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Research Article

Optimization of Green Synthesis Formulation of Selenium Nanoparticles (SeNPs) Using Peach Tree Leaf Extract and Investigating its Properties and Stability

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Background: Selenium nanoparticles (SeNPs) are highly sought after in diverse industries for their distinct properties and advantages. SeNPs can be synthesized via several methods, including the use of microwave, bain-marie, autoclave, and heater.

Objective: The objective is to optimize the SeNP synthesis formulation, emphasizing stability, concentration, particle size minimization, and uniformity using central composite design.

Materials and methods: The method involves autoclave heating at 121 °C under 1.5 bar pressure for 15 minutes. Prunus persica tree leaf extract and Aloe Vera gel serve as a regenerating agent and stabilizer, respectively. Four responses including SeNPs concentration, average particle size, zeta potential, and dispersion index (PDI), were assessed according to the experimental design. The optimal synthesis point was determined and evaluated for SeNP imaging, antioxidant, and antifungal properties.

Results: Results indicate that the optimal SeNPs formulation includes 5.73 mL of Prunus persica tree leaf extract, 13.45 mL of sodium selenite salt solution, and 0.80 mL of Aloe Vera gel.

Conclusion: The optimal formulation of selenium nanoparticles (SeNPs) achieved in this study, using Prunus persica tree leaf extract as a reducing agent and Aloe Vera gel as a stabilizer, demonstrates superior properties including high stability, a small average particle size, and a favorable zeta potential. These characteristics make the SeNPs well-suited for applications requiring enhanced antioxidant and antifungal activities. The findings underscore the importance of optimizing synthesis parameters to maximize the functional properties of SeNPs.

Keywords: Aloe Vera gel stabilizer, Antifungal, Antioxi, Optimization, Peach tree leaf extract, SeNPs

1. Background

In recent years, nanotechnology has played a crucial role in numerous fields, including physics, chemistry, biotechnology, and medicine. This innovative technology utilizes materials at the nanoscale to offer new possibilities and potentials in various industries (1, 2). Recent advancements in nanotechnology have enabled the production of metal nanoparticles (NPs) with high surface area-to-volume ratios in the nanometer range, expanding their range of applications (3, 4). In recent years, green chemistry and biological techniques have enabled the rapid synthesis of various types of nanoparticles, including selenium (Se), gold, silver, copper oxide, zinc oxide, and metallic NPs. These nanoparticles are produced in an environmentally-friendly, non-toxic, and pure manner, utilizing high-energy renewable materials. This approach improves the safety and performance of nanoparticle development processes (5, 6). Metal nanoparticles have attracted much interest due to their potential as antimicrobial agents (7-9). Due to the excellent biocompatibility and low toxicity of SeNPs, they have gained popularity and are frequently used in biomedicine and food science (10). Potent antioxidant and antibacterial action, high absorption, and minimal toxicity have all been documented for SeNPs (11). The use of plant extracts, microbes, and green chemistry for synthesizing SeNPs presents a promising alternative to physical and chemical processes (12, 13). Studies have indicated that using SeNPs as a powerful antioxidant is significantly safer than using bulk Se (14). SeNPs are produced using various techniques, including chemical, physical, and biological methods. Chemical and physical processes are typically not preferred due to their difficult synthesis conditions, production of toxic byproducts, bio-safety concerns, and high cost (15).The removal of selenite and selenate from effluents is connected to the biosynthesis of SeNPs, which is a cost-effective and advantageous process. Consequently, the biological production of NPs has drawn increased interest (12). When fabricating metal and metal oxide NPs, green synthesis processes have several advantages over physicochemical-based methods (16, 17). Compared to other approaches, biological or green methods for the production of inorganic NPs have several advantages. For instance, biosynthesis techniques are economical, eco-friendly, single-step, and clean procedures that minimize or eliminate environmental toxicity and pollution (18). Plant extract-mediated NPs improve SeNPs production by supplying reducing/stabilizing agents and herbal capping. Additionally, bioactive components such as steroids, tannins, glycosides, quinine, proteins, carbohydrates, saponins, phenols, alkaloids, and flavonoids found in plant extracts can speed up the production of SeNPs in a single step (19). Prunus persica leaves extract is one of the critical reducing agents for synthesizing SeNPs (20). Optimization generally aims to identify the factors that result in the optimal output. Traditionally, optimization has been done by examining each variable separately. However, the main drawback of this method is that it takes much time, and if significant interactions exist between the factors under study, the results could be misinterpreted. As a result, methods for factor optimization using multivariate techniques such as central composite design, Box-Behnken design, and mixture design have been promoted because they are quicker, more practical, and allow for the simultaneous optimization of multiple variables. The simplex centroid mixture design is typically used to investigate correlations between the proportions of different factors and their corresponding responses. It can estimate each component of the mixture along with their interactions, create a continuous surface model of variables, and optimize the component elements to find the best ingredient ratio (21, 22).

2. Objectives

This study centers on three primary objectives: firstly, the synthesis of Selenium nanoparticles (SeNPs) utilizing Prunus persica leaf extract; secondly, the optimization of SeNP formulation, encompassing the quantities of the reducing agent (sodium selenite solution), Prunus persica leaf extract, and Aloe Vera gel, with a specific focus on investigating stability; and thirdly, an in-depth exploration of the physicochemical, antioxidant, and antimicrobial properties inherent in the synthesized SeNPs.

3. Materials and Methods

3.1. Materials

Sodium selenite salt was purchased from Sigma Aldrich. Prunus persica leaves were obtained from local gardens in Ardabil, while the Penicillium digitatum (p.digitatium) fungus with the number PTCC 5251

was obtained from the Microbial Bank of Iran. PCA (Plate Count Agar) medium was purchased from Pars Oxoid, while the antioxidant substance1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich. The distilled water used in the study was obtained from Marwarid Pars Company.

