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Green Synthesis of Selenium Nanoparticles from Broccoli, Characterization, Application and Toxicity

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Abstract

Plant-based diets and phytochemicals present in plants can be used to decrease the risk of cancer. This article deals with Brassica species and broccoli in particular which are associated with reduced risk of several important cancers. These plants mainly contain selenium (Se) covalently bound in a number of different chemical forms which is mainly responsible for its biological activity. Further, several enzymes contain selenium in the form of the unusual selenocysteine amino acid which act on free radicals. Broccoli has the ability to accumulate Se many-fold beyond the concentration of Se present in the soil. Reflecting on the above it can be suggested that development of methods to increase the natural accumulation of Se in broccoli may greatly enhance its health-promoting properties. The present article details about green synthesis of selenium nanoparticles and their methods of characterizations. Green synthesis is a biological procedure which can be achieved can be achieved by using plant extracts. This method is capable of producing SeNPs in a size range of about 50-150 nm, under ambient conditions. It was found that SeNPs are able to inhibit the cell growth by dose-dependent manner. In addition, combination of SeNPs and doxorubicin shows better anticancer effect than individual treatments. The present review also brings to light about the causes and implications of toxic effects posed by Se nanoparticles as well as applications of Se nanoparticles in medicine.

Keywords: Selenium; Nanoparticles; Nanotechnology; Green synthesis; Broccoli

Introduction

In the twenty-first century nanotechnology has become one of the most promising approaches for innovations that lead to fulfilment of the human needs. Research on the synthesis of Nano sized material is of great interest because of their unique properties like optoelectronic, magnetic, mechanical, photo responsive, catalytic properties [1-4] and biomedical application [5,6] which differs from bulk. In nanotechnology, a particle is defined as a small object with size ranges between 1 and 100 that behaves as a whole unit in terms of its transport and properties.

NP's are mainly used in the drug delivery system as their particle size and surface characteristics can be easily manipulated to achieve both passive and active drug targeting. Further, they can be used to provide control and sustain release of the drug during the transportation at the site of action. They alter organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects. We have focused on Se mainly because of its unique properties and great potential applications.

Selenium is one of the essential trace elements in the body in due to its anti-oxidative as well as pro-oxidative effect and has great importance in nourishment and medicine [7]. Se has one of the narrowest ranges between dietary deficiency (40 lg day^{-1}) and toxic levels (400 lg day^{-1}). The Se field is expanding at a rapid pace and has grown dramatically in the last years. Selenium is a key player in cellular metabolism, an essential component of enzymes that protect the body against free radical species and has important roles in metabolism of thyroid, human fertility and many other vital functions. All aspects of Se in biology have advanced in various fields such as genetic, biochemical, molecular, and health areas. Many stable organic selenium compounds have been successfully synthesized which are used as antioxidants, enzyme inhibitors, anti-tumor, anti-infective agents, cytokine inducers and immuno-modulators [8,9]. Nanoparticles of selenium act as a potential chemo-preventive agent with reduced toxicity [10-12]. For example it has been reported that the redness selenium nanoparticles has high biological activities and low toxicity [13,14]. Thus selenium nanoparticles caused the great interest of researchers and a variety of synthesis methods have been exploited [12].

Se Nanoparticles from Plants

Plants containing Se may be grouped into two broad categories:

- Those that accumulate Se in direct proportion to the amount of Se available from the soil (e.g. wheat)
- Those that actively accumulate Se in orders of magnitude greater than the Se concentrations in the soil (e.g. *Astragalus* spp.) [15].

Different plant species often contain different chemical forms of Se, and the chemical form of Se often determines its bioactivity [16]. Forms of Se can be safely stored in membrane-bound structures within the plant. A key enzyme necessary for synthesis of many of these methylated compounds is selenocysteine-specific methyltransferase.

Selenium in broccoli

Broccoli belongs to the Brassicaceae family, originated in the Mediterranean regions and distributed in Europe and in the United States. Broccoli is rich in micronutrients such as carotene, vitamin C and folic acid-fibers, phytochemicals such as glucosinolates (GLs) and

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polyphenols [17,18]. These phytochemicals are responsible for the induction of phase-2 detoxication enzymes as well as other antioxidant activities [19,20]. The phytochemicals in broccoli have recently gathered great interest in their potential for the maintenance of human health. Isothiocyanates (ITCs), obtained from myrosinase hydrolysis of GLs (by chewing, cutting or processing the vegetable), are mainly responsible for the protective effects of Brassica vegetable [21,22].

