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Research Article

Green Synthesis and Comprehensive Characterization of Selenium Nanoparticles: Towards Sustainable Biomedical and Environmental Applications

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ABSTRACT

Background and Rationale: Nanotechnology has revolutionized various scientific disciplines, with Selenium Nanoparticles (SeNPs) demonstrating exceptional potential due to their unique physicochemical properties and applications in medicine, agriculture, and environmental sciences. However, conventional synthesis methods often employ toxic chemicals, posing significant environmental and health risks. Green synthesis of nanoparticles using plant extracts offers a sustainable and eco-friendly alternative. This study focuses on the green synthesis of SeNPs utilizing the aqueous leaf extract of *Anacardium occidentale*, optimizing the synthesis conditions, and characterizing the resulting nanoparticles. **Materials and Methods:** The green synthesis of SeNPs was conducted using aqueous leaf extract of *Anacardium occidentale* as a reducing and stabilizing agent. The synthesis process was optimized using the orthogonal test of the Taguchi design experiment. Characterization techniques, including UV-vis spectroscopy, Fourier-Transform Infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Zeta potential analysis was done to confirm the synthesis and stability of the Selenium Nanoparticles. **Results and Discussion:** The optimal conditions for the green synthesis of Selenium Nanoparticles were a reaction time of 1 hr, citric acid concentration of 0.04 M, aqueous leaf extract concentration of 1.0 mg/mL, and pH 7.5, yielding nanoparticles with an average particle size of 121.9 nm. The UV-vis spectroscopy results highlighted the surface plasmon resonance at 270 nm, indicative of small nanoparticle size. FTIR analysis confirmed the involvement of specific functional groups from the leaf extract in the reduction and stabilization processes. SEM and TEM analyses corroborated the spherical shape and uniform size distribution of the Selenium Nanoparticles. The zeta potential value of -23.5 mV indicated good colloidal stability. These findings demonstrate the effectiveness of *Anacardium occidentale* leaf extract in producing stable, spherical SeNPs via an eco-friendly approach. **Conclusion:** Green synthesis of SeNPs from *Anacardium occidentale* leaf extract minimizes adverse environmental and health impacts associated with conventional methods.

Keywords: Green Synthesis, Selenium Nanoparticles, Characterisation study, Condition optimisation.

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INTRODUCTION

Nanotechnology has marked the beginning of a paradigm shift in various scientific disciplines, offering unprecedented avenues for innovation and advancement. Within the myriads of nanomaterials, Selenium Nanoparticles (SeNPs) with remarkable properties applicable across diverse fields, including medicine, catalysis, and environmental science has garnered significant attention. Selenium is a critical trace element with numerous health benefits primarily mediated through its incorporation into selenoproteins. These selenoproteins play a vital role in maintaining the body's antioxidant defence systems, protecting cells from oxidative stress, and reducing inflammation.[1] Selenium supports thyroid hormone metabolism, which is essential for regulating growth, development, and energy metabolism. It also plays a crucial role in reproductive health, ensuring proper sperm function and overall fertility. In addition, selenium has neuroprotective effects, helping to safeguard brain function and potentially lowering the risk of neurodegenerative diseases. Its role in immune modulation enhances the body's ability to fight infections and supports recovery from illnesses. Research also highlights selenium's potential in cancer prevention by influencing genetic and molecular pathways involved in tumor

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suppression.[2] Overall, selenium is indispensable for maintaining cellular health, preventing oxidative damage, and supporting key physiological processes.

Selenium, a trace element essential for human health, exhibits remarkable properties when engineered into nanoparticles.[3] However, reports indicate that consuming selenium in high quantities can cause serious harm. Therefore, researchers are focusing on synthesizing SeNPs to harness its benefits while avoiding adverse effects.[4,5] Selenium Nanoparticles possess unique characteristics such as high surface area to volume ratio, enhanced reactivity, and biocompatibility, rendering them promising for various biomedical applications including anticancer therapy, antimicrobial agents, and antioxidant supplements.[6-7] Their unique physicochemical properties, along with their biocompatibility, make them particularly suitable for drug delivery, bioimaging, and therapeutic interventions.

Traditional methods for the synthesis of nanoparticles often involve the use of toxic chemicals and harsh conditions, raising concerns about environmental sustainability and human wellbeing. The synthesis of Selenium Nanoparticles through green methods, utilising the reducing potential of natural extracts, represents a sustainable and environmentally friendly approach that circumvents the drawbacks associated with conventional methods.[8]

Comprehensive characterization of the synthesized Selenium Nanoparticles is imperative to understand their physicochemical properties, including size, morphology, surface charge, and composition. Characterization study provides insights into the stability, dispersibility, and interaction of nanoparticles with biological systems, which are crucial for their therapeutic application. Various analytical techniques employed to elucidate the structural, morphological, and elemental characteristics of metal nanoparticles are UV-vis spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR), Energy-Dispersive X-ray spectroscopy (EDX), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM).[9]