3.2. Preparation of Prunus persica Tree Leaf Extract

After washing and drying the Prunus persica leaves for ten days, 5 grams of the powdered leaves were added to 100 mL of boiling water and left to extract for 15 minutes. The extract was then obtained by filtering it with Whatman paper No. 1 and kept refrigerated.

3.3. Synthesis of SeNPs

Based on previous sources, the concentration of sodium selenite salt required for the green synthesis of NPs is 10 mM. For this experiment, 0.263 grams of sodium selenite salt was added to 100 mL of double-distilled water. Aloe Vera gel was used as a stabilizing agent for the synthesized SeNPs. Using the Design of Experiments (DOE) method, varying amounts of the three selected materials were tested to identify the optimal formulation of NPs. Specifically, 0 to 8 mL of Prunus persica tree leaf extract, 10 to 20 mL of sodium selenite salt solution, and 0 to 2 mL of Aloe Vera gel were considered. After conducting the 13 runs of DOE, all the samples were placed in a hydrothermal autoclave under the same conditions of 121 °C and 1.5 bar pressure for 15 minutes.

3.4. Analyzes

3.4.1. Experimental Design and Data Statistical Analysis

The interaction effects between the absorbance (Y_1, a.u.), mean particle size (Y_2, nm), PDI (Y_3), and zeta potential (Y_4, mV) of the synthesized NPs were studied using an enhanced central composite design with 13 points (**Table 1**). The amounts of Prunus persica tree leaf extract (X_1, 0.0- 8.0 mL), sodium selenite salt solution (X_2, 10.0-20.0 mL), and Aloe Vera gel (X_3) were selected as independent variables. The significance of the model terms, estimated coefficients, and coefficient of determination were evaluated using mixture analysis (R2). To generate the distinctive cubic models, the following multiple regression coefficients were calculated (Eq. **2**):

$$Yi=\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{123}X_1X_2\ X_3 \quad \textbf{(2}$$

β_{123}, β_{12}, β13, and β_{23} are the interaction terms for ternary and binary effects, Yi represents the expected response, and β_1, β_2, and β3 are the regression coefficients for each linear effect term (23).The significance of the determined regression coefficients was evaluated using the T-value at a probability (p) of 0.1(23, 24). The contour plots illustrate the relationship between the response and the three stabilizer components as suggested by the models. The entire experimental design, data analysis, regression, and optimization processes were performed using the Minitab v.14 (Minitab Inc., PA, USA).

3.4.2. Specifications of the Extracted Se Essential Oil

The primary functional groups present in the SeNPs were identified using FTIR spectroscopy (FTIR 8400s, Shimadzu Co, Kyoto, Japan) with KBr pellets in the 4000-400 cm^{-1} range.

3.4.3. Physico-Chemical Specifications of the Prepared SeNPs

The particle size, polydispersity index (PDI), and zeta potential of the produced Se O/W NPs were measured using a particle size analyzer based on dynamic light scattering (DLS) (Malvern Instruments, Zetasizer Nano ZS, Worcestershire, UK). FTIR spectroscopy (FTIR 8400S, Shimadzu Co., Kyoto, Japan) was used to identify the major functional groups present in the synthesized NPs. To assess the morphological characteristics of the synthesized NP, transmission electron microscopy (TEM, CM120, Philips, Amsterdam, Netherlands) was used with an acceleration voltage of 120 kV.

3.4.4. Fungicidal Properties of the Made SeNPs

The samples' fungicidal activity was evaluated based on the inhibition of the P. digitatum strain's growth in the mycelia. In this procedure, a potato dextrose agar disk with a pure culture of the fungus was obtained, placed in the center of provided plates, with PDA (Potato Dextrose Agar) serving as the control sample. The plates were then amended with the prepared NP and extracted essential oil. The plates were then incubated at 26 °C for ten days. Daily measurements of radial mycelial growth for the samples and the control were taken, and the extent of fungal mycelial growth inhibition caused by the samples› fungicidal effects was reported (in mm).

Table 1. Matrix of an augmented simplex-centroid design

Independent variables Amount of Materials / mL					Independent variables (Response)		
Sample No.	Amount of Peach tree leaf extract	Amount of Sodium selenite salt solution	Amount of Aloe Vera Gel	Absorbance / a.u.	Mean Particle Size /nm	PDI	Zeta Potential / mV
1	0.0	20.0	0.0	0.01	303	0.621	0.2
2	0.0	19.0	1.0	0.09	312	1.000	2.9
3	8.0	12.0	0.0	2.59	79	0.398	2.4
4	2.0	16.5	1.5	1.29	125	0.801	18.8
5	4.0	14.0	2.0	1.69	81	0.694	27.8
6	4.0	15.0	1.0	1.87	45	0.595	19.9
7	2.0	17.5	0.5	1.25	89	0.735	9.4
8	6.0	12.5	1.5	2.61	51	0.732	25.1
9	6.0	13.5	0.5	2.75	38	0.492	11.5
10	8.0	10.0	2.0	2.31	108	0.768	28.4
11	4.0	16.0	0.0	1.82	40	0.398	6.5
12	8.0	11.0	1.0	2.56	79	0.678	17.9
13	0.0	18.0	2.0	0.07	353	1.000	3.1

3.4.5. Antioxidant Activity of the Prepared SeNPs

The antioxidant activity of the synthesized SeNPswas measured using a method based on free radical-scavenging activity. 100 µL of the samples were mixed with 5 mL of a 50% methanol solution containing DPPH radicals (1 mM). As a control sample, a solution consisting of pure DPPH and methanol at a ratio of 1:1 (V/V) was used. After vigorous shaking, the samples and controls were kept in a dark environment at 27 °C for 30 minutes. Using a UV-Visible spectrophotometer (250-800 nm, Perkin Elmer Co., Rodgau, Germany), the maximum absorbance of the samples was measured at 517 nm, and their scavenging capacity was calculated using Eq. **(1**:

$$I\% = ((A_{control} - A_{sample}) / A_{control}) \times 100 \quad (1$$

Where $A_{control}$, and A_{sample} are the absorbance of the control and the sample, respectively.