Broccoli is a Se accumulator [23] and is known to contain fairly [24] large quantity of selenium as methyl selenocysteine. Broccoli may contain Se in a form that is especially chemo protective against certain cancers. Se from broccoli does not accumulate efficiently in man or rats [25,26] because it's major part as selenium methyl selenocysteine is perhaps metabolised to methyl selenol and enters the excretory pathway [27,28]. Experiments have revealed that methyl substituted forms of Se is an effective anticancer agent than the other derivatives of organo-Se compounds [29]. Numerous studies have shown that consumption of cruciferous vegetables lead to a consequent decrease in the incidence of many cancers [30]. Decrease in the risk of bladder cancer is also directly related to broccoli intake [31]. The effectiveness of Se in broccoli against cancer, the inclusion of many other anticancer compounds, and the general acceptability of broccoli by the public should make this vegetable an excellent source of supplemental dietary Se.

Therefore, there is renewed interest in the development of new generation of pharmaceuticals derived from natural products. Based on our pioneering efforts in green nanotechnology and Nano medicine, Chanda et al. [32], Katti et al. [33], Shukla et al. [34], Mukherjee et al. [35], are exploring to enhance the therapeutic payloads of biologically active phytochemicals in broccoli. Nanotechnology in herbal medicine would therefore allow storage, efficient transport and delivery of reservoir of phytochemical cocktails into the cellular matrix.

Green Synthesis of Selenium Nanoparticles

The complete understanding of the synthesis mechanism of nanoparticles using the biological agents has not been devised. The biological synthesis mechanism include both intra and extracellular of nanoparticles which are different for various biological agents and different biomolecules responsible for the synthesis of these nanoparticles. Biological agents used for nanoparticles synthesis represent mainly microbes including bacteria, fungi, algae and yeast and plants which react differently with metal ions [13].

Biochemicals can be used for the synthesis of nanomaterials, however the biogenic synthetic route is frequently used due to its ease and simplicity. In addition there are no hazardous and toxic residues released in the environment [36,37]. It has been established that the Se nanoparticles prepared from biological material are less toxic than the bulk Se nanoparticles prepared from chemicals. The biomolecules present in the extract act both as reducing agent and stabilizers of Se nanoparticles. Green synthesis of selenium nanoparticles from selenious acid was achieved by dried extract of raisin (*Vitis vinifera*) [38] (Figure 1).

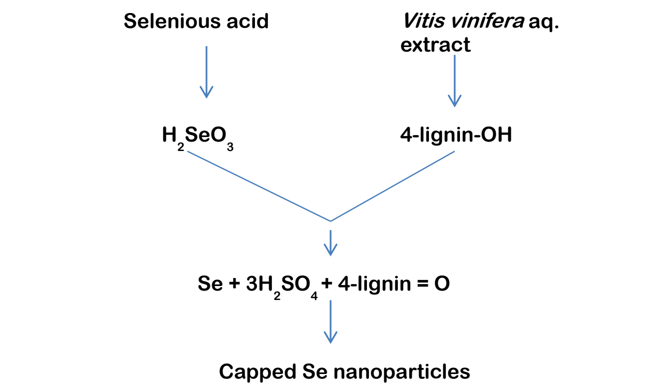

Figure 1: Se nanoparticle synthesis using *Vitis vinifera* [40].

A variety of Se nanoparticles can be produced by using H_2SeO_3 (thiosulfuric acid) to treat plant extracts for example, α-Se nanoparticles have been generated from *Capsicum annum* extract in aqueous medium at low pH and at optimum temperature [39]. The light green extract of *C. annum* turns pale after 5 h of the addition of H_2SeO_3 and then gradually turned red after 12h. This red colour is the characteristic indication of Se nanoparticles. The Se nanoparticles synthesized from fenugreek seed extract in aqueous medium at room temperature are between 50-150 nm and have been found to be active against human breast cancer cells [41].

Se Nanoparticles Characterization [42]

An initial characterization of the test substance is imperative before any toxicity screening is commenced. A more extensive and complete characterization, including size distribution, shape, surface area, surface chemistry, crystallinity, porosity, agglomeration state, surface charge, solubility, etc., is recommended for nanomaterials in order to determine the correct correlation between their physicochemical properties and the biological effects they elicit [43-46].