The anticancer and antimicrobial activity exhibited by *Anacardium occidentale* L. can be attributed to its high anti-oxidant property.[10,11] Cajado *et al.*, in their study demonstrated the antimicrobial activity of aqueous and hydroalcoholic leaf extract of *Anacardium occidentale*.[12] Muraina IA *et al.*, demonstrated the anti-mycoplasm activity of *Anacardium* leaf extract.[13] Extracts and phytochemicals derived from *Anacardium occidentale* show anesthetic, anti-inflammatory, anti-bactericidal properties as demonstrated by various studies.[14-15] Nanoparticles synthesized through green methods offer a wide range of applications owing to their biocompatibility and capacity for controlled substance release.[16,17] Thus, the synthesis of Selenium Nanoparticles from *Anacardium occidentale* L. leaf extract may potentially enhance the bioactivity of SeNPs. Understanding the physicochemical properties of Selenium Nanoparticles synthesized via green methods is pivotal for their application in medicine and environmental remediation. By elucidating the synthesis and characterization of SeNPs via green synthesis using *Anacardium occidentale* L. leaf extract, this study contributes to the emerging field of green nanotechnology.

MATERIALS AND METHODS

Preparation of Leaf Extract

Fresh *Anacardium occidentale* leaves were collected from Sawyerpuram, Thoothukudi. The plant material was identified and confirmed by the voucher specimen [Botanical Survey of India, Indian Virtual Herbarium Barcode: [BSID0008257]. The aqueous extract of *Anacardium occidentale* leaves were obtained using the method described by Tafinta *et al.*[18] The leaves were thoroughly washed with sterile distilled water to eliminate dust particles. 100 grams of ground *Anacardium occidentale* leaves were mixed with 1000 mL of sterile distilled water in a conical flask to prepare of aqueous extract. The conical flask with the mixture was covered with cotton wool and aluminium foil in order to prevent contaminations. The mixture was agitated and left to stand at room temperature for 48 hr after which they were filtered through a muslin cloth. The resulting filtrate was then evaporated to dryness in an oven at 37°C to yield crude extracts with a yield percentage of 10%. The obtained crude extracts were stored in a sterile container at room temperature until further use.

Green Synthesis of SeNPs-Condition Optimisation

SeNPs were green synthesized using aqueous extract of *Anacardium occidentale* L. leaf. 50 mL of aqueous extract of *Anacardium occidentale* leaf (1 mg/mL) was mixed with aqueous solution of sodium selenite (50 mL, 0.04 M). Freshly prepared solution of 50 mL of citric acid (0.04 M) was added slowly to the mixture under continuous magnetic stirring (500 rpm) at room temperature for 1 hr. The pH of the reaction system was adjusted to 7.5 using 1 M Sodium Hydroxide (NaOH) and 1 M glacial Acetic acid (HAc). The presence of SeNPs was validated through a gradual transition of the solution's colour to orange. Subsequently, the solution was centrifuged at 9000 rpm to isolate the Selenium Nanoparticles, which were then cleaned by rinsing the sample thrice with distilled water and twice with 100% ethanol.[19] The cleaned SeNPs were stored overnight at 50°C, resulting in a dry, brown-colored powder.

Optimization of parameters for the green synthesis of Selenium Nanoparticles

Several trials and single parameter experiments were conducted for optimising the best conditions for Selenium Nanoparticles synthesis. Taguchi design methods were used to optimize the parameters for the synthesis of green synthesis of Selenium Nanoparticles using aqueous leaf extract of *A. occidentale*.

Different nanoparticle formulations were tested using varying reaction time, citric acid concentration (Vc), concentration of *A. occidentale* leaf aqueous extract, and pH, which were denoted as variables A, B, C and D respectively. The concentration of sodium selenite (0.04 M) remained constant throughout the experiments. These variables were identified as having a significant impact on the diameter of the synthesized SeNPs compared to other variables. In this study, an orthogonal array L9 of the Taguchi design was employed to optimize the synthesis conditions for green-synthesized SeNPs. The experiment involved four factors, each with three levels, while maintaining a constant concentration of sodium selenite (0.01 M). Table 1 shows the four variables (A, B, C, and D) and their corresponding levels (Levels 1, 2, and 3) for each variable selected for the optimization experiment.

Characterization of Selenium Nanoparticles

The synthesized Selenium Nanoparticles were characterized using analytical techniques to understand the structural and morphological characteristics of SeNPs. Including UV-vis spectroscopy, X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Fourier-Transform Infrared Spectroscopy (FTIR), and zeta potential analysis.

The characterization methods utilized include Bio spec Nano (Shimadzu), which is used to measure the surface plasmon resonance of the nanoparticles. ATR-FTIR spectrometer was employed to determine the functional group of extracts responsible for reducing the selenium and forming the nanoparticle (Shimadzu IR affinity).[20] The nanoparticles were analyzed for their crystalline nature using the X-ray diffractometer (XPERT-PRO).[21] The microscopic nature and surface morphology of the nanoparticles were identified using a Scanning Electron Microscope (FESEM, TESCAN) and Transmission Electron Microscope (JEOL Ltd., Japan). The particle size and zeta potential of selenium nanoparticles in liquid suspension were analyzed using a Zetasizer Nano ZS (Malvern Instruments, UK).[22]

RESULTS

In this study, the optimization of Selenium nanoparticles (SeNPs) synthesis using an aqueous extract of *Anacardium occidentale* leaves was performed using the Taguchi method, followed by characterization through various analytical techniques to evaluate their size, stability, and surface properties.

