SELENIUM NANOPARTICLES: PRODUCTION, CHARACTERIZATION AND POSSIBLE APPLICATIONS IN BIOMEDICINE AND FOOD SCIENCE

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Nowadays, an important research direction in biotechnology is considered the nanoparticles production by controlling the size and morphology using physical, chemical or biological methods. 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. 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 algae, bacteria, fungi, viruses, yeasts and plants in a number of biochemical and biophysical processes. The biological synthesis via nanobiotechnology processes have a significant potential to boost nanoparticles production without the use of harsh, toxic, and expensive chemicals commonly used in conventional physical and chemical processes. On the other hand, biosynthesis of selenium nanoparticles by different plant extracts or different bacteria strains aiming to produce selenium nanoparticles, demonstrated that each bacteria strain (or vegetal source) produce selenium spheres indifferent size ranges. 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. By chemical route, elemental nano-selenium 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. In the present work, the synthesis route, the structure and morphology of selenium produced by various methods, are described, on the basis of our recent developments. Possible applications of selenium nanoparticles in biomedicine and food technology are discussed.

Keywords: selenium nanoparticles; biosynthesis; hydrothermal reaction; SEM; TEM; DLS.

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1. Introduction

Selenium is an element whose trace amounts are essential for life and has gained a considerable attention in both human, technical and agricultural field of science due to its piezoelectric, photoelectrical, semiconducting, catalytic activities but also its bioactivity [1, 2]. In mammals, at least30 selenoproteins have been identified and it has been found that humans have 25 selenoproteins that play important roles in the body [3]. Increasing deficiency of selenium in humans and animals was confirmed in geographical regions where soils are characterized by low content of this micro nutrient. Selenium deficiency leads to degeneration of many organs and tissues, for example disorders related to heart muscle and joints, prostate cancer, thyroiditis or astma [4]. On the other hand, the synthesis of nanosized materials has received great interest because of their unique properties, which differ largely from those of the bulk materials. Owing to the versatile properties of selenium, as well as the quantum-size effect, nano-selenium was also being considered as an excellent material, which could be used for producing novel devices with new types of applications or greatly enhancing the performance of existing devices [5-12].Selenium nanoparticles (SeNPs) have been exploited for medical purposes such as antimicrobial, antifungal, antidiabetic and anticancer agent [13-16], but these nanomaterials have also been reported to be used in nanowire electronics, sensors, and more-efficient solar cells [17].

Chemical synthesis ofelemental nano-selenium employs the reduction of a selenium salt with a reducing agent, usually in the presence of a stabilizing agent to prevent the clusters of selenium atoms from growing and to obtain stabilized nanoparticles in colloidal suspension. Many other strategies, including physical evaporation approaches, hydrothermal processes, gama-radiolytic reduction and sonochemical processes, have been employed as the syntheticprocesses [18-20].On the other hand, biogenic synthesis of Se nanoparticles is frequently achieved by reduction of selenate/selenite inpresence of bacterial proteins or plant extracts containing phenols, flavonoids amines, alcohols, proteins and aldehydes [21]. Over 16 different species of bacteria and viruses have been found to reduce colourless selenite and selenite to red elemental Se of different shape and size [22,23]. Plants, fungi and microbes may act as producers and protectors of the environment when they are properly used. Bacteria, 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.

Selenium may exist in many crystalline and amorphous forms, but the shape, size and structure of the selenium nanoparticles depend on different factors like the concentration, temperature, nature of biomolecules and pH of the reaction mixture. For this reason, the properties of Se nanoparticles varies with size and shape. For example, Se nanosphereshas been proved to have high biologicalactivity and low toxicity while Se nanowires have high photoconductivity [18,23].

In the present work, the structure and morphology of SeNPs produced by both hydrothermal synthesis and biological reduction, were characterized using SEM/TEM (transmission/scanning electron microscopy) and DLS (dynamic light scattering). According to their properties in terms of morphology, size and shape, possible applications of SeNPs in biomedicine and food technology are discussed in the present paper.

