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The Formation of Selenium Nanoparticles Using Ascorbic Acid With the Support of Pomelo-Derived Pectin as a Green Stabilizer

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ABSTRACT

Selenium nanoparticles (Se NPs) were reported as a source of trace selenium element that is essential for biological activities and nutrition for health. However, their practical applications were hindered by the relatively low stability due to aggregation. In this research, Se NPs were synthesized by chemical reduction using ascorbic acid with the support of the surface capping agent of pectin. The resultant Se NPs possess a hydrodynamic diameter of 140.8 nm (PDI = 0.034) and zeta potential of -15.07 mV through the dynamic light scattering (DLS) method. They showed an absorbance peak at 270 nm, indicating the presence of surface plasmon resonance (SPR) or electronic transitions related to selenium and pectin, and the stability and monodispersed size distribution of the nanoparticles. Besides, SEM-EDX also confirmed the spherical morphology and the existence of selenium in the composition of Se NPs. In conclusion, the creation of Se NPs will open a platform for biomedical, cosmetics, and food supplement research with intensive future investigations by *in vitro* and *in vivo*.

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1. Introduction

Selenium is an indispensable candidate for the maintenance of health and growth because it is a trace element with a massive function in the biological system [1]. For human intake, the British Nutrition Foundation reported the range of 60-70 µg per day throughout our lives [2]. Its form at the nanoscale expresses safer and better platforms for biological activities, which are presented in many applications in anticancer, antioxidant, antibacterial, and so on. For instance, in 2024, a research group from Vietnam produced selenium nanoparticles using ascorbic acid as a reducing agent while employing *Tithonia diversifolia* pectin as a capping agent. They showed the size of 100-200 nm with an ear edema anti-inflammation of 34.8%, 43.3% and 46.7%, respectively, at 30, 60, and 90 min after injection [2]. Besides, in 2020, a selenium nanoparticle-lysozyme nanohybrid system was created to attain the potential antibacterial property against *Staphylococcus aureus*, with the MIC value found to be 82 µg/mL, which proved the feasible applications in medicine, biomedicine, food safety, and healthcare products of selenium nanoparticles [1]. Finally, in 2019, pectin-stabilized selenium nanoparticles were designed for loading curcumin and evaluated the physicochemical properties as well as biological activities. Pectin-based selenium nanoparticles and curcumin-loaded nanosystem got the size of about 61 and 119 nm, respectively, with an encapsulation efficacy of 60.6% (loading content ~ 7.4%) and 500-fold water solubility enhancement compared with free curcumin. The antioxidant, antitumor abilities of curcumin-loaded Se NPs were better than those of pectin-based Se NPs [3].

As pointed out in much research, aggregation is a core reason that depresses the use of nanoparticles in biological systems. Therefore, it is crucial to maintain the stability of nano selenium for fully exhibiting bioactivity, bioavailability, and biocompatibility. There are various methods for synthesizing Se NPs, including chemical, physical, biological, and green chemistry approaches. In particular, the chemical method provides precise reaction conditions, allowing for fast reaction rates, high control over particle size and shape, high yield, as well as good scalability and reproducibility, making it applicable