Condition Optimisation of green synthesis of Selenium Nanoparticles

For optimizing the conditions for Selenium Nanoparticles, a Taguchi experimental analysis utilizing an orthogonal array (L9) was conducted after collecting response values for synthesis parameters such as reaction time (A), concentration of citric acid (B), concentration of aqueous extract of *Anacardium occidentale* leaves (C) and pH (D). Table 2 shows the recorded average particle size distribution for the green synthesis of Selenium Nanoparticles (SeNPs) using an aqueous extract of *Anacardium occidentale* leaves for each combination, as measured by a Dynamic Light Scattering (DLS) particle size analyser. It was observed that the lowest and highest mean size of the SeNPs was obtained from experiments number two and seven and it was found to be 124.59 nm and 141.44 nm, respectively. As shown in Figure 1, the results demonstrate that the effect of concentration of citric acid has a larger influential factor on the diameter of green synthesized SeNPs followed by concentration of aqueous extract, reaction time and a smaller effect due to pH was observed in the order of RB (8.8)>RC (4.1)>RA (3.9)>RD (2.8), which has been illustrated from response means (Table 3). Therefore, the concentration of citric acid has a significant effect on the diameter of green synthesized Selenium Nanoparticles from aqueous extract of *Anacardium occidentale* leaf.

For the synthesis of Selenium Nanoparticles, the results show that particle size was smaller in level 2 having 0.04 M concentration of citric acid compared to level 1 (0.02 M) and level 3 (0.06 M). Therefore level 2 (0.04 M) concentration of citric acid provides a more efficient reducing environment to accelerate the formation of crystal nuclei which can help in the stabilization. Hence it is considered the optimal condition for green synthesis of Selenium Nanoparticles. The diameter of Selenium Nanoparticles obtained at pH 7.5 (level 1) was smaller when compared to those obtained at level 1 (pH 6.5) and level 2 (pH 7). Thus, optimum pH for the synthesis of Selenium Nanoparticles from aqueous extract of *Anacardium occidentale* leaves was considered as 7.5 (Level 3). The optimal reaction time for the synthesis of Selenium Nanoparticles was found as 1 hr (Level 2). Longer period of reaction time resulted in the agglomeration of SeNPs and the

Table 1: Selected Variables and Corresponding Levels for Orthogonal Experiment.

Levels	Experimental Factors and their Corresponding Levels for Optimization of Reaction Conditions			
	A	B	C	D
	Reaction time (Hours)	Concentration of Citric acid (M)	Concentration of *Anacardium occidentale* aqueous leaf extract (mg/mL)	pH
1	0.5	0.02	0.5	6.5
2	1	0.04	1	7
3	1.5	0.06	1.5	7.5

Table 3: Response table results for Means by Taguchi design experiments.

Level		Experimental factors			
		Reaction Time (Hours)	Concentration of Citric Acid (M)	Concentration of Aqueous *Anacardium occidentale* leaf extract (mg/mL)	pH
Mean Diameter (nm)	1	129.1	136.7	131.3	132.1
	2	131.3	127.9	129.0	132.0
	3	133.0	128.7	133.1	129.3
Delta		3.9	8.8	4.1	2.8
Rank		3	1	2	4

Table 2: Orthogonal experimental results for the particle size measurements of Selenium Nanoparticles by DLS measurements (Mean±SD of triplicate analysis).

Numbers	Experimental Factors				Mean Diameter (nm)
	A	B	C	D	
	Reaction time (hours)	Concentration of Citric Acid (M)	Concentration of Aqueous extract of *A. occidentale* leaves (mg/mL)	pH	
1	0.5	0.02	0.5	6.5	135.87 ±0.56
2	0.5	0.04	1	7	124.59 ±0.53
3	0.5	0.06	1.5	7.5	126.86±0.51
4	1	0.02	1	7.5	132.93±0.57
5	1	0.04	1.5	6.5	131.03±1.26
6	1	0.06	0.5	7	129.28±1.38
7	1.5	0.02	1.5	7	141.44±0.91
8	1.5	0.04	0.5	7.5	128.12±1.22
9	1.5	0.06	1	6.5	129.37±0.92

size of the SeNPs were also larger. The diameter of SeNPs was found to be smaller at level 2 (1 mg/mL) of aqueous leaf extract concentration compared to level 1 (0.5 mg/mL) and level 3 (1.5 mg/mL).

