# Green Synthesis, Optimization and Characterization of Carrot Extract Silver Nanoparticles

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*Original Research Article*

# Green Synthesis, Optimization and Characterization of Carrot Extract Silver Nanoparticles

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ABSTRACT

Silver nanoparticles (AgNPs) are currently being used in the field of nanomedicine. Carrots are plants with phytochemicals that act as reducing and capping agents for nanoparticles. The use of plant extracts is considered a safe, non-toxic, and effective therapeutic option for various diseases. The aim of this study was to synthesis, optimize, and characterize silver nanoparticles using carrot extract. The green synthesis of carrot extract AgNPs was carried out by mixing ethanol extract of carrot with silver nitrate ($AgNO_3$) at concentrations ranging from 1 to 3 mM and pH 6 - 8. Carrot extract silver nanoparticles (CE-AgNPs) was characterized using UV-Vis spectrophotometry, particle size analysis (PSA), Field Emission Scanning Electron Microscopy (FE-SEM), and X-ray diffractometry (XRD). The results showed that the optimal synthesis of carrot extract silver nanoparticles is done using $AgNO_3$ 1 mM concentration, and pH 7 which produced the smallest particle size. FE-SEM indicated the formation of spherical silver nanoparticles. XRD analysis indicated that the nanoparticles contain silver components. In conclusion, the conditions for carrot extract silver nanoparticles production can affect the particle size and morphology of the nanoparticles.

*Keywords:* Green synthesis, Silver nanoparticles, Carrot extract, Optimization.

## Introduction

Nanotechnology is a science that continues to be developed due to its huge potential in various fields. Its potentials can be harnessed in fields of cosmetics, renewable energy, environmental science, health, and medicine.[1-2] Nanotechnology can also be employed in drug encapsulation and targeted drug delivery, increasing drug effectiveness and reducing toxicity.[2] Nanoparticles can be made using chemical, physical, and biological methods. Chemical method produces nanoparticles in large quantities and in a short time, but releases hazardous and toxic by-products that impact human health and the environment.[3–5] Physical method uses special equipment that requires high pressure and temperature, requiring large energy, expensive operational costs, and also produces small quantities of nanoparticles.[4] Biological method is an ideal way to produce nanoparticles because it is simple and harmless.[3]
There are two approaches to the synthesis of nanoparticles: the top-down and bottom-up methods. The top-down approach breaks down the material into small particles, while the bottom-up method forms nanostructures from small particles.[4] The chemical and biological methods of making nanoparticles use the bottom-up approach, while the physical methods use the top-down approach.

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The biological method of manufacture of nanoparticles uses green synthesis, which aims to minimize toxicity to humans and the environment. This is because the green synthesis method uses enzymes, microorganisms, plants or plant extracts as alternatives to chemical substances to produce nanoparticles.[2] The use of plants result in a more stable nanoparticles and a faster synthesis rate compared to other organisms.[6] The advantages of green synthesis are that it is more environmentally friendly compared to chemical methods, less energy is used compared to chemical and physical methods, it is cheaper, it can be produced in large quantities, and it is efficient.[4,7,8] Silver nanoparticles (AgNPs) are nanoparticles that are often used in the development of nanomedicines.[2] AgNPs are commonly used because they have distinctive properties such as good conductivity, stability, and have the potential for antibacterial, antidiabetic, antifungal, anticancer, antioxidant, antiviral, and anti-inflammatory activities.[4,9–11] Plant extracts act as reducing agents and capping agents in nanoparticles.[3-4] Plants contain phytochemicals such as tannins, flavonoids, polyphenols, ascorbic acid, and terpenoids, which function as reducing agents to convert $Ag^+$ to $Ag^0$ in the production of AgNPs.[2-4] Plant extracts are considered as safe, and effective therapeutic option for various diseases.[2]
Silver nanoparticles (AgNPs) are influenced by conditions such as pH, temperature, silver concentration, metal interaction with reducing agents, and adsorption of capping agents.[7] These parameters affect the size, shape and morphology of silver nanoparticles.[2] Therefore, it is important to develop an optimal conditions for the green synthesis of silver nanoparticles. In recent years, research on silver nanoparticles using various types of plants has been successfully carried out.[2] Many studies have shown that the use of plant-based silver nanoparticles can treat various diseases, such as diabetes, high blood pressure and immunological disorders.[3]
Carrots contain carotenoids, vitamins, polyphenols, polyacetylene, ascorbic acid, potassium, fibre, and minerals that are useful in reducing the risk of cardiovascular disease due to their antioxidant properties.[12,13] Carrots also have antihypertensive and diuretic activities.[15,16] A major component of carrots is carotenoids (β-carotene) comprising about 80%