4. Results

4.1. Analysis of Response Models

Experimental data was analyzed using mixture regression, utilizing a unique cubic model to predict absorbance, particle size, PDI, and zeta potential empirically. In **Table 2**, the regression coefficients and determination coefficients (R2 and R2-adj) are presented.

Table 3 shows the interaction effects of stabilizer compounds, as well as the p-value and T-value for regression coefficients, in the final reduced special cubic polynomial models for the particles. The significant interaction terms in the special cubic model of absorbance, particle size, PDI, and zeta potential indicate that these responses are influenced by either the individual stabilizer compounds or by the binary and ternary interactions. According to the data presented in **Tables 2** and **3**, *Aloe Vera* gel had the most significant influence (b3, b2 and b1) on the three examined responses.

4.2. Influences of Preparation Parameters on Responses of the Made SeNPs

The average particle size of NPs is one of the most critical factors in their evaluation, as those with smaller particles have higher surface-to-volume ratios and exhibit significantly higher activity levels. As per the experiment's design, the average NP size ranged from 38 to 353 nm, as shown in **Table 1**. These values were particularly intriguing because the average particle size of NPs is typically less than 1000 nm.

Table 2. Regression coefficients, R2, adjusted R2, and probability values for the final reduced models

Regression Coefficients	Absorbance /a.u.	Mean Particle Size / nm	PDI	Zeta Potential / mV
	Y_1	Y_2	Y_3	Y_4
b_1	-28.1	20.97	24.86	-127.8
b_2	-0.49	2.93	6.67	-1.35
b_3	-78.8	78.64	-411.8	-643.4
b_{12}	157.3	-38.63	-41.96	217.7
b_{13}	860.2	-123.6	467.5	1568.8
b_{23}	794.1	-78.13	501.8	782.6
R-square (%)	98.55	98.13	95.52	97.32

Table 3. The significance probability (p-value, t value) of interaction coefficients in final reduced polynomial models

Linear effects	X1X2	X1X3	X2X3
Absorbance / a.u.			
P-Value	0.000	0.047	0.095
Linear effects	X1X2	X1X3	X2X3
F-Value	37.30	5.30	3.72
Mean Particle Size / nm			
P-Value	0.000	0.042	0.179
F-Value	139.74	7.46	2.23
PDI			
P-Value	0.002	0.012	0.008
F-Value	24.02	11.44	13.36
Zeta Potential / mV			
P-Value	0.000	0.028	0.048
F-Value	38.19	7.63	4.98

4.2.1. Effect of Formulation on the Absorption Rate

The impact of the formulation of three substances on the amount of synthesized SeNPs absorption is presented (**Fig**. **1A (a and b)**). The DOE synthesized samples resulted in most having a minimum absorption value of over 1.5. The results indicate that a higher amount of the chosen extract leads to a higher concentration of synthesized SeNPs, resulting in a higher absorption number (**Fig**. **1A (a)**). However, due to the lack of synthesis and NP formation at low levels of Prunus persica tree leaf extract, the concentration is insufficient. The bold area corresponds to NPs with high concentration, resulting from the optimal mixture of sodium selenite salt and the extract (**Fig**. **1A (b)**). At low levels and in the absence of the extract, the concentration of SeNPs was the lowest, as indicated by the pale color (**Fig**. **1A (b)**). As can be seen in **Table 3**, the p-value of the interaction between the extract and sodium selenite salt solution is the lowest (highest effect). **Table 1** presents the results obtained from the DOE experiment, which are justifiable. The maximum absorption (concentration) of synthesized SeNPs can be attributed to the combination of 6 mL and 8 mL of Prunus persica tree leaf extract, while the lowest absorption was observed for 0 and 2 mL of extract. High levels of sodium selenite salt may not result in the synthesis and reduction of the materials, leading to either no formation of NPs or the formation of NPs