As shown in Table 3, the result of the Taguchi experiments gave the best optimal combinations for the synthesis of Selenium Nanoparticles which was A2B2C2D3 (factors-A, B, C, and D; levels 1, 2, and 3). Thereafter, these combination verification tests were conducted parallelly in triplicate experiments. The results show that the diameter of optimized SeNPs obtained through DLS measurements was smaller than all experimental combinations of Taguchi experiments which indicates that the SeNPs are well stabilized by Aqueous extract of *A. occidentale* leaves. Therefore, the optimal preparation conditions of SeNPs were 1 hr of reaction time with 0.04 M concentration of citric acid, 1.0 mg/mL of aqueous leaf extract, and the 7.5 pH of the overall reaction mixture which gave nanoparticles with particle size of 121.9 nm.

Characterisation study of green synthesized Selenium Nanoparticles

UV-vis spectroscopy analysis of Selenium Nanoparticle

In our study, we investigated the reduction of metallic selenium ions by analysing the UV-vis spectrum within the 250 nm to 700 nm wavelength range. We observed a distinct absorption peak at 270 nm, which signifies the presence of Surface Plasmon Resonance (SPR), a characteristic feature of SeNPs (Figure 2A).

Fourier Transform Infrared Spectroscopy (FTIR) of Selenium Nanoparticles

The study utilized Fourier-Transform Infrared Spectroscopy (FTIR) to examine the functional groups on the surface of synthesized Selenium Nanoparticles. FTIR allows us to identify specific vibrational modes of chemical bonds, and in this investigation, spectral bands were observed in the range of 4000-400 cm^{-1}. The FTIR spectrum of SeNPs showed a notable peak at 578.64 cm^{-1}, indicating a stretching vibration associated with C-I bonds. Additionally, a common feature across all spectra was the broad peaks around 3826.77 cm^{-1}, corresponding to the stretching vibration of Hydroxyl (OH) groups (Figure 2B).

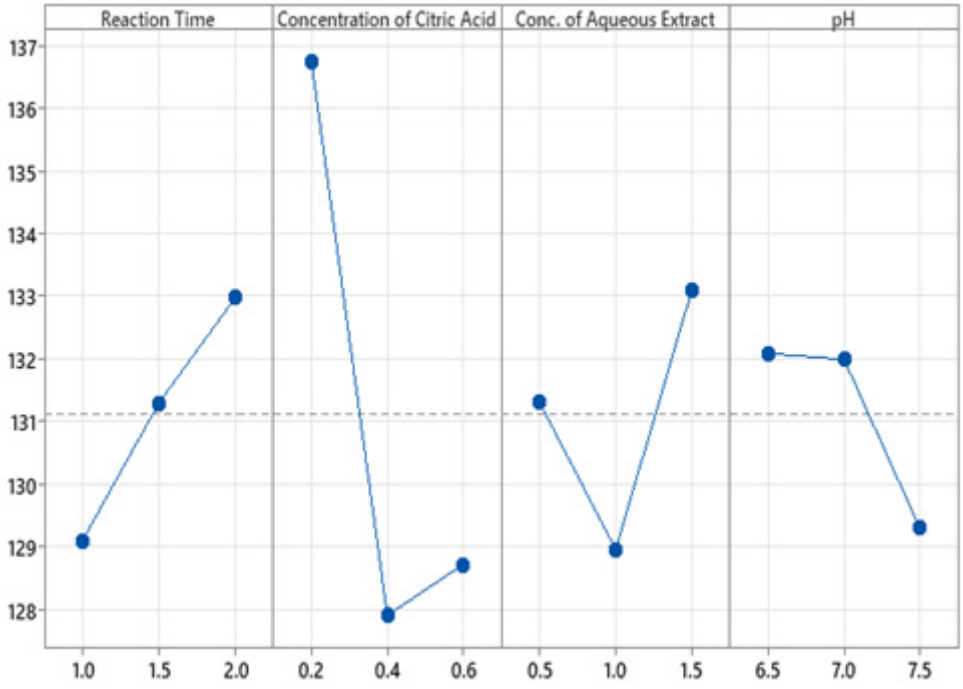

Figure 1: Main effects plot for Means of each factor versus diameter of Selenium Nanoparticles.

Energy-Dispersive X-ray spectroscopy (EDX)

In our research, we performed an elemental analysis of Selenium Nanoparticles (SeNPs) produced through a green synthesis method. We utilized Energy-Dispersive X-ray spectroscopy (EDX) with a detector, examining the nanoparticles deposited on a carbon film. In our study, we identified specific elemental peaks in the SeNPs, including strong absorption peaks for metallic selenium ions at 1.35-40 keV, 11.20 keV, and 12.30 keV. Peaks for Na and O were also detected, likely due to mixed components from the plant extract used in the synthesis. Notably, a distinct peak around 0.2 keV on the left side of the spectrum indicated the presence of carbon, while a less visible peak at 0.5 keV was associated with the characteristic oxygen line. Additionally, copper peaks at 1.2 keV, 8.1 keV, and 8.9 keV were observed, attributed to the Cu support grid. A peak at 1.2 keV, attributed to Na (sodium) and K (potassium), along with peaks at 0.2 keV and 3.3 keV, indicated the presence of these elements (Figure 2C and 2D). The absence of peaks for other elements, coupled with a high concentration of selenium, confirmed the purity of the selenium metal in the final product. Vyas *et al.*, in their study opine that the presence of carbon and oxygen spots in the samples confirmed the existence of stabilizers with alkyl chains.[23]

Scanning Electron Microscope (SEM) analysis of Selenium Nanoparticles

Scanning Electron Microscopy (SEM) is a powerful imaging technique used to evaluate the structure and surface appearance of various materials at high magnifications. In the present study, SEM imaging reveals that the use of plant extracts results in the formation of tightly packed SeNPs (Figure 2E).