of the total components.[12] Generally, carrots contain about 2000 $\mu$g of carotenoids/100 grams dry weight.[16] Carotenoids have been shown to have free radical scavenging, and anti-mutagenic activities, and also boost immunity.[14] The phytochemicals in carrots, in addition to their health benefits, can also act as reducing and capping agent for the synthesis of silver nanoparticles (AgNPs).[3] The synthesis of silver nanoparticles has been carried out previously using carrot juice and carrot aqueous extract, but the manufacture of silver nanoparticles using carrot ethanol extract has not been reported.[3,17]

This study aims to optimize and characterize silver nanoparticles using carrot ethanol extract. Nanoparticle production was optimized at different pH and $AgNO_3$ concentrations. The characterization of the formed nanoparticles was done using UV-Vis spectrophotometry, particle size analysis (PSA), Field Emission Scanning Electron Microscopy (FE-SEM), and X-ray diffractometry (XRD).

## Material and Methods

*Plant material and chemicals*

Carrot (*Daucus carota* L.) was collected from Batu, East Java, Indonesia. Other materials used includes $AgNO_3$ (Merck, Germany), ethanol (Emsure, Germany), NaOH 0,1 N (Merck, Germany), and demineralized water (Water One, Indonesia).

*Preparation of carrot extract*

Carrots were washed with water until clean. Then, the carrots were thinly sliced and put into an oven (Yenaco) at a temperature of 50°C for 4-5 days until dry. Then, the carrots were ground into a fine powder.[18] The extraction process was carried out by mixing the dried powdered carrot with 70% ethanol at a ratio of 1:20 (w:v). The mixture was placed in an ultrasonicator (Biobase), and sonicated at 3[illegible]°C for 17 minutes.[19] Then, the extract was filtered using Whatmann No.1 filter paper. The carrot ethanol extract was stored at 4°C until needed for use.[3]

*Biosynthesis of carrot extract silver nanoparticle (CE-AgNPs)*

Ethanol extract of carrots (10 mL) was mixed with a $AgNO_3$ solution (90 mL). Furthermore, the solution was stored overnight in a dark container to prevent photo activity. Thereafter, the silver nanoparticle solution was centrifuged at 10000 rpm for 20 minutes. The supernatant was discarded, and the pellets were washed with demineralized water to remove impurities. Then, the pellets were dried in an oven at 50°C.[2-3]

*Optimization of carrot extract silver nanoparticle (CE-AgNPs)*

The synthesis of silver nanoparticles used silver nitrate at concentrations of 1 mM, 2 mM, and 3 mM and pH of 6, 7, and 8. NaOH (0.1 N) was used to create the different pH conditions at which the nanoparticles were synthesized.[3] The synthesis of carrot extract silver nanoparticles at various concentrations of $AgNO_3$ was carried out at pH 7, while the synthesis at various pH was carried out using an $AgNO_3$ concentration of 1 mM.

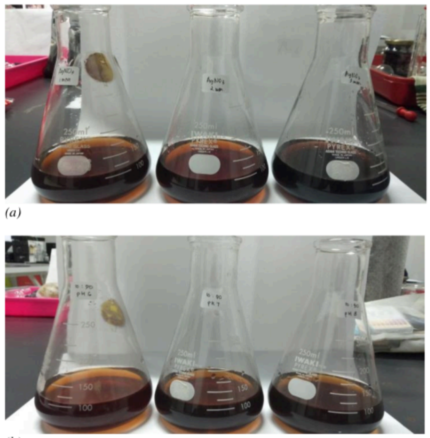
*Characterization of carrot extract silver nanoparticle (CE-AgNPs)*

