solution at different concentrations, SeO_2 and Vc were added, which are called as a sub phase (IV), (III), (II), respectively.

All the sub phases are filtered using a microvoid filter film of 0.22 μm and later shifted into beakers, where small fragments of crystal coverslips were placed. The concentrations of SeO_2 and Vc in sub phase (III) and (II) are 30 and 60 $\mu\text{mol L}^{-1}$, respectively. The concentrations of SeNPs in the sub phase (IV) are 1, 5 and 30 $\mu\text{mol L}^{-1}$, accordingly. Later the sub phases with pH of 5.5 to 6.5 are permitted to standstill for about 72 h at a temperature of $25 \pm 2^\circ\text{C}$ being maintained.

The crystals of CaC_2O_4 were raised in sub phase (I) to (IV) after 3 days followed by accumulating on the coverslips. The CaC_2O_4 crystals along with small fragments of glass coverslips were carefully transferred into a drier containing silica-gel. The crystals of CaC_2O_4 which raised correspondingly in sub phase (I) to (IV) are called as (I) to (IV) crystals. Scanning Electron Microscopy (SEM) was employed to examine the (I) to (IV) crystals.

Characterization

The green reduction of Selenium salt to SeNPs was examined by aliquots periodic sampling and then the NPs absorbance was measured. A Perkin Elmer Lambda 35 model, Waltham, MA 02451 USA instrument was used for UV–Visible optical absorption measurements. Aliquots wavelength was scanned at regular intervals within 300–700 nm of range to determine the maximum absorbance–wavelength along with the time taken for fabrication. After the visual authorization and the UV–visible investigation of SeNPs, the sample was centrifuged for 10 min at the rate of 10,000 rpm and then cleaned thrice using Milli Q water, followed by pellet drying. The final pellet was deposited for further characterization. The characterization of synthesized NPs was done using transmission electron microscopy (TEM) to investigate the particles dispersity and morphology. A JEOL JEM 2100 HR-TEM, Japan have been used for TEM analysis. The energy dispersive spectroscopy (EDS), an elemental investigation technique that would give the details of elemental composition can be measured with the same TEM instrument. X-ray diffraction analysis was performed using a Bruker D8 Advance Diffractometer (Bruker AXS, Germany) with Cu $\text{K}\alpha$ radiation ($k = 1.54 \text{ \AA}$). The instrument was recorded over a 2θ range of 10° – 90° with a scanning rate of 4° min^{-1} and with a step size of 0.02° . FTIR is a surface analytical technique

that was performed using Shimadzu Instrument, Japan with IR Affinity-1 model, 400–4000 cm^{-1} of wavelength range and a resolution of 4–8 cm s^{-1} . Fourier-transform infrared spectroscopy calculates the infrared intensity against the mentioned wavelengths and provides details regarding the probable functional group interactions, which were involved in the reduction procedure. Powdered SeNPs and the extract were totally dried followed by mixing with KBr for obtaining the pellet (Yu et al. 2016a, b; Gautam et al. 2017; Cremonini et al. 2016; Srivastava and Kowshik 2016).

Results and discussion

Change in the color of the reaction mixture depending on time was noticed in the sample, which was incubated at 30 °C for about 48 h, as represented in Fig. 1a. The original color of the solution, which was light yellow slowly transformed to red with time. After incubating for 48 h, no additional color change was noticed indicating the completion of SeNPs formation. The change in color was because of the surface plasmon vibrations excitation of SeNPs (Sofer et al. 2016). In the control experiments, no color change of the sample was noticed, which states that the biogenic SeNPs formation was only because of plant polyphenols. The protein absorbance peak was obtained at 330 nm using UV spectrometric investigation (as shown in Fig. 1b). The absorption peak intensity was declined according to time because of the use of polyphenols that were produced by *O. tenuiflorum* leaf extract in the reaction for reducing Se^0 from SeO_3^{2-} .