2. Bacteria assisted selenium nanoparticles

The synthesis mechanism of Se nanoparticles using the biological agents is still under consideration and not fully understood. Biological agents used for nanoparticles synthesis are mainly microbes including bacteria, fungi, algae, yeast and plants [24-26]. The biological methods used for nanoparticles synthesis include both extra- and intra-cellular methods. In addition, thesynthesis mechanism for both intra- and extracellular ofnanoparticles is totally different in various biological agents, because these biological agents react differently with metalions and also there are different biomolecules responsible forthe synthesis of these nanoparticles [21]. Both selenite and selenite forms of selenium salts are able to act as precursors in bio-synthesis of selenium nanoparticles. Microorganisms reduce the toxic, selenate and seleniteoxoanions into nontoxic elemental selenium which isinsoluble in water. Different types of the Se nanoparticles were reported to be synthesized from *Rhizobium selenireducens sp*., *Dechlorosoma sp., Pseudomonas sp., Paracoccus sp*., *Enterobactor sp*., *Thaurea sp.,Sulfurospirillium sp*., *Desulfovibrio sp*.,*Shewanella sp.,Bacillus sp*.[27-29].

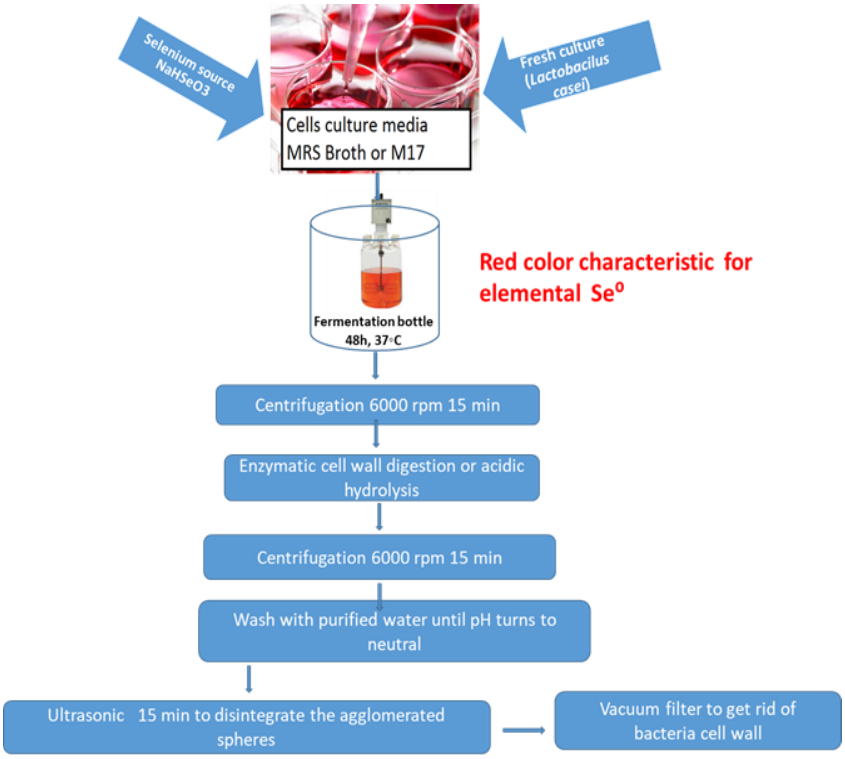
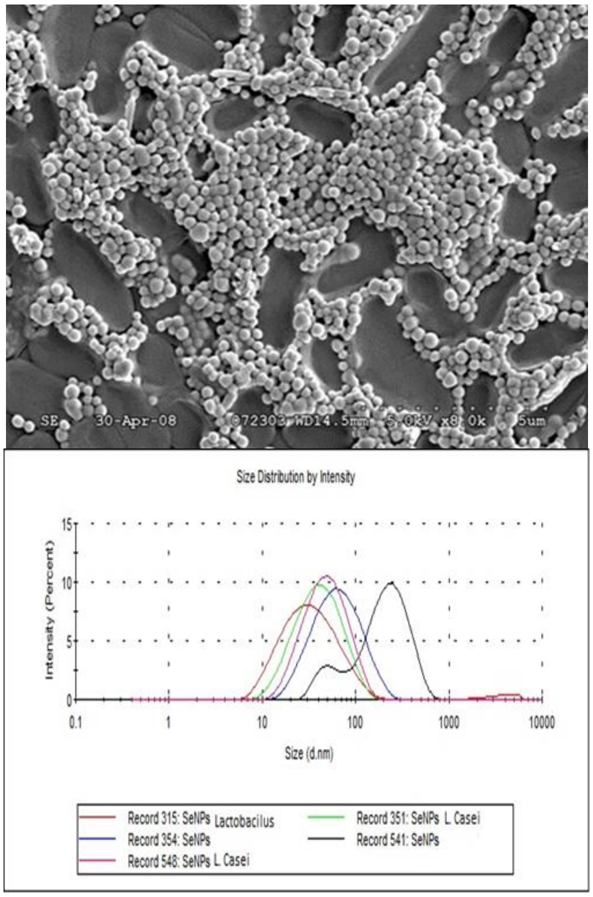


Fig. 1 Flow chart of SeNPS production using $NaHSeO_3$ and *Lactobaciluscasei* as starting biomaterials.