for industrial production. However, this method sometimes faces environmental and health issues, such as the usage of toxic reducing agents (e.g., sodium borohydride, hydrazine), a requirement of strict pH and temperature control, and the possibility of residual contaminant production that reduces biocompatibility [4]. On the contrary, the physical approach precisely controls nanoparticle formation via laser ablation and ultrasonication methods, producing high-purity Se NPs regardless of chemical reagents. Nevertheless, it possesses high energy consumption and equipment expense, low yield, limited scalability, and a shortage of surface functionalization, reducing biomedical applicability [4]. On the other hand, the biological strategy uses microorganisms (such as bacteria, fungi, algae) or plant extracts as reducing agents, thus producing eco-friendly Se NPs with natural surface coatings that enhance stability, functionality, and biocompatibility, ideal for medical applications. Particularly, plant extracts stemmed from *Syzygium aromaticum* (Clove), *Calluna vulgaris* (Heather), *Aloe vera*, *Camellia sinensis* (green tea), *Azadirachta indica* (neem), *Eryngium foetidum*, olive pomace, and enzymatically bioreducing bacteria, fungi, and algae, including *Bacillus sp.*, *Saccharomyces cerevisiae*, and *Chlorella vulgaris* can be used in the synthesis of selenium nanoparticles [5]-[7]. These plant extracts not only enhance particle stability through stabilizing capability but also exhibit strong antioxidant and antimicrobial activities. Although it encounters several drawbacks, such as slow reaction rate and low yield, complex biological residue purification, and variability in nanoparticle properties due to inconsistent biological sources [8]. To surmount these disadvantages, the green synthesis method emerges, which utilizes plant extracts (e.g., polyphenols, flavonoids) as reductants and stabilizers, and can produce Se NPs with enhanced antioxidant and antimicrobial properties. It is non-toxic, sustainable, and energy efficient. Notwithstanding, its scalability and reproducibility are compromised by the difficulty in standardizing plant extract composition and additional purification requirements to remove organic residues [4].

To overcome the inherent limitations of green synthesis, the combination of chemical synthesis and green synthesis is considered a solution. Specifically, it can be demonstrated by the combination of eco-friendly reducing agents (e.g., ascorbic acid, alginate) and plant-extract stabilizing agents (such as mandarin peel-derived pectin, pomegranate peel extracts) [9]-[11]. Among them, natural antioxidants, such as ascorbic acid (vitamin C), can simultaneously act as reducing agents that help convert selenium salts (e.g., Na_2SeO_3) into selenium nanoparticles, and as capping agents to improve stability and bioactivity [7]. In addition, high-methoxyl pectin, originating from pomelo (*Citrus maxima*) peel, can act as both a reducing and stabilizing agent [12].

In this research, nano selenium (Se NPs) was produced by ascorbic acid with the support of pomelo (*Citrus maxima*) peel-derived pectin in the aqueous medium. On the other hand, the core parameters influencing the size and zeta potential of Se NPs, such as ascorbic acid, pectin, and Na_2SeO_3 doses, were also investigated for the process optimization. This is a green method for Se NPs synthesis, which is an alternative to chemical and physical approaches owing to the use of green reductant and stabilizer.

2. Materials and Methods

2.1. Materials

L-Ascorbic acid and sodium selenite (Na_2SeO_3, 99%) were purchased from Sigma. Pectin was obtained from pomelo peel, which was done by the Department of Organic-Environmental Technology at the Institute of Advanced Technology [13]. Double-distilled (DD) water was produced at the Department of Organic-Environmental Technology without any purification before use.

2.2. Synthesis of selenium nanoparticles (Se NPs)

The process of Se NPs formation was conducted by the participation of ascorbic acid as a reductant and pomelo pectin as a stabilizer. In brief, 2.5 mg of pectin was added to 8.9 mL of water, which was preheated at 70 °C to dissolve completely and obtain a homogenous solution. Afterward, the volume of Na_2SeO_3 20 mM was dropwise added to this solution. After 2 minutes, 0.8 mL of ascorbic acid 2% was added slowly, and the solution changed from colorless to orange. The reaction occurred for 2 hours.

2.3. Physicochemical characterization

A volume of 100 µL of Se NPs was diluted 100-fold with double-distilled water to determine particle size, zeta potential, and polydispersity index (PDI) at 25 °C using the Horiba SZ-100 (Horiba, Japan).

500 µL of Se NPs was diluted to 2 mL by DD water, and absorbance was measured from 200 to 350 nm with 1 nm resolution by Jasco V-770 UV-Vis spectrophotometry (Jasco, Japan).

Fourier-transform infrared spectroscopy was employed to investigate the potential functional groups involved in the reduction and stabilization of the nanoparticles. Briefly, Na_2SeO_3, ascorbic acid, pectin, and Se NP samples were scanned using an FT-IR spectrometer (Bruker Tensor 27, Bruker, Germany) in the 4000-500 cm^{-1} wavenumber range.