Figure 2 represented the XRD pattern obtained from selenium sample. In the XRD pattern the diffraction peaks can be indicated as trigonal Se. Using the XRD pattern, measurements of lattice were calculated to be $c=0.498$ nm, $a=0.440$ nm, corresponding to the constants of trigonal Se ($c=0.495$ nm, $a=0.434$ nm) described in the literature

Fig. 1 Time dependent color change (a) and UV-Visible spectra of SeNPs at 12, 24 and 48 h (b)

Fig. 2 XRD pattern of biosynthesized SeNPs

(Stuke et al. 1974). Additionally, XRD pattern also reflect that the obtained Se NPs are amorphous in nature. This amorphous nature of Se NPs may be due to the presence of surface *O. tenuiflorum* leaf extract polyphenols (Johnson et al. 1999).

The existence of strong signals proportional to elemental Se was exhibited by EDX spectrum. The signal of strong principal absorption, 1.37 keV was obtained for SeNPs. The existence of elemental Se was clearly represented by the peaks at 11.2 keV and 1.37 keV. Thus, the green reduction of selenium salt to elemental form was confirmed by the existence of elemental Se. The additional peaks identified were due to Nitrogen (6%), Carbon (30%), Oxygen (31%), Selenium (21%) and Sodium (10%) and the percentage of Se obtained was higher when matched with other reports like D.Cui which resulted with a percentage of Oxygen (16%), Se (5%), Sodium (2%), Carbon (75%), Oxygen (5%) and Selenium (94%) (Yu et al. 2016a, b). The additional peaks subsequent to nitrogen, oxygen and carbon were due to the bioactive constituents like polysaccharides, flavonoids and phenolics, which were bounded to the surface of SeNPs. Energy dispersive X-ray spectrum of green synthesized SeNPs, validates the existence of signals like Nitrogen, elemental Se, Sodium, Oxygen and Carbon as represented in the Fig. 3. The above results are in good agreement with previous reports (Cui et al. 2018; Husen and Siddiqi 2014).

The FTIR analysis of synthesized SeNPs was studied for finding the possible reducing biomolecules inside the leaf

Fig. 3 Energy dispersive X-ray of SeNPs fabricated from the *O. tenuiflorum* extract

Fig. 4 FTIR spectrum of SeNPs

extract of *O. tenuiflorum*, FTIR results are represented in Fig. 4. The absorption peaks of FTIR were noticed around 1738, 1647, 1517, 1460, 1380, 1110, and 1023 cm^{-1} . The peak emerged at 1023 cm^{-1} could be due to stretching vibrations of -C-O alcoholic groups. The absorption peak located at 1380 cm^{-1} may be because of the stretching modes of -C-O-C . The peaks emerged at 1460 and 1110 cm^{-1} were due to the hydroxyl groups in phenols and polyphenols, respectively. The band found at around 1380 cm^{-1} may be due to stretching vibration of C-N or bending vibrations of O-H . The peak located at 1738 cm^{-1} is attributed to -C=O stretching vibration representing the carboxylic acids group. The existence of peak at 1647 cm^{-1} indicates that the SeNPs being surrounded by proteins through carboxylate group. The -C-C- stretching vibration in aromatic ring results an absorption peak at around 1517 cm^{-1} . All these bands and stretching vibrations indicate that the carboxylic acids, proteins, polyphenols and alcoholic groups were bounded to the surface of SeNPs.

TEM images of SeNPs were shown in Fig. 5. These images represented the formation of uniform monodispersed and spherical SeNPs that are stable for a minimum of half a year. It was also found that the majority of particles existed in the range from 15 to 20 nm and the average particle size was found to be 18 nm. Further, the stabilization of SeNPs in polysaccharides is prominent for food and medicinal applications.