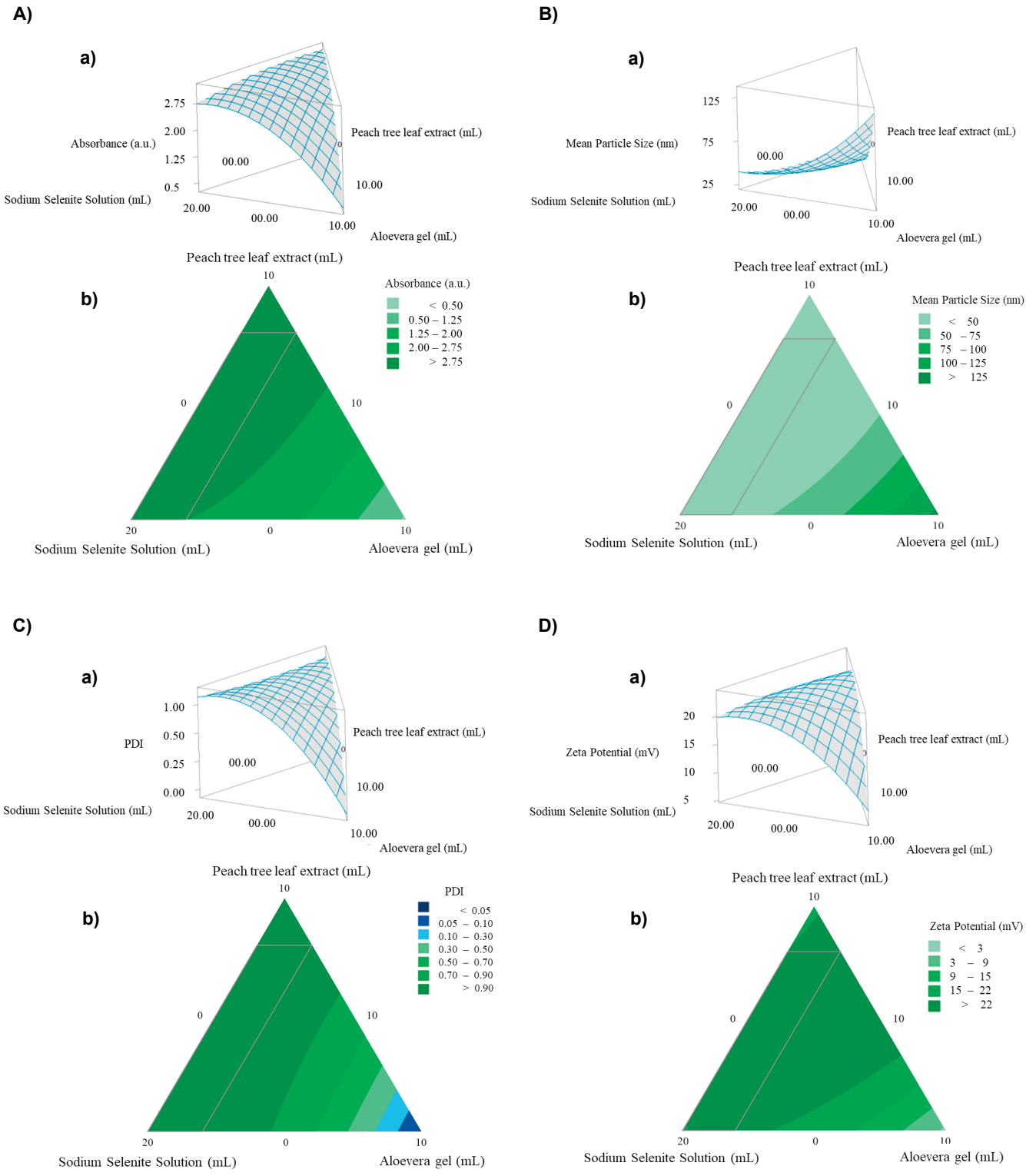

Figure 1. Effect of a mixture of sodium selenite salt, Prunus persica leaf extract, and Aloe Vera gel on: A) absorption rate (concentration measurement), **B)** average particle size, **C)** PDI, and **D)** zeta potential (a measure of stability).

with low concentration. **Table 2** reports the regression coefficients and predicted values resulting from the DOE for the concentration of synthesized NPs.

4.2.2. Effect of Formulation on Average Particle Size

The impact of the synthesis formulation on the average particle size of SeNPs is illustrated (**Fig**. **1B (a and b)**). The 3D view of the average particle size from the 13 tests conducted is displayed (**Fig**. **1B (a)**). Opting for the optimal formulation of Prunus persica tree leaf extract and sodium selenite salt will result in obtaining low values of the average particle size. High and low amounts of plant extract and high amounts of sodium selenite salt result in an average particle size exceeding 100 nm, and in some samples, exceeding 300 nm. Varying ranges of average particle size are depicted with different colors, where bold colors indicate high average particle sizes and pale colors correspond to sizes less than 50 nm (**Fig**. **1B (b)**). **Table 3** reports p-values indicating that the interactions between the sodium selenite salt solution and the extract, as well as the sodium selenite salt solution and the *Aloe Vera* gel, have a significant impact on this response. **Table 1** presents the results obtained from DOE, indicating that the smallest average particle size is 38 nm. This size can be achieved with 6 mL of extract, 13.5 mL of sodium selenite salt, and 0.5 mL of Aloe Vera gel. On the other hand, the largest average particle size (values exceeding 300 nm) is related to the absence of the extract and varying amounts of sodium selenite salt and *Aloe Vera* gel. It is natural and justifiable that in the absence of Prunus persica tree leaf extract, there is no regenerating agent in the environment, and NPs will not form. **Table 2** reports the regression coefficients and predictions for various numbers obtained from the DOE, along with the average size of the synthesized NPs.

4.2.3. Effect of the Formulation on the PDI

The impact of sodium selenite salt, extract, and Aloe Vera gel on the PDI is presented, revealing that only a few samples achieve a suitable PDI below 0.5, while the majority have a PDI above this threshold. The two-dimensional representation of these effects shows numerical values ranging from 0 to 1 (**Fig**. **1C (a and b)**). The blue area and its range correspond to the most favorable PDI, while the bold area represents the highest PDI values. The samples without Prunus persica tree leaf extract exhibit a substantially high PDI value of 1. This is due to the disparity between the sizes of sodium selenite salt and *Aloe Vera* gel, resulting in extremely varied particle size measurements. Consequently, the dispersion index is significantly elevated. Upon comparing the outcomes presented in **Table 3** with the reported p-values, it is evident that all potential interactions scrutinized to evaluate the PDI parameter hold significance. The corresponding values for these interactions are below 0.05. Based on the data provided in **Table 1** and a thorough comparison of the findings, the minimum PDI value of 0.398 corresponds to the application of 4 and 8 mL of extract, 16 and 12 mL of sodium selenite solution, and the absence of *Aloe Vera* gel. **Table 2** presents the regression and prediction coefficients.