Transmission Electron Microscopic analysis (TEM)

In the present study, Transmission Electron Microscopy analysis of the liquid solution showed the presence of SeNPs. Figure 2F illustrates the sizes of the particles produced using a plant extract, which ranged from 14.65 nm to 17.82 nm and from 0.28859 nm to 8.78534 nm. This provides a visual representation of the dimensions of the nanoparticles generated through our process.[24]

Zeta potential analysis of Selenium Nanoparticles

Zeta potential is a measure of the electrostatic potential at the surface of particles, commonly used to assess the stability of colloidal dispersions, including SeNPs.[25] SeNPs green synthesized in the present study were confirmed to have a negative surface charge with a zeta potential value of -23.5 mV. At 25°C, the stability and size of the SeNPs were assessed using a zeta sizer, revealing a particle size of 121.9 nm (Figure 3).

DISCUSSION

Nanoparticles can be synthesized through a variety of techniques, generally categorized into chemical and biological methods. Chemical methods include techniques such as sol-gel processes, precipitation, steam condensation, and micro-emulsion methods.[26] On the other hand, biological methods leverage natural sources such as algae, plants, fungi, yeast, bacteria, and isolated sugars (including cellulose, starch, and glucose) to produce nanoparticles.[27]

Plant extracts have garnered significant attention for the green and sustainable synthesis of nanoparticles due to their abundance, safety, and broad array of metabolites that act as reducing and