Fig. 5 TEM morphological images of SeNPs

The calcium oxalate crystals morphologies are represented in Fig. 6. The images of crystals (I) to (IV) are represented in the Fig. 6a–d, respectively. The three-dimensional hexagonal crystals in Fig. 6a are the morphologies of crystals (I). The aggregation of crystals (II) to (IV) was reduced when compared with crystals (I) as shown in Fig. 6b–d. When the sub phases of supersaturated CaC_2O_4 were added with SeNPs, few of the crystals transformed to spherical two-dimensional hexagonal plate shaped crystals from three-dimensional hexagonal shape (as shown in Fig. 6d).

Further, there is a need to study the influence of SeNPs solutions concentration on the morphologies of CaC_2O_4 crystals. The SeNPs influence at various absorption peaks on the morphologies and the evolution of calcium oxalate crystals was represented in Fig. 7. When the SeNPs solutions concentration was 1 $\mu\text{mol L}^{-1}$, maximum number of the crystals were three-dimensional hexagonal in shape (as shown in Fig. 7a). While the SeNPs solutions concentration was of 5 $\mu\text{mol L}^{-1}$, few of the particles were aggregated and these NPs were two-dimensional whereas the rest were well separated (Fig. 7b). In addition, while the SeNPs solutions concentration was of 30 $\mu\text{mol L}^{-1}$, majority number of the particles was isolated (as shown in Fig. 7c). With the increase in SeNPs solutions concentration, there was a decrease in the aggregation of CaC_2O_4 crystals (as shown in Fig. 7a–c). All these results represented that the chemical changes in the morphologies of CaC_2O_4 crystals by SeNPs revealed the effects of concentration.

Conclusions

In this work, we have showed the green synthesis of SeNPs using the *O. tenuiflorum* plant extract as reducing agent. Also, we studied the effect of SeNPs on the chemical transition of CaC_2O_4 crystal's morphology using SEM. SeNPs inhibited the aggregation and growth of CaC_2O_4 monohydrate (COM) crystals and hence the prepared SeNPs could have important prospects in medical and pharmaceutical applications as a potential inhibitor of CaC_2O_4 urinary stones.

Fig. 6 Ca_2O_4 crystals SEM images (a, b) indicates crystals (I–IV), respectively

Fig. 7 Ca_2O_4 crystals SEM images in the SeNPs presence (a–c) where SeNPs = 1, 5 and 30 $\mu\text{mol L}^{-1}$, respectively

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References

- Clement IP (1998) Lessons from basic research in selenium and cancer prevention. *J Nutr* 128:1845–1854
- Cremonini E, Zonaro E, Donini M, Lampis S, Boaretti M, Dusi S, Melotti P, Lleo MM, Vallini G (2016) Biogenic selenium nanoparticles: characterization, antimicrobial activity and effects on human dendritic cells and fibroblasts. *Microb Biotechnol* 9:758–771
- Cui D, Yan C, Miao J, Zhang X, Chen J, Sun L, Meng L, Liang T, Li Q (2018) Synthesis, characterization and antitumor properties of selenium nanoparticles coupling with ferulic acid. *Mater Sci Eng* 90:104–112
- Dhanjal S, Cameotra SS (2010) Aerobic biogenesis of selenium nanospheres by *Bacillus cereus* isolated from coalmine soil. *Microb Cell Fact* 9:52–62
- Ganesan V (2015) Biogenic synthesis and characterization of selenium nanoparticles using the flower of *Bougainvillea spectabilis* Willd. *Int J Sci Res* 4:690–695
- Gautam PK, Kumar S, Tomar MS, Singh RK, Acharya A, Kumar S, Ram B (2017) Selenium nanoparticles induce suppressed function of tumor associated macrophages and inhibit Dalton's lymphoma proliferation. *Biochem Biophys Rep* 12:172–184