Scanning electron microscopy (SEM) was set up to capture the morphology of Se NPs. This instrument was equipped with an energy-dispersive X-ray (EDX) to accumulate information about elemental composition.

3. Results and Discussion

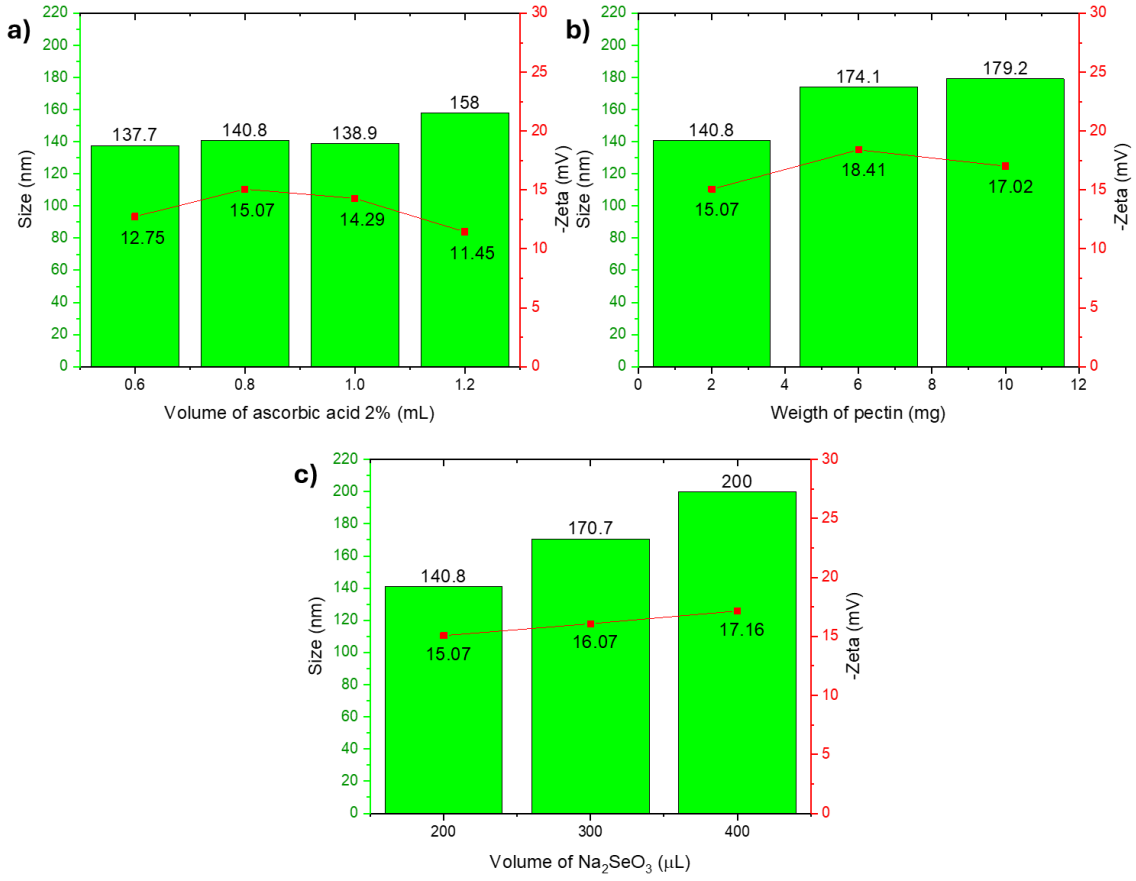

Figure 1. *The optimization of Se NPs formation by the evaluation of a) the volume of ascorbic acid 2%, b) the weight of pectin, and c) the volume of Na_2SeO_3 20 mM.*

The formation of Se NPs was conducted by the chemical reduction of ascorbic acid, along with the presence of a natural stabilizer like pectin. In our research, we have established the reaction conditions in terms of the input of either ascorbic acid or pectin, and Na_2SeO_3 concentration to optimize the efficacy through the size and zeta potential of the formed Se NPs. This kind of nanomaterial plays an important role in nutritional supplements owing to its good bioactivity, bioavailability, and low toxicity without aggregation under physiological conditions [5]. Figure 1 depicts the characteristics of Se NPs in terms of size and zeta potential, which were investigated by the impact of different reaction conditions. First, in Figure 1a, the increase of ascorbic acid seems like it did not significantly change the size of Se NPs.