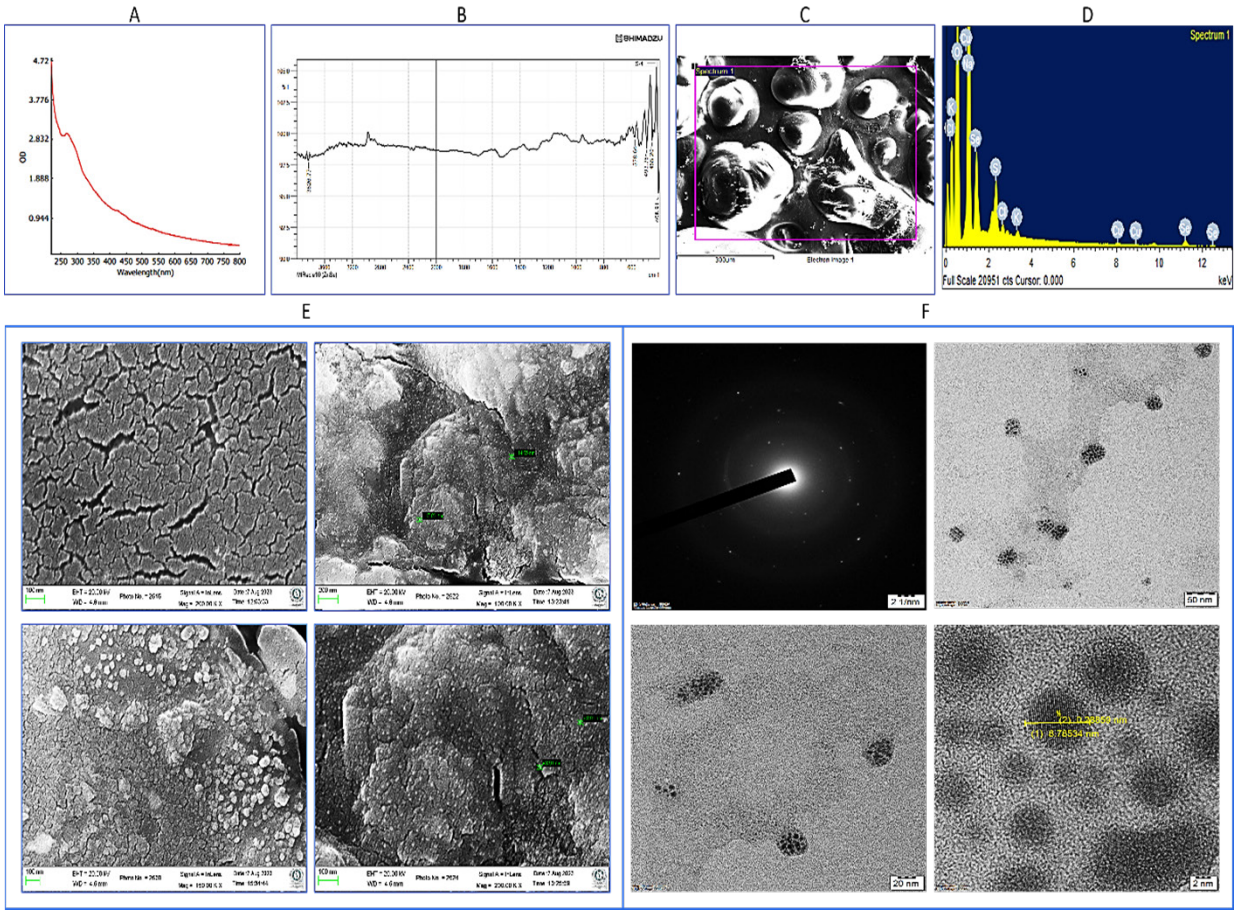

Figure 2: A-UV-visible spectra of SeNPs; B-FTIR spectrum analysis of Selenium Nanoparticles; C and D-EDX analysis of Selenium Nanoparticles; E-SEM images of SeNPs and F-TEM images of SeNPs.

capping agents, providing stability to metal nanoparticles. They are easily accessible, cost-effective, and faster in synthesis compared to microbes. Plants' ability to accumulate and detoxify heavy metals is well-documented, and their rich phytochemical content, including steroids, saponins, carbohydrates, and flavonoids, facilitates nanoparticle formation. The eco-friendly synthesis process using plant extracts eliminates toxic chemicals and harsh conditions, making it an environmentally safe method. These bioactive compounds act as reducing and stabilizing agents, donating electrons to metal ions and facilitating nanoparticle formation with precise size, shape, and composition. Additionally, nanoparticles derived from plants are generally safer for humans compared to those synthesized chemically and possess significant biological potential. These plant-based nanoparticles have applications in agriculture, food science, bioengineering, cosmetics, nanomedicine, and human health protection.[28] The synthesis of SeNPs through green methods, using aqueous extracts from *Anacardium occidentale* leaves, offers an eco-friendly and cost-effective alternative to traditional chemical processes.

Biomolecules like proteins and polysaccharides serve as capping agents, preventing aggregation and influencing nanoparticle size and shape.[29,30] Optimal pH, temperature conditions, concentration of precursor ions, reaction time and stirring conditions are crucial, as they affect the reduction, nucleation, and growth of nanoparticles.[31,32] The specific mechanisms for green synthesis of nanoparticles can vary depending on the type of plant extract, metal precursor, and reaction conditions, highlighting the need for further research to understand these processes in detail.

The optimization of conditions for the green synthesis of Selenium Nanoparticles (SeNPs) is crucial, as it significantly influences the size, stability, and overall characteristics of the nanoparticles. The need for condition optimization arises from the inherent sensitivity of nanoparticle properties to various synthesis parameters. Factors such as the concentration of reducing agents, the ratio of precursor to stabilizer, pH of the reaction mixture, and reaction time can all influence the nanoparticle size and its stability. Nanoparticles of specific sizes are desired for various applications, such as in drug delivery, biomedical imaging, and catalysis, as these properties directly affect their functionalization and bioactivity. Therefore, it is essential to optimize the reaction conditions to consistently produce SeNPs with the desired characteristics.

The current study focused on optimizing the conditions for the green synthesis of Selenium Nanoparticles (SeNPs) using *Anacardium occidentale* leaf extract. A Taguchi experimental design with an orthogonal array (L9) was employed to examine the effects of reaction time, citric acid concentration, aqueous extract concentration, and pH. The results revealed that the concentration of citric acid had the most significant impact on the particle size, followed by aqueous extract concentration, reaction time, and pH. Optimal conditions were found to be 1 hour of reaction time, 0.04 M citric acid concentration, 1.0 mg/mL aqueous extract concentration, and pH 7.5, which resulted in Selenium Nanoparticles with a particle size of 121.9 nm. These findings suggest that the optimized conditions enhance nanoparticle stability and can be applied for scalable SeNP synthesis.

In the study by Jha N *et al.*, the optimal conditions for synthesizing RMLP-SeNPs from polysaccharide of mangrove *Rhizophora mucronata* were 2 hr of reaction time, 0.04 M of Vitamin C concentration, 1 mg/mL of RMLP, and pH 7.5.[33] The synthesized SeNPs had a particle size of around 54.85 nm, as measured by DLS. In contrast, Diko CS *et al.*, synthesized SeNPs through a simple, environmentally benign SeO2-reduction process using *Trichoderma* sp. WL-Go culture broth. The optimal conditions for this synthesis were pH 8, an inoculation time of 24 hours, and a SeO2 concentration of 2 mM.[34] Moreover, Bahig El- deep *et al.*, used a bacterial strain, *Streptomyces* sp., isolated from soil around the roots of *Alhagi graecorum* in desert soil. The optimal conditions for Selenium Nanoparticle synthesis in this study were pH 7 and 32°C, with Selenium Nanoparticles being produced sustainably within a week.[35]

Analysis of UV-vis spectrum within the 250 nm to 700 nm wavelength range helps us understand the optical properties of Selenium Nanoparticles (SeNPs). The size and shape of these nanoparticles significantly influence their behaviour. The distinct absorption peak at 270 nm observed in the present study signifies the presence of Surface Plasmon Resonance (SPR), a characteristic feature of Selenium Nanoparticles. This peak at 270 nm suggests that the SeNPs are likely smaller in size. Interestingly, as the absorption peak diminishes, it indicates that the nanoparticles are aggregating. In simpler terms, the UV-vis spectrum changes demonstrate the initial formation of small Selenium Nanoparticles through ion reduction, followed by their