4.2.4 Effect of Formulation on Zeta Potential

The effect of three different material formulations used for synthesizing SeNPs on the zeta potential, which measures stability, is analyzed in the study (**Fig**. **1D (a and b)**). The three-dimensional view of the effects of the formulation on the zeta potential is shown through the data analysis (**Fig**. **1D (a)**). Most of the synthesized samples exhibit high values of zeta potential, which can be attributed to several factors. The primary factor is the presence of *Aloe Vera* gel in the environment, which creates a spatial barrier between the NPs and prevents them from communicating with each other. As a result, the NPs maintain their size and state. The low values of zeta potential (3-9 mV) are associated with the absence of *Aloe Vera* gel in the environment. In such cases, the size of the NPs increases after a certain period, and instability eventually becomes apparent in the NPs. Another innovative aspect of this article is the utilization of *Aloe Vera* gel to ensure maximum stability of NPs for an extended period. The impact of the formulation on three other parameters, including absorption rate, average particle size, and PDI, was also explained. The presence of *Aloe Vera* gel was found to have a negative effect on some of these parameters, leading to inappropriate results. In certain cases, the presence of *Aloe Vera* gel is ineffective and does not yield positive results. However, without *Aloe Vera* gel, the synthesized NPs will only have limited application for a few days and become unstable after a while. To observe the combined effects of these factors and obtain the best combination of percentage and formulation, optimization and DOE were performed in a single

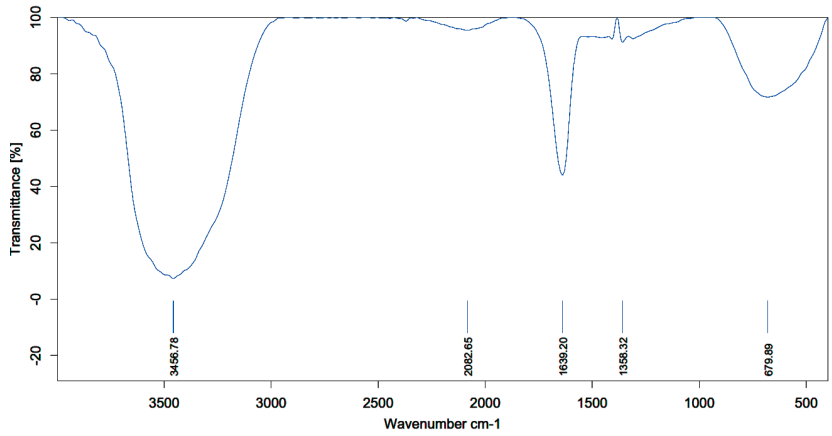

Figure 2. FTIR analysis of Prunus persica leaf aqueous extract.

step. A large area is highlighted with a bold color, representing samples with a suitable zeta potential. Out of the 13 tested samples, 10 included Aloe Vera gel in their formulation (**Fig**. **1D (b)**). The presence of *Aloe Vera* gel creates a suitable surface charge, ultimately leading to the stability of the NPs. Comparison of the results obtained from **Table 3** with the reported p-values shows that all the interactions of the variables considered to verify the PDI parameter are significant. The corresponding values for these interactions are less than 0.05. By comparing the different p-values related to the interaction with the amount of *Aloe Vera* gel, it is evident that this factor has a significant effect in this section. **Table 1** reports the numerical values of the 13 tests. The value of 28.4 mV corresponds to the formulation containing 8 mL of extract, 10 mL of sodium selenite salt solution, and 2 mL of *Aloe Vera* gel. The lowest zeta potential value of 0.2 corresponds to the formulation with 20 mL of only sodium selenite salt. The absence of *Aloe Vera* gel can lead to instability in the synthesized NPs. **Table 2** reports the regression coefficients and predicted values of various parameters resulting from the DOE, including the synthesized zeta potential.

4.3. FTIR Analysis

The analysis of the functional groups in the aqueous extract of *Prunus persica* tree leaves reveal five indicative peaks, with prominent peaks observed at wave numbers 3456 and 1639, corresponding to OH functional groups (**Fig**. **2**). As well as, the peak obtained indices are related to wave numbers 2082, 1358 and 679, related to functional groups of terpene and flavonoid substances, which have a high regenerative effect. As mentioned in the introduction section, the leaves of the Prunus persica tree have substances with regenerative properties, and by extracting them, these substances will be extracted and their regenerative properties will be strengthened, that is why the extract of this plant was used in this research(3).

4.4. Optimization of Processing Parameters for NPs Preparation

The results of numerical multiple optimizations also showed that the best conditions for preparing SeNPs in subcritical water were 6.0 mL of Prunus persica tree leaf extract, 13.5 mL of sodium selenite salt solution, and 0.5 mL of *Aloe Vera* gel. SeNPs produced under these ideal conditions had nanodroplet sizes of 38 nm and PDI values of 0.492, which were the smallest among all.

4.4.1. Graphical and Numerical Optimization

The results of the optimization indicate an optimal area, as outlined by the figure guide (**Fig**. **3**). This corresponds to the highest absorption rate, the lowest average particle size, the lowest PDI, and the highest zeta potential. The optimal area falls in the white range, which is close to the guidelines with appropriate values. Numerical optimization was performed based