Briefly, 0.6, 0.8, and 1 mL of ascorbic acid showed the sizes from 137.7 nm (PDI = 0.071), 140.8 nm (PDI = 0.034), and 138.9 nm (PDI = 0.086), respectively, and slightly rose to 158 nm (PDI = 0.77) by using 1.2 mL of reductant. However, the zeta potential expressed the most stable situation of -15.07 mV by using 0.8 mL of ascorbic acid; therefore, this value was utilized for the next experiment. In Figure 1b, the increase in weight of pectin would increase the size of Se NPs, from 140.8 nm (PDI = 0.034) to 174.1 nm (PDI = 0.113), followed by a plateau state of about 179.2 nm (PDI = 0.153). The zeta potential also draws a similar situation. In Figure 1c, by using 2 mg of pectin and 0.8 mL of ascorbic acid from previous investigations, an increase in the input of Na_2SeO_3 would enhance either the size of Se NPs or the zeta potential values.

In summary, we have optimized the best conditions for the Se NPs formation as: 8.9 mL of water will dissolve 2 mg of pectin with the addition of 0.8 mL ascorbic acid 2% and 200 µL of Na_2SeO_3 20 mM under the temperature of about 70 ^oC for 2 hours of reaction. The resulting Se NPs had a size of 140.8 nm (PDI = 0.034) associated with the zeta potential of -15.07 mV, in which the low PDI reflects the narrow particle size contribution, and a negative zeta potential will ensure the stability of Se NPs by electrostatic repulsion [14].