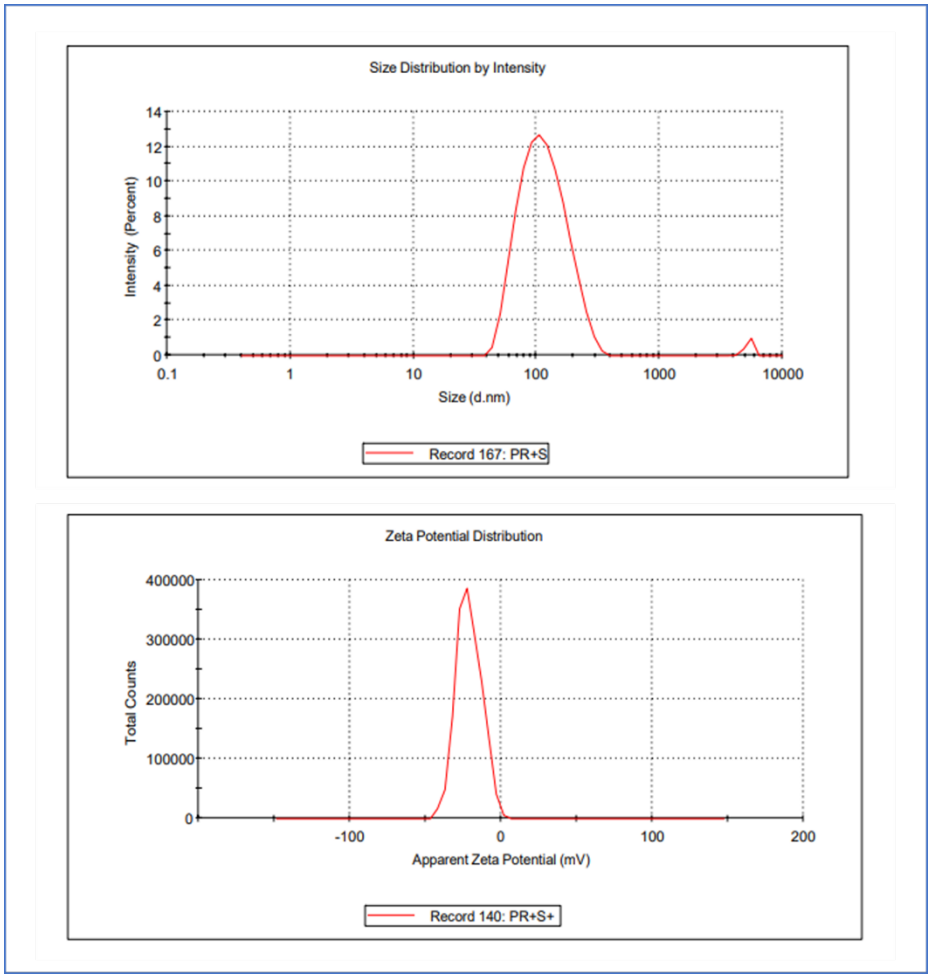

Figure 3: Size distribution and Zeta Potential distribution analysis of the synthesized Selenium Nanoparticles.

aggregation. This information is essential for comprehending the behaviour of Selenium Nanoparticles in the study.[36]

The FTIR spectrum of Selenium Nanoparticles in the current study showed a notable peak at 578.64 cm^{-1}, indicating a stretching vibration associated with C-I bonds. This provides insights into the chemical structure of the Selenium Nanoparticles. Additionally, a common feature across all spectra was the broad peaks around 3826.77 cm^{-1}, corresponding to the stretching vibration of Hydroxyl (OH) groups. This suggests the involvement of OH groups, possibly related to the surface characteristics or stabilization of the Selenium Nanoparticles. If stabilizing agents or capping molecules were used during synthesis, FTIR analysis allowed for the identification of specific functional groups associated with these molecules on the nanoparticle surface. This information is crucial for understanding the chemical composition and surface modifications of SeNPs in the study.[37]

Energy-dispersive X-ray spectroscopy is a widely used technique for determining the elemental composition of materials, providing insights into the presence and distribution of different elements.[38] The EDX analysis produced a spectrum showing peaks corresponding to the elements in the sample. For SeNPs, a prominent peak was expected at the energy associated with Selenium (Se). The detection of additional peaks indicated the possible presence of impurities, stabilizing agents, or elements from the synthesis process. The absence of peaks for other elements, coupled with a high concentration of selenium, confirmed the purity of the selenium metal in the final product. Vyas *et al.*, in their study opine that the presence of carbon and oxygen spots in the samples confirmed the existence of stabilizers with alkyl chains.[39]

Scanning Electron Microscopy (SEM) is a powerful imaging technique used to evaluate the structure and surface appearance of various materials at high magnifications. SEM analysis allows us to observe the size, shape, distribution, and overall characteristics of Selenium Nanoparticles (SeNPs) derived from plant materials.[40,41] The SEM images reveal that Selenium Nanoparticles synthesized using plant extracts form tightly packed nanoparticles, indicating efficient particle formation and stabilization. Transmission Electron Microscopy (TEM) is an advanced imaging technique that allows for the detailed examination of the structure and shape of materials at an extremely small scale, such as SeNPs.[42,43] The sizes of the Selenium Nanoparticles in the current study ranged from 14.65 nm to 17.82 nm, showcasing the small and uniform dimensions of the particles produced through this green synthesis process.