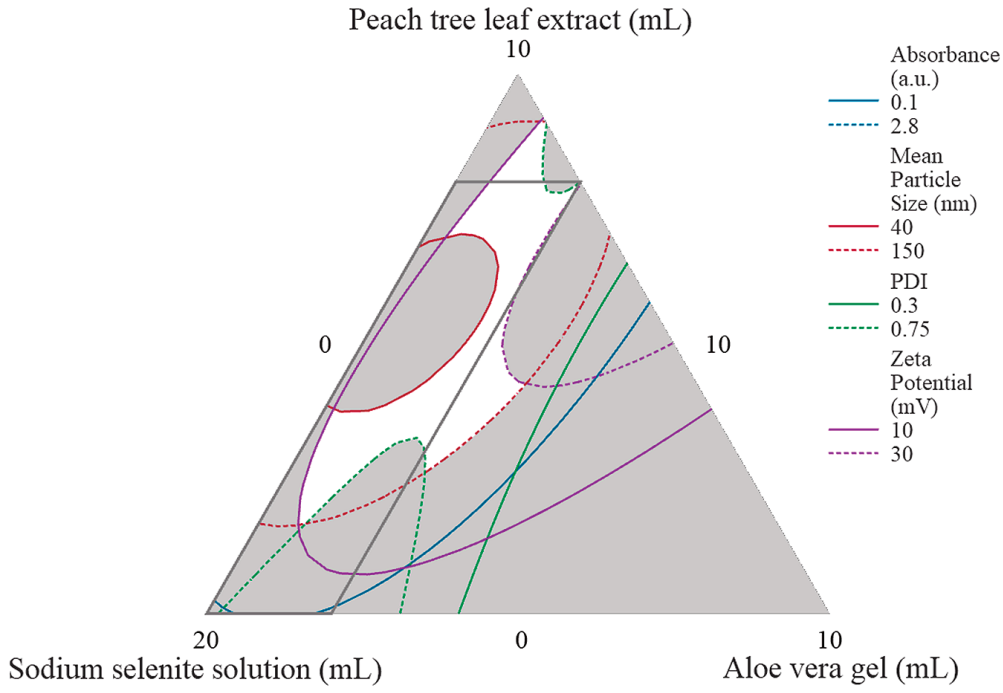

Figure 3. Optimization results for the synthesis of SeNPs.

on the upper range that was optimally obtained in graphic mode. The results of the optimization are as follows: if the optimal point is obtained using the values of independent variables and formulation as follows: 5.73 mL of Prunus persica tree leaf extract, 13.45 mL of sodium selenite salt solution, and 0.80 mL of *Aloe Vera* gel to synthesize SeNPs, then the selected output parameters, including absorption rate (2.7897 absorption number), average particle size (36.75 nm), PDI (0.4846), and zeta potential (26.14 mV) will be achieved. These results are depicted in the data presented (**Fig**. **4**).

4.4.2. DLS and Zeta Potential Analysis of the Optimal Sample

Since some of the output variables are determined based on the average particle size and PDI, it is essential to perform this analysis to validate the results of the experimental design and statistical analysis. The size distribution curve of SeNPs under optimal conditions indicates a consistent particle size within the desired range (**Fig**. **5A**). The particle size distribution falls within the range of 30 to 40 nm, as demonstrated by the data (**Fig**. **5B**). By performing this analysis with three repetitions, an exact value of 38 ± 2 nm was obtained, which had a slight difference from the optimal value obtained from the experimental design (36.75 nm). Another parameter that was obtained by DLS analysis is the PDI, which had a value of 0.51±0.02. This value is close to the optimal value reported from the statistical analysis (0.484).

Through three repetitions, zeta potential was determined to be 25 ± 0.7 mV, which was very close to the optimal value obtained from the experimental design and statistical analysis (26.14 mV). After obtaining the optimal points of the independent variables and conducting the experiments, and reviewing and analyzing the analyses performed, including the amount of absorption, average particle size, particle dispersion index, zeta potential, and several other analyses, the results indicated that there was no significant difference between the predicted results and the results obtained from the laboratory. The value is ($p>0.05$), confirming that the obtained models have correctly determined the effects of independent variables on dependent variables in the desired range.

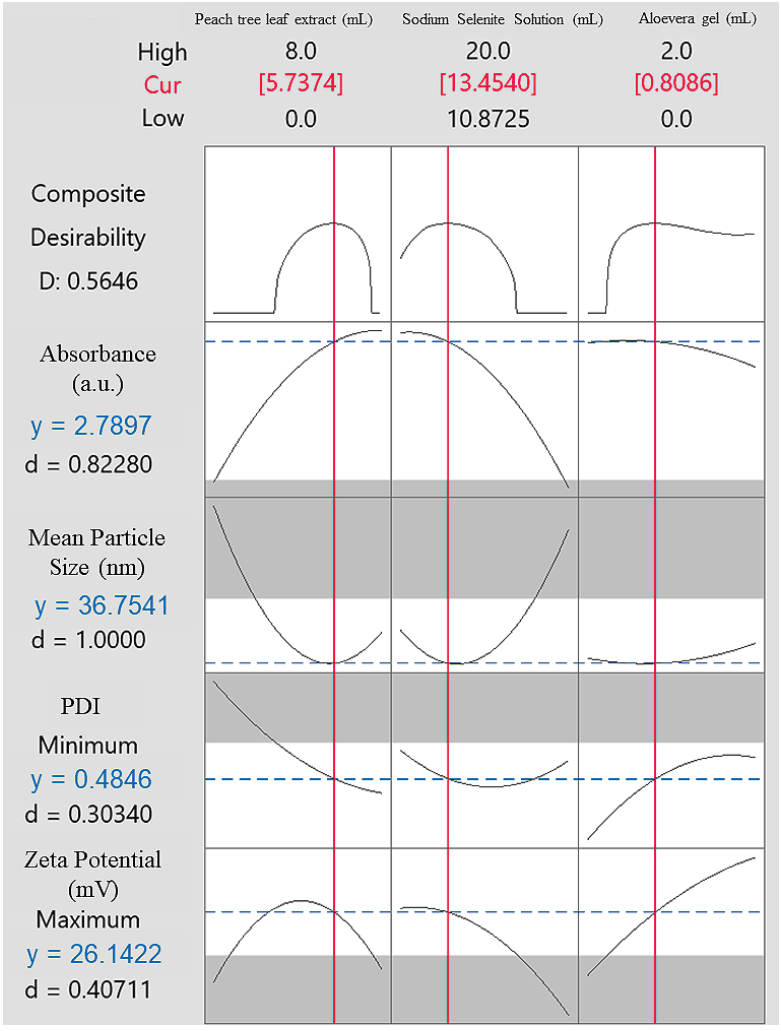

Figure 4. Adsorption rate, average particle size, particle dispersion index, and zeta potential at the optimal point.