- Husen A, Siddiqi KS (2014) Plants and microbes assisted selenium nanoparticles: characterization and application. *J Nanobiotechnol* 12:1–10
- Johnson JA, Saboungi M-L, Thiyagarajan P, Csencsits R, Meisel D (1999) Selenium nanoparticles: a small-angle neutron scattering study. *J Phys Chem B* 103:59–63
- Knekt P, Marniemi J, Teppo L, Heliövaara M, Aromaa A (1998) Is low selenium status a risk factor for lung cancer. *Am J Epidemiol* 148:975–982
- Li Q, Chen T, Yang F, Liu J, Zheng W (2010) Facile and controllable one-step fabrication of selenium nanoparticles assisted by L-cysteine. *Mater Lett* 64:614–617
- Rayman MP (2000) The importance of selenium to human health. *Lancet* 356:233–241
- Rayman MP (2005) Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proc Nutr Soc* 64:527–542
- Shin Y, Blackwood JM, Bae I, Arey BW, Exarhos GJ (2007) Synthesis and stabilization of selenium nanoparticles on cellulose nanocrystals. *Mater Lett* 61:4297–4300
- Sireesh Babu M (2017) Green synthesis of biocompatible Au–Cu₂–xSe heterodimer nanoparticles and their in vitro photothermal assay. *Environ Toxicol Pharmacol* 53:29–33
- Sireesh Babu M, Badal Kumar M, Shivendu R, Nandita D (2015) Diastase assisted green synthesis of size controllable gold nanoparticles. *RSC Adv* 5:26727–26733
- Sireesh Babu M, Badal Kumar M, Kiran Kumar A (2017a) Environment friendly approach for size controllable synthesis of biocompatible silver nanoparticles using diastase. *Environ Toxicol Pharmacol* 49:131–136
- Sireesh Babu M, Badal Kumar M, Kiran Kumar A (2017b) Tyrosine assisted size controlled synthesis of silver nanoparticles and their catalytic and in vitro cytotoxicity evaluation. *Environ Toxicol Pharmacol* 51:23–29
- Sireesh Babu M, Jegatheeswaran S, Serap Suzu K Y, Guobin H, Yurong C, Junkuo G, Qingqing N, Yao Juming (2018) Silk sericin induced fabrication of reduced graphene oxide and its in vitro cytotoxicity, photothermal evaluation. *J Photochem Photobiol B* 186:189–196
- Sofer Z, Bartůňek V, Junková J, Ulbrich P, Babuněk M, Kuchař M (2016) Synthesis of spherical amorphous selenium nano and microparticles with tunable sizes. *Micro Nano Lett* 11:91–93
- Srivastava P, Kowshik M (2016) Anti-neoplastic selenium nanoparticles from *Idiomarina* sp. PR58-8. *Enzyme Microb Technol* 95:192–200
- Stuke J, Zingaro RA, Cooper WC (1974) Selenium. Van Nostrand Reinhold, New York, p 177
- Wang T, Yang L, Zhang B, Liu J (2010) Extracellular biosynthesis and transformation of selenium nanoparticles and application in H₂O₂ biosensor. *Colloids Surf B* 80:94–102
- Xia YY (2007) Synthesis of selenium nanoparticles in the presence of silk fibronin. *Mater Lett* 61:4321–4324
- Yu B, Liu T, Du Y, Luo Z, Zheng W, Chen T (2016a) X-ray-responsive selenium nanoparticles for enhanced cancer chemo-radiotherapy. *Colloids Surf B* 139:180–189
- Yu B, You P, Song M, Zhou Y, Yu F, Zheng W (2016b) A facile and fast synthetic approach to create selenium nanoparticles with diverse shapes and their antioxidation ability. *New J Chem* 40:1118–1123
- Zeng H, Combs GF (2008) Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion. *J Nutr Biochem* 19:1–7
- Zhang W, Chen Z, Liu H, Zhang L, Gao P, Li D (2011) Biosynthesis and structural characteristics of selenium nanoparticles by *Pseudomonas alcaliphila*. *Colloids Surf B* 88:196–201