In fig. 1 is presented the flow chart of SeNPS production using sodium selenite as precursor and *Lactobaciluscasei* as biological reducing agent. By this procedure, we were able to synthesize perfectly spherical shape SeNPs, with the maximum size distribution ranging from 40 nm to 200 nm, depending on the experimental conditions (temperature, pH, reactants concentration). The morphology and DLS analysis of as prepared SeNPS is presented in fig. 2(a,b).

a



b

Fig. 2SEM morphological aspect (a) and DLS analysis (b) of SeNPs synthesized using *Lactobaciluscasei* as microbiological reducing agent (adapted from reference [1]).

The mechanism of nano-selenium formation in this case is related to SeO_3^{2-}reduction that can be catalyzed by reductases, includingthe periplasmic nitrite reductase, sulfite reductase or dimethyl sulfoxide (DMSO) reductase. A number of thiol-mediated reactions have also been observed to reduce selenite to elemental selenium [1, 30]. Microbial reduction of Se may not only be exploited in Se bioremediation but also in the production of selenium nanoparticles for biotechnological applications; however, the mechanisms involved in the formation of nanoparticles and their physical and chemical properties are yet to be fully elucidated.

3. Synthesis of Se nanoparticles using saccharide reducing agents

The development of a simple, efficient and green strategy for the production and stabilization of SeNPs has attracted increasing attention, because biomacromolecules, especially polysaccharides, have been used recently for the preparation and stabilization of well-dispersed SeNPs in aqueous medium [31]. Chitosan, glucose and starch are the most frequently used saccharide for metallic nanoparticles production. The size of the produced NPs with this method could be controlled in the range of 10–200 nm, in a hydrothermal process, achieving interesting assembles of particles, such as cubes, triangles, wires and spheres. Different types of saccharides with different molecular properties and chain conformations were used as stabilizer and capping agent to fabricate stable and water-dispersible selenium nanoparticles [32].

In fig. 3, the flow chart of SeNPs production via different saccharide as reducing agent is presented.