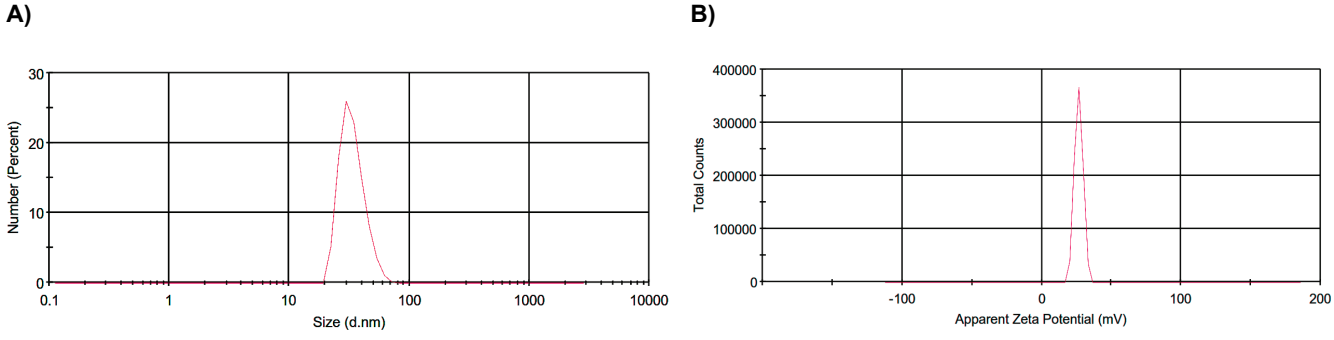

Figure 5. Comprehensive characterization of the optimal SeNPs sample through A) DLS, and **B)** Zeta potential analysis.

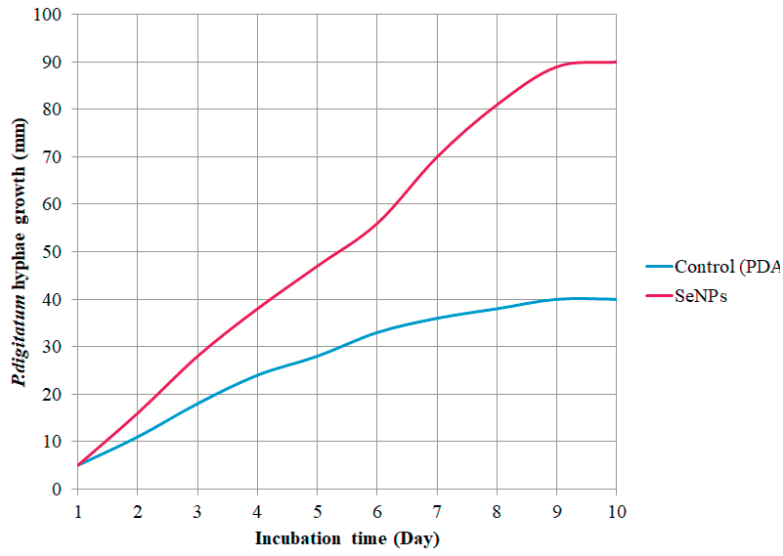

Figure 6. Growth of Penicillium digitatum fungus in the presence and absence of SeNPs after ten days.

4.4.3. Antifungal and Antioxidant Properties of SENPs Synthesized in Optimal Conditions

The growth of *P. digitatium* fungus was observed over ten days, both in the presence and absence of SeNPs, allowing for a direct comparison of the effects (**Fig. 6**). To accurately calculate the antifungal property, the growth of fungus was measured daily by recording and plotting the fungi growth diameter (in millimeters) against the growth time (in days) for each of the plates (plates containing SeNPs and those without the presence of NPs). The following equation was used to calculate its inhibition percentage.

$$\textit{Antifungulactivity}\ (\%) Y = ((D_C - D_S)/D_C) \times 100 \qquad (3$$

$$\textit{Antifungulactivity}\ (\%) Y = ((90-41)/90) \times 100 = 54.44\% \qquad (4$$

Since the diameter of the plates used was 90 mm, after ten days, the fungus grew entirely in the control sample (plate without NPs). The growth rate of the plate containing SeNPs after ten days was 41 mm. Finally, by using the equation, the inhibition percentage of the produced product was calculated to be 54.44%. This is a good percentage for the anti-fungal properties of the material. It appears that the antifungal property of the produced product and its mechanism of effect on the growth of fungus are related to the change in the physical structure of the fungi cells. The SeNPs, which have been converted into tiny particles, can easily pass through the cell wall and cytoplasmic membrane. As a result, not only does radial growth stop, but sporulation is also inhibited. Ultimately, this leads to inactivation and inhibition of cell growth and proliferation. Moreover, the synthesized SeNPs, with their high surface-to-volume ratio, can effectively control the cell membrane and alter its permeability (3).The antioxidant property of materials is of great importance and if a material has this property, it will work well in inhibiting free radicals (18), by evaluating the antioxidant property of selenium nanoparticles synthesized in the optimal state by the DPPH method, A value of 14.35% was obtained, which will be considered a relatively acceptable property in inhibiting free radicals. In order to check the effect and antioxidant property of the synthesized product, the antioxidant property of sodium selenite solution and peach tree leaf extract was also evaluated and this property was obtained for 4.4% extract and 6.6% sodium selenite solution, by comparing the obtained results. For selenium nanoparticles, the extracted extract and sodium selenite solution, it is concluded that the antioxidant properties of selenium nanoparticles are produced due to their small and nanometer dimensions, which ultimately lead to the best inhibition of free radicals.