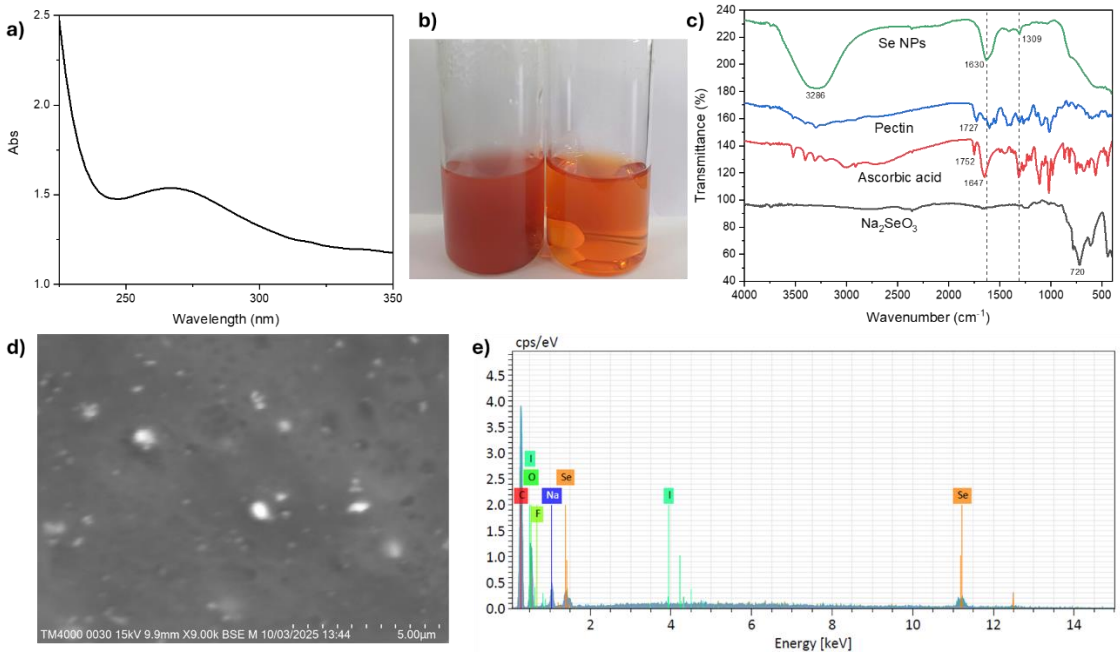

Figure 2. *The physicochemical characterization of the Se NPs by a) UV-Vis spectrum, b) the appearance of Se NPs without (left) and with (right) pectin, c) FTIR measurement, d) SEM image, and e) EDX scanning, respectively.*

It is utmost necessary to define the physicochemical specifications of Se NPs to discover the potential applications. In Figure 2a, Se NPs depict the maximum absorption wavelength at 270 nm, which corresponds to localized surface plasmon resonance of itself [3]. Besides, we also conducted a comparison between without (left) and with (right) pomelo peel pectin in Figure 2b for the synthesis of Se NPs, in which both utilized ascorbic acid as a reducing agent. As we can see, the color of the left sample was significantly darker orange compared with the right one, which showed a transparent orange solution. Due to the presence of pectin, it can stabilize the resulting Se NPs without aggregation. On the other hand, Figure 2c shows the specific functional groups on the surface of the formed nanoparticles. Regarding Se NPs, the strong signals at 3286 and 1630 cm^{-1} corresponded to -OH stretching and C=O stretching [15], which may arise from functional groups from pectin by chemical interaction [16]. Pecctin revealed that the vibrations at 1727 cm^{-1} and 1602 cm^{-1} were assigned for the C=O stretching vibration of the ester and the stretching vibration of the methyl esterified carboxyl group COO-R [13]. With respect to ascorbic acid, there are some significant vibrations at 1752, 1647, 1314, 1042, and 1021 cm^{-1} that are assigned to C=O stretching, C=C stretching, C-O-C asymmetric stretching, -OH in-plane

bending, and C-O-C symmetric stretching [17]. The FTIR spectrum of sodium selenite expressed only the strong vibration at 720 cm^{-1}, which corresponds to Se-O stretching. Both ascorbic acid and Na_2SeO_3 vibrations were attenuated in Se NPs, indicating the reduction to Se^0 during synthesis [14]. Consequently, the graph fully distinguished the formation of Se NPs from selenium salt by the specific vibrations, which indicates the presence of functional groups from both ascorbic acid and pectin on the surface of the resulting nanoparticles.

Figure 2d captures the morphology of Se NPs by SEM measurement. Herein, they were monodispersed, spherical nanoparticles with a size ranging from 100 to 250 nm. On the other hand, from the SEM picture, the EDX measurement in Figure 2e exhibits the composition of the particles. We can observe that carbon got the highest percentage of about 59.21% which originated from either ascorbic acid or pectin on the surface of nanoparticles, followed by the share of oxygen element by 33.70%. Besides, the shares of sodium and selenium were 3.16% and 3.93%, respectively. This indicates that the formation of Se NPs was strongly promoted by ascorbic acid surrounded by a pectin network for significant stability.

4. Conclusions

The green process was applied to form the Se NPs by using ascorbic acid as a reductant and pectin as a surface capping agent. The obtained Se NPs showed an average size of 140.8 nm (PDI = 0.034) and surface charge of -15.07 mV via the DLS technique. The SEM photograph further confirmed the spherical shape and nanoparticle size of 100-250 nm. These Se NPs presented an absorbance peak at 270 nm, attributed to the existence of surface plasmon resonance or electronic transitions involving selenium and pectin. In addition, the EDX spectrum further confirms the presence of selenium in the nanoparticles. This research is a platform for deeper exploitation of the future use of Se NPs in diverse applications like biomedical, cosmetics, and food supplements. On the other hand, intensive biological activities, as well as *in vitro* and *in vivo* tests, should be carried out to support the evidence for potential research.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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