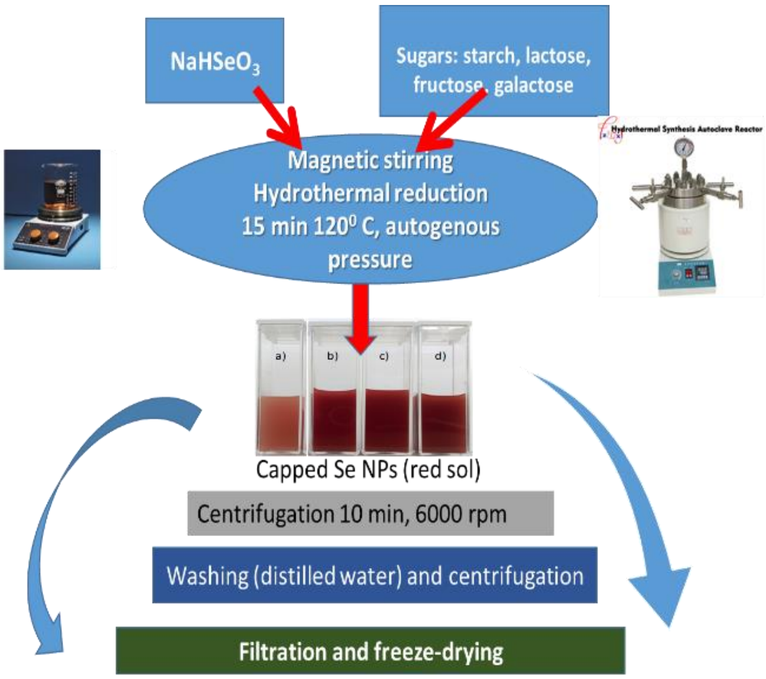
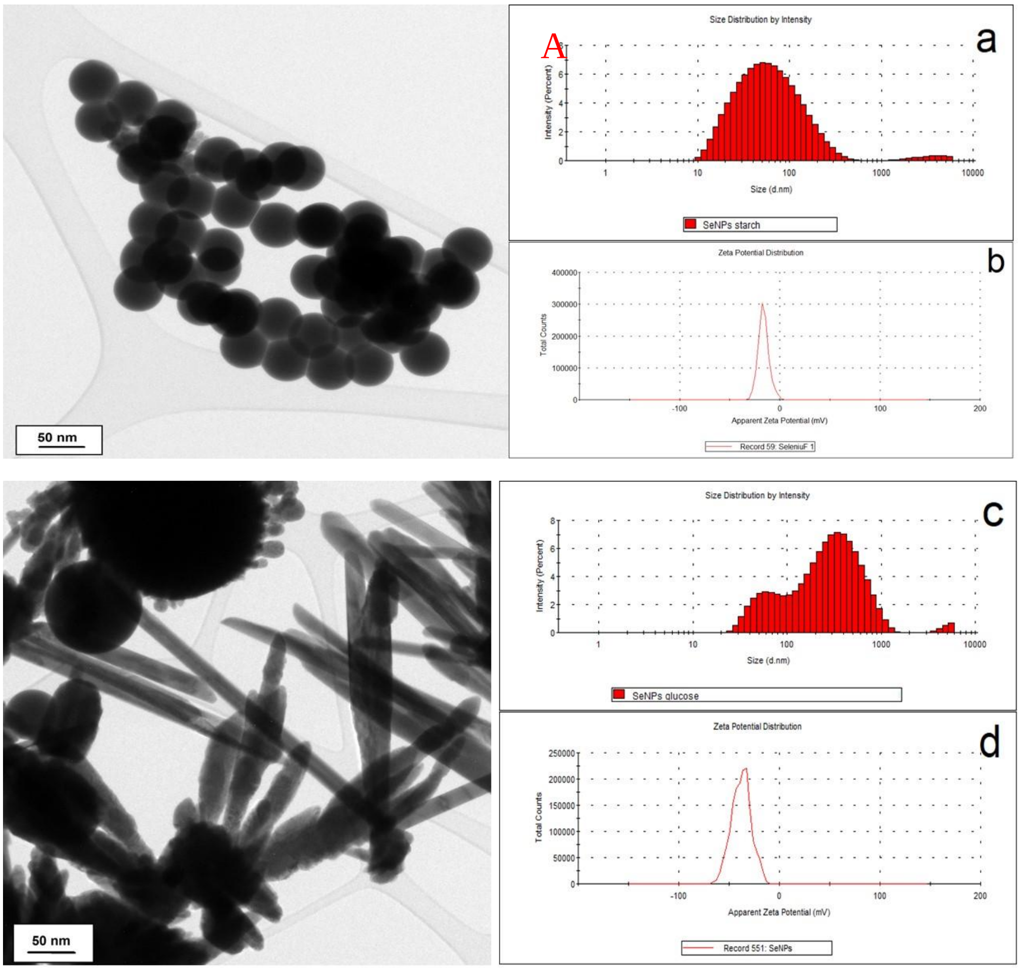


Fig. 3 The flow chart of SeNPS production in a hydrothermal reaction, using $NaHSeO_3$ as precursor and different saccharides as reducing agent.

In our study, we used both polysaccharide(starch) and mono-saccharide (glucose and galactose) as reducing agents in reaction with sodiumhydrogen selenite precursor. The reaction occurred rapidly in alkaline solution and displayed a time-dependent color change during the heating procedure, in all the three cases, but the final red color of the selenium sol remained stable for longer time (30 days) [33].The formation of nanoparticles was confirmed by laser diffraction (DLS), revealing that particle size distribution obtained from each highly dispersed mixture was maximum 55 nm in the case of starch, 50 nm and

290 nm for glucose, and 35 nm for galactose reducing agent. Moreover, these nanoparticles were very stable, the zeta potential measurement indicates negative values, from -22 mV to -37 mV. 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.



B

Fig. 4. The morphology (TEM) of SeNPS produced in a hydrothermal reaction, using $NaHSeO_3$ as precursor and different saccharides as reducing agent: starch (A) and glucose (B), along with the respective DLS measurement (adapted from ref [5]).

As revealed by electron microscopy images (Fig. 4), the size and shape of selenium nanoparticles is strongly influenced by the type of saccharide reducing agent. By using starch (a polysaccharidesugar), a regular spherical shape was observed, with diameter of about 60 nm. In the case ofmono-saccharides, a different behavior was noticed, as glucose gave rise of a mixture of nano-rods (with the thickness of about 40 nm) and large spherical particles (with diameter ranging from 100 to about 200 nm). However, in all cases, the reducing agent was strong enough to ensure complete conversion of the precursor molecules into nano- sized selenium particles.