X ray diffraction (XRD)

The confirmation of formation of Se nanoparticles can be achieved from the energy dispersive x-ray spectroscopy. The XRD analysis can be used to analyse the morphology of Se nanoparticles. The particle size is calculated by using Debye Scherrer equation: $Dp=0.9\lambda/\beta \cos\theta$, Where Dp corresponds to the particle width in A°, λ is the X ray wavelength, θ is the Bragg angle, and β corresponds to the full width at half maximum (fwhm, in radians) of the peak under consideration. When the samples are heated to 200°C for 3 h, the peaks become well-defined and sharp due to increased crystallinity [47-50].

SEM (scanning electron microscope) and TEM (transmission electron microscope)

SEM and TEM images of the Se are taken for the analysis of size and shape of SeNPs. TEM/SEM analysis is generally performed in vacuum. Moreover, it requires much less laboratory space than TEM/SEM and is simpler to operate. A closer look at the highly magnified field emission scanning electron microscopy (FESEM) image are useful in suggesting the composition of the nanoparticles as well as to observe the nature of the surface of the nanoparticles. It is quite likely that Se nanoparticles are held by van der Waals forces.

Fourier transform infrared spectroscopy (FTIR) analysis

For FTIR measurements, the air-dried powder form of the samples is grinded with KBr pellets and analyzed on a Thermo Nicolet spectrum instrument in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}. The peaks obtained were plotted as % transmittance in X axis and wave number (cm^{-1}) in Y axis.

UV-Vis absorbance spectroscopy analysis

Sample is taken in aliquots (2 ml) and the bio reduction of the selenious acid in solution is monitored periodically and measured the UV-Vis spectra of the solution with a Shimadzu 1,700 UV-Vis spectrophotometer at wavelength ranging between 200 and 1,000 nm with a scanning speed of 1,856 nm/min. The readings are recorded at 5, 30, 60, 180, 720 and 1,440 min.

Brunauer-emett-teller (BET) method

The BET method is typically used to calculate the surface areas of solids through the physical adsorption of gas molecules onto the solid surface. It involves adsorbing a liquid nitrogen monolayer onto the surfaces of particles and then measuring the amount of nitrogen released upon vaporizing that layer. Thus, the BET surface represents the surface area that is freely accessible to gases. The primary particle diameter (assumed to be the equivalent sphere diameter) is then calculated from the specific surface area and the density of the particles-data that are already available [51,52].

Atomic force microscope

An atomic force microscope (AFM) is a cost effective instrument that has several advantages in the characterization of nanoparticles.

Qualitative analysis: Using the AFM, individual particles as well as groups of particles can be resolved. The AFM offers visualization in three dimensions.

Quantitative analysis: quantitative information from individual or groups of nanoparticles can be generated by using software based image processing of AFM data. For individual particles, size information (length, width and height) and other physical properties (such as morphology and surface texture) can be measured [53].

Dynamic light scattering (DLS)

DLS measures time dependent fluctuations in scattering intensity produced by particles in Brownian motion. Using the Stokes-Einstein relation, DLS helps in the measurement of the particle size. The size obtained by DLS is usually greater than that measured by other techniques, like TEM, BET, etc. This is because DLS measures Brownian motion and the subsequent size distribution of an ensemble of particles in solution and yields the mean hydrodynamic diameter, which is usually larger than the BET or TEM diameter [37]. During DLS measurements, there is a tendency of particles to aggregate in the aqueous state, so this method gives the sizes of aggregates rather than individual particles. DLS reports an intensity weighted average hydrodynamic diameter of a collection of particles, so any sample polydispersity will skew the average diameter towards larger particle sizes [54].

Nanoparticle tracking analysis (NTA)

A more recently developed system based on the Brownian motion of nanoparticles is known as nanoparticle tracking and analysis (NTA). This helps in the visualization of nanoparticles individually with simultaneous analysis of their Brownian motion. The particle size distribution can be obtained on a particle-by-particle basis which offers higher resolution and therefore a better understanding of aggregation as compared to methods like DLS. It avoids any intensity bias towards large particles that could result in a small number of large particles/agglomerates masking the presence of a greater number of nanoscale particles, as seen with other light-scattering techniques (e.g. DLS). NTA can be used to identify and count nanoparticle aggregates/agglomerates due to its ability to visualize the particles individually [55].