SeNPs green synthesized in the present study exhibited a zeta potential of -23.5 mV, indicating a negatively charged surface and suggesting good stability in the colloidal solution, as the repulsion between particles helps prevent aggregation. The zeta potential results provide insights into the surface charge and potential interactions between nanoparticles in a solution. Zeta potential, a key metric for surface charge potential, was emphasized as a critical parameter in evaluating the stability of nanoparticles within solutions (Figure 3).

CONCLUSION

The green synthesis of SeNPs (SeNPs) using *Anacardium occidentale* leaf aqueous extract proved to be effective and efficient. The synthesis conditions for SeNPs were optimized using the orthogonal test of the Taguchi design experiment, resulting in a particle size of approximately 121.9 nm. The UV-vis spectroscopy data demonstrated an absorbance peak at 270 nm, indicating the presence of surface plasmon resonance and suggesting the formation of small-sized SeNPs. Fourier-Transform Infrared spectroscopy (FTIR) analysis provided detailed insights into the functional groups involved in the reduction process during synthesis, highlighting the chemical interactions on the nanoparticle surface. Scanning Electron Microscopy (SEM) analysis revealed that the synthesized SeNPs are spherical in shape and well-distributed. These characterization studies collectively confirm the successful synthesis and stability of SeNPs, emphasizing their potential for applications with minimized adverse effects. However, challenges such as variability in extract composition, complexity of bioactive components, batch-to-batch variations, and limited stability of plant extracts must be addressed. Despite these challenges, the ease of synthesis and broad applications make plant extracts a promising avenue for nanoparticle production, warranting further research and optimization to maximize their potential.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

A. occidentale: *Anacardium occidentale*; **XRD:** X-ray Diffraction; **EDX:** Energy Dispersive; **SPR:** Surface Plasmon Resonance; **HRTEM:** High Resolution Transmission Electron Microscope; **FTIR:** Fourier Transform Infrared Spectroscopy; **FESEM:** Field Emission Scanning Electron Microscopy; **DLS:** Dynamic Light Scattering; **SeNP'S:** Selenium Nanoparticles; **NP's:** Nanoparticles.

SUMMARY

Selenium Nanoparticles (SeNPs) were green synthesized using Anacardium occidentale L., leaf extract. The synthesis of Selenium Nanoparticles (SeNPs) via green methods involves several factors

that significantly influence the size, stability, and overall quality of the nanoparticles. To ensure optimal production of Selenium nanoparticles, various synthesis parameters must be carefully optimized. In this study, the optimization of conditions for the green synthesis of Selenium nanoparticles was carried out using Taguchi design method. Four key variables namely A (Reaction Time) B (Citric Acid Concentration), C (Concentration of Aqueous Leaf Extract) and D (pH) were selected for optimization. An orthogonal array L9 of the Taguchi design was employed to test different combinations of these variables, each with three levels (Levels 1, 2, and 3). Sodium selenite (0.04 M) was kept constant as the precursor for all experiments. By varying the levels of each variable in different experiments, the impact of each factor on the size of the synthesized SeNPs was assessed. The best conditions for the green synthesis of SeNPs were determined to be 1-hour reaction time, 0.04 M citric acid concentration, 1 mg/mL leaf extract concentration, and a pH of 7.5. Under these conditions, selenium nanoparticles with an average size of 121.9 nm were obtained, indicating that the nanoparticles were well-stabilized and suitable for further characterization and application studies. In this study, Selenium nanoparticles (SeNPs) characterization was done using various techniques. UV-vis spectroscopy confirmed nanoparticle formation with an SPR peak at 270 nm. This peak is consistent with previously reported studies, which observed similar spectral behaviour for selenium nanoparticles, indicative of their successful synthesis. FTIR analysis revealed bioactive compounds like hydroxyl, carboxyl, and amine groups aiding in Selenium Nanoparticle reduction and stabilization. EDX confirmed selenium presence, while SEM and TEM showed spherical nanoparticles with sizes around 120-150 nm, which corroborates the findings from Dynamic Light Scattering (DLS) measurements. The clear lattice fringes observed in the TEM images indicate the crystalline nature of the selenium. Zeta potential analysis demonstrated good colloidal stability with a value of -30.4 mV, indicating effective particle dispersion and stability in suspension. This comprehensive characterization confirmed the successful synthesis of selenium nanoparticles using the green method, with optimal physicochemical properties suitable for potential applications in biomedicine and environmental science.

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