4. Possible applications of SeNPs in biomedicine and food science

Years ago, it was generally considered about elemental selenium to be biologically inert, but recently, some researchers proved that nano-Se has similar bioavailability to other selenium forms [1,34,35] and reported that nano-Se not only has a higher efficiency in up-regulating seleno-enzymes but also seem to be less toxic comparing to selenite. These results indicated that nano-Se can serve as an antioxidant with reduced risk of Se toxicity, showing a better absorption into plants, animals, humans and microorganisms. Moreover, one of the most important applications of selenium nanoparticles is its chemo-preventive property, by immunological stimulation [3, 11]. Hence, the challenge to use nano-Se as food supplements is related to find an appropriate matrix as floating microspheres, to obtain prolonged and sustained release of selenium in gastrointestinal tract. For this purpose, in a recent study, alginate and chitosan were chosen to prepare different formulations based on alginate and alginate/chitosan as controlled delivery matrices for nano-selenium [1].Nano-selenium maintained a low level of crystallinity within the final composites, which implies an advantageous formulation for the purpose of controlled release. These results demonstrated that nano-Se release depends upon the nature of the polymer matrix as well as pH of the media, the mixture alginate/chitosan being a convenient matrix to be used for nano-selenium delivery in duodenum, caecum and colon.

SeNP loaded with an anticancer molecule offer a new strategy for cancer treatment. In a recent study [27] it was reported application of anisomycin-loadedselenium nanoparticles (SeNP-Am) synthesized by conjugating anisomycin to the surface of SeNP for improving anticancer efficacy of anisomycin against human hepatocellularcarcinoma (HepG2) cells.

Se-enriched Lactobacillus had been reported as animmunostimulatory agent that can be used to increase the lifespan of cancer-bearing animals. Lactic acid bacteria can reduce Se ions to elemental SeNP and deposit them in intracellular spaces [36]. So, this property can be used in food science, in order to produce functional food designed as a potent antioxidant agent. Another application is related to the protective effect of SeNPs against toxicity with heavy metals like lead, cadmium and mercury or arsenic derivatives. Recent studies [37] investigated the role of specific probiotic bacterial strains (*Lactobacillus rhamnosus* and *Propionibacterium freudenreichii*) as cadmium and lead removal tools aiming to characterize the capacity of food grade probiotic strains to bind heavy metals and to identify factors that may affect the binding capacity such as pH, contact time and pretreatment and demonstrating the ability of S-layers to bind metal ions and its consequence on the secondary structure of the S-layer proteins. Other studies reported the protective effect of biogenic SeNP against arsenite-induced cytotoxic and genotoxic effect on human lymphocytes [38-40].

One of our novel approach was to made and characterized a nanostructured surface on titanium meshfor cranioplasty, by adhesion of selenium nanoparticles (SeNPs) in situ, in a hydrothermal

Reaction, revealing that selenium nanoparticles adherence on titanium mesh surface had the best result in the case of starch-derived SeNPs, as demonstrated by SEM/EDX analysis [5]. The proposed improvement of the surfaces in the case of titanium mesh for cranioplasty may offer important benefits in terms of osteointegration, without using additional screws for fixation and closure procedure. According to literature, adherence properties of SeNPs was also demonstrated when polymeric substrate was used [41,42]. Medical polymeric substrates can be coated with selenium nanoparticles in situ, aiming to develop a novel antimicrobial coating to inhibit bacterial growth on polyvinyl chloride, polyurethane and silicone based medical devices. The reduction of bacteria growth is directly correlated with the density of Se nanoparticles on the coated substrate surfaces. Moreover, nanoparticulate selenium surface is able to promote a better osteoblast adhesion onto the surface of orthopedic implants [43].

5. Conclusions and future perspectives

The bio-therapeutic relevance of SeNPs has emerged innovative opportunities for biotechnology and engineering in terms of the fabrication of superior and effective diagnostic, treatment and remedial devices for biomedical functions. The actual information permits the synthesis of new selenium redox active compounds that may be added to existing drugs, or targeted with specific molecules, such as monoclonal antibodies.The strategically promising solution toward improving Se’s efficacy andreducing its toxicity seems to be fulfilled by the novel approach of nanotechnology.On the other hand, nanoscience and nanotechnologies are very widely seen as vital sectors having huge potential to bring a lot of benefits, not only in the in the area of drug development, water decontamination, information and communication technologies, but also related to the production of stronger, reliable, durable and useful life-saving materials.

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