Applications of Selenium Nanoparticles

Nanoparticles have proved to be a means of providing enhanced therapeutic bioavailability of drugs and also targeting therapeutic agents to particular organs. This has been achieved due to the high surface reactivity and advantage of size effect of nanoparticles [56]. It has been experimentally proved that nanoparticles showed new characteristics of transport and uptake and exhibited higher absorption efficiencies [57].

It is well documented that Se contains properties that make it a unique element relative to other metals and metalloids. For several reasons, researchers chose Se because of its application in ecological, ecotoxicological, and radio ecological sciences. Se may exert diverse beneficial effects at low concentrations including growth promoting activities of higher plants [58-61], tolerance of plants by enhancing their antioxidative capacity [62-64] and increasing plant resistance against oxidative stress [65-68].

Different Se forms including organic and some salts have been used in studying its biological effects several years ago, whereas recently, nanoparticles of elemental selenium (SeO) have gained the attention as a possible source of this beneficial element [69]. However, there is little data on intestinal absorption and Se retention of nano-Se [70]. Nano-Se, which is bright red, soluble, highly stable, and nano-defined size in the redox state of zero (Se0), has been manufactured for use in both of the nutritional supplements and developed for applications in medical therapy [71]. It is reported that nano-Se at 20-60 nm had a similar bioavailability to sodium selenite [72]. It was suggested that the biological activities of nano-Se may come from the special properties of these nanoparticles and demonstrated that nano-Se has comparable efficacy to selenite in up-regulating selenoenzymes and Se levels in tissue, but is less toxic [73,74]. These results indicated that nano-Se can serve as an antioxidant with reduced risk of Se toxicity [75].

Toxicity of Selenium Nanoparticles

Since smaller nanoparticles have higher surface area and particle number per unit mass in comparison to larger particles thus they may lead to adverse effects. Even toxicological studies have shown that small nanoparticles (<100 nm) cause adverse respiratory health effects, causing more inflammation than larger particles made from the same material [76-80]. The body will react differently to the same mass dose consisting of billions of nanoparticles compared to several micro particles. Larger the surface area more is the reactivity [81] and this leads to an increased source of reactive oxygen species, as demonstrated by in vitro experiments [82].

The toxicity of nanoparticles may be determined by their aggregation as it has been noted that a more effective macrophage clearance is observed in the case of larger particles compared to smaller ones. This leads to reduced toxicity of nanoparticle aggregates larger than 100-200 nm [83]. High concentration of nanoparticles would induce particle aggregation [83] and this would subsequently cause reduce toxic effects compared to lower concentrations of nanoparticles [84]. Most aggregates are observed to be larger than 100 nm which is assumed to be a threshold for many of the adverse health effects of small particles.

Concerning the phytotoxicity of selenium, Se at higher concentration more than 1 mg/kg is considered toxic for most of the plants and gets accumulated in the plant tissues to affect the cellular metabolism. It is found that plants growing on seleniferous soils showed chlorosis, reduced growth and low yields as well as senescence [85]. Due to the phytotoxicity of Se, it causes generation of oxidative stress and other metabolic abnormalities as Se replaces S in proteins and other molecules resulting in their functional impairment [86]. Concerning Se detoxification mechanisms, metallothioneins and total thiols as well as glutathione-s-transferase activity are involved, which increased at lower Se concentrations, whereas at excessive Se concentrations markedly decreased. At low Se concentrations, a rise in these molecules possibly decreased the toxic effects of Se by conjugating it and/or scavenging the reactive oxygen species arising due to Se stress [87], which are in conformity with earlier findings on the Se tolerance mechanisms [88]. At high Se concentrations, all these mechanisms probably fail or become limited due to overall cellular metabolic dysfunction, which may hinder the growth of stressed plants.