5. Discussion

SeNPs are among the most important nanoparticles used in various industries. Green technology has been employed in this article. Leaf extract from the Prunus persica tree was used as a reducing agent, and *Aloe vera* gel was used as a stabilizer for the synthesis of SeNPs. An experimental design was conducted to optimize the synthesis formula for nanoparticles with the highest stability, highest concentration, smallest average particle size, and complete uniformity using the mixed-design method.

The results indicate that as the selected extract amount increases, the synthesized selenium nanoparticles have a higher concentration and will yield a higher absorption value. However, at low amounts of Prunus persica tree leaf extract, since no synthesis takes place and nanoparticles won't form, the concentration is very low. At very low amounts and in the absence of extracted extract, the concentration of selenium nanoparticles has been the lowest. In high amounts of sodium selenite salt, due to the possibility of non-synthesis and the lack of reduction of these substances, nanoparticles either do not form or form with a low concentration.

Similarly, Alizadeh *et al*. investigated antimicrobial, cytotoxicity, and catalytic activities of biosynthesized SeNPs using Crocus caspius extract. SeNPs were synthesized by Crocus caspius aqueous extract. Biomolecules found in C. caspius extracts were attributed to reducing Se4+ to Se (0). The DLS particle size analyzer revealed an average size of 266.3 nm by number and zeta potential of −44.75 mV for C@ SeNPs. Synthesized NPs demonstrated effective antifungal activity against several fungi strains (MIC = 0.53 $\mu g.mL^{-1}$) and antileishmanial activity against promastigotes (IC50 = 42.81 $\mu g.mL^{-1}$)(25).

Hussein Ali et al. synthesized SeNPs by using aqueous extract of peel Solanum Melongena L. They synthesized SeNPs from an aqueous extract of eggplant peel. SeNPs were produced in a single step utilizing an aqueous extract of peel Solanum melongena L. as a reducing and capping agent. NPs sized were between 50 and 150 nm were observed using a SEM(26).

Cittrarasu *et al*. explored how SeNPs could be produced naturally using an extract from Ceropegia bulbosa Roxb. Their research found that the SeNPs had an IC50 value of 34 $\mu g.\ mL^{-1}$ against MDA-MB-231 cells over a 48-hour period. Additionally, these SeNPs demonstrated inhibitory effects on the growth of certain clinical pathogens like Bacillus subtilis and *Escherichia coli*. They also showed significant larvicidal activity against Aedes albopitus mosquito larvae, with a maximum mortality concentration of 250 $g.\ mL^{-1}$ after 24 hours of exposure. Furthermore, computational analysis indicated that SeNPs maintained consistent stability in relation to the breast cancer protein BRCA2. Overall, their discoveries suggest that SeNPs could serve as a powerful disruptor against MDA-MB-231 cells, specific pathogens, mosquito larvae, and could enhance the degradation of photocatalytic dye(27).

Salem et al. conducted a study on the eco-friendly biosynthesis of selenium nanoparticles (SeNPs) using discarded orange peel waste. The research focused on utilizing orange peel waste (OPW) as a green and environmentally friendly method for the synthesis of Se-NPs, exploring their potential applications as agents with antibacterial and antibiofilm properties. The findings indicated that Se-NPs displayed significant antibacterial effects against both Gram-positive bacteria (including S. aureus ATCC 29213 and biofilm-producing clinical isolates of S. aureus) and Gram-negative bacteria (such as Pseudomonas aeruginosa PAO1, multi-drug-resistant strains, biofilm-forming variants, and quorum-sensing-producing clinical isolates of multi-drug-resistant P. aeruginosa, multi-drug-resistant *E. coli*, and K. pneumonia) (28).

One of the parameters that will increase the average particle size is the presence of *Aloe vera* gel. Due to the larger molecular size and higher viscosity of *Aloe vera* gel compared to sodium selenite salt and extracted leaf extract, it appears that the presence of this substance in the environment leads to an increase in the average particle size. Therefore, in determining the average size of selenium particles, the optimal ratio of Prunus persica tree leaf extract to sodium selenite salt is one of the main influential factors, and lower amounts of *Aloe vera* gel will improve and reduce the average particle size.

The presence of *Aloe vera* gel in the environment creates a spatial barrier between the formed nanoparticles, preventing them from coming into contact with each other, ultimately maintaining their sizes. Low zeta potential values (3-9 mV) also correspond to a state where *Aloe vera* gel is not present in the environment, and the formed nanoparticles will increase in size after a specific period, ultimately leading to instability in the nanoparticles. The innovation of this paper lies in

the use of *Aloe vera* gel for the long-term stability of nanoparticles.

The product produced was also evaluated for its antifungal properties, and the fungus P. digitatum was used as the test sample.

The antifungal properties of the produced product and their mechanism of action on fungal growth are related to changes in the physical structure of fungal cells. The synthesized selenium nanoparticles, with their high surface-to-volume ratio, exert complete control over the fungal cell membrane, altering its permeability significantly.

6. Conclusion

This study successfully optimized the synthesis formulation of SeNPs, yielding nanoparticles with desirable properties such as a high absorption peak, small particle size, and low polydispersity index. The use of Prunus persica leaf extract and Aloe Vera gel proved to be effective in producing stable SeNPs with a zeta potential indicative of good colloidal stability. The hydrothermal method, specifically autoclave heating, was confirmed to be a reliable technique for achieving uniform particle characteristics. The synthesized SeNPs exhibited potent antioxidant and antifungal activities, highlighting their potential for diverse industrial applications. Future research should explore alternative green synthesis methods, such as microwave or heater-stirrer techniques, to further enhance the properties of SeNPs and expand their applicability. These findings not only advance the field of nanoparticle synthesis but also open avenues for the development of SeNPs with tailored properties for specific industrial uses.

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Competing interests

The authors declare that they have no competing interests.

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