Intake of selenium higher than the upper limit range 350-400 μg/d [89,90] of the RDA is also of major concern to humans and animals since it can result in selenium toxicity or selenosis. Upon ingestion of 17.2 μg/mL selenium due to incorrect dosage in animal feed, pigs showed signs of acute selenium toxicity, including paralysis, hyperesthesia, anorexia, and tremors [91,92]. Signs of acute selenosis in buffalo include anorexia, alopecia, mild convulsions and lowered body temperature [93,94]. Symptoms of chronic selenosis include hair loss, deformation or cracks on the skin, horns, and hooves of animals, resulting in the sloughing of hooves and staggering [95]. In humans, signs of selenosis include garlic breath, hair and nail loss, thickened and brittle nails, teeth deformation, skin lesions and lowered haemoglobin levels upon dietary selenium intake of 5 mg/d.

Selenium Deficiency

As inorganic forms of selenium effectively increase selenium levels in plant crops and prevent selenium deficiency diseases in people that consume them, it is important to understand the effect of selenite and selenate supplementation in crops. Plants more efficiently absorb selenate, as indicated by the higher concentrations of selenium in plants supplemented with selenate as compared to selenite supplementation, but selenite may be safer to use in fertilizers, since there are fewer adverse effects with supplementation at high concentrations [96]. To better treat selenium deficiency and to prevent selenium toxicity, an accurate evaluation of the effects of inorganic selenium compounds in fertilizers is required. Selenite and selenate-enriched pastures and salt licks are also used to increase selenium concentrations in livestock [97]. It is therefore also important to understand the effects of this supplementation on animals, and further studies are necessary to determine the appropriate levels and forms of inorganic selenium supplementation that are most effective.

In humans, selenium deficiency causes poor immune response by reducing T-cell counts and impairing lymphocyte proliferation and response [98]. Studies have shown that human supplementation of 200 μg/d of sodium selenite over an eight-week period resulted in enhanced T-lymphocyte response [99]. In HIV and AIDS patients, selenium deficiency is associated with decreased immune cell count, higher rates of disease progression, and increased risk of death [100,101]. Additionally, selenium was found to protect cells from oxidative stress, resulting in slower progression of this disease [102]. Numerous studies indicate that selenium also plays an important role in chemotherapies [103,104]. In a random, double-blind cancer prevention trial, the incidence of prostate cancer was reduced by 63% compared to the placebo group upon selenium supplementation of 200 μg/d as selenium-enriched yeast. Similar studies also showed a noticeable decline in lung and colorectal cancers, as well as in total cancer mortality rates [105,106]. In a separate trial, patients with uterine cervical carcinoma were found to have low glutathione peroxidases and selenium levels [106].

Conclusion

Selenium, the essential poison, is becoming more and more insufficient in food crops. It is biologically essential because it is an essential constituent of several enzymes as well as proven to reduce the risk of many cancers. This essentiality of this nutrient was first recognized in the late 1950s. Since that time, Se has become a subject of several investigations in many parts all over the world. This review focuses on the plant nutrition of selenium and nano-Se including Se bioavaialability, Se phytotoxicity and deficiency in humans, Se application methods, and nano-selenium in plant nutrition and in cancer treatments. The complete understanding of Se and nano-Se metabolism in plants requires that more detailed biochemical studies. Molecular studies and the overexpression of genes encoding proteins involved in the uptake, transport, and assimilation of both Se and nano-Se will expand our understanding of the close relationship between these two Se forms. The appropriate concentration of exogenous Se and nano-Se is still a matter of intensive research. The importance, production, and biological effects of this element and its use in the sustainable development were still an attractive issue. On the other hand, Se nanoparticles can be synthesized using different chemical, physical, and biological methods. The biological method is an environmental friendly method compared with other methods.

Investigating the antioxidant and anticancer properties of inorganic selenium and oxo-sulfur compounds in the treatment of diseases such as cancer, aging and neurodegenerative diseases generated from reactive oxygen species is an active and promising area of research. In the case of inorganic selenium compounds, sodium selenite is the compound of choice for both antioxidant and anticancer studies. While the mechanism for its antioxidant activity is unclear, it has been proposed that the ability of selenocysteine containing enzymes such as GPx and thioredoxin reductases to prevent radical formation as a possible mechanism [106].

Broccoli is a popular vegetable, and consumption of broccoli also reduces cancer risk. Broccoli has the ability to accumulate Se and to convert it into a form that is especially chemo protective against cancer. There is also evidence that other substances in broccoli may turn on selenoproteins that may regulate cell growth. Development of agricultural methods for broccoli varieties that can increase the accumulation of Se under natural agricultural conditions may further enhance the health-promoting properties of this already beneficial vegetable.

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