The formation of silver nanoparticles was observed visually by a change in colour of the $AgNO_3$ solution on addition of carrot extract. Furthermore, the wavelength of maximum absorption ($\lambda_{max}$) of the synthesized CE-AgNPs was determined using UV-Visible spectrophotometer (Shimadzu UV-1780). The UV-Vis spectrum was recorded at wavelength range of 300 - 500 nm. The particle size was determined using a particle size analyzer (PSA) (Biobase BK 802N). Field Emission Scanning Electron Microscope (Hitachi Regulus 8220), and X-ray diffractometer (X Ray Diffraction Rigaku Miniflex 600) were used to determine the morphology, structure and composition of the CE-AgNPs.[2-3]

## Results and Discussion

*Ultraviolet-Visible Spectroscopy Spectrum of CE-AgNPs*

The formation of carrot extract silver nanoparticles was indicated by a change in colour from light yellow to brown to dark brown. This observation is similar to that found with synthesis of silver nanoparticles from carrot aqueous extract.[3] When the solution was stored overnight, the colour became darker. These results were similar to results from previous studies which showed that overnight storage of silver nanoparticles produces darker nanoparticle solutions (Figure 1).[20] The colour change indicates the reduction of $Ag^+$ to $Ag^0$.[2] The solution with a silver nitrate concentration of 3 mM had the most intense brown colour compared to solutions with 1 mM and 2 mM silver nitrate. On the other hand, nanoparticle solutions at various pHs showed no difference in their colour intensities.



(a)

(b)

**Figure 1:** Carrot extract-silver nanoparticle solution at various (a) concentration $AgNO_3$ and (b) pH

UV-Visible spectrophotometry was used to identify the wavelength of maximum absorption ($\lambda_{max}$) as indicated by changes in surface plasmon resonance (SPR). In this study, the $\lambda_{max}$ obtained was 400-425 nm. The UV-Vis spectra of carrot extract-silver nanoparticles at the optimization conditions (various concentrations of $AgNO_3$ and pH) are presented in Figures 2 and 3. Nanoparticles produced with $AgNO_3$ concentration of 1 mM had the highest absorbance compared to other $AgNO_3$ concentrations (2 and 3 mM). Hence, 1 mM $AgNO_3$ solution is the optimum concentration needed for the synthesis of silver nanoparticles using carrot extract. This finding is similar to findings from previous studies which showed that optimum silver nanoparticles is synthesized using $AgNO_3$ at concentration of 1 mM.[2,4] In the synthesis of silver nanoparticles using cyanobacteria as reductant, it was observed that $AgNO_3$ concentrations greater than 1 mM resulted in a decrease in the intensity of the SPR band.[21] Other studies also showed that the synthesis of silver nanoparticles with $AgNO_3$ concentration of 0.5 mM was not sufficient to form nanoparticles. In comparison, the use of $AgNO_3$ concentration of 2 mM was less suitable for producing silver nanoparticles, so the optimal concentration was 1 mM.[2] The synthesis of silver nanoparticles with β-carotene was successfully done using $AgNO_3$ concentration of 1 mM.[1]

Carrot extract-silver nanoparticle solution at pH 6, 7 and 8 did not show any significant difference in $\lambda_{max}$, but the absorbance at pH 8 was higher when compared to pH 6 and 7. The addition of NaOH has the function of increasing the reduction of $Ag^+$ to $Ag^0$ until silver nanoparticles are formed. If the synthesis of nanoparticles is carried out in an acidic environment, or the amount of $H^+$ is high, the reduction reaction will not occure.[20] According to previous studies, pH 7 (neutral) is the optimal pH for the formation of nanoparticles. Alkaline pH can cause a shift in surface plasmon resonance, which may prevent the formation of nanoparticles.[2]

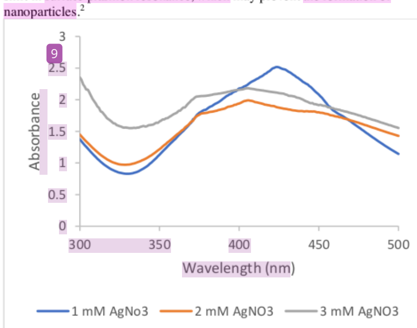



**Figure 2:** UV-visible absorption spectra of carrot extract-silver nanoparticle (CE-AgNPs) at different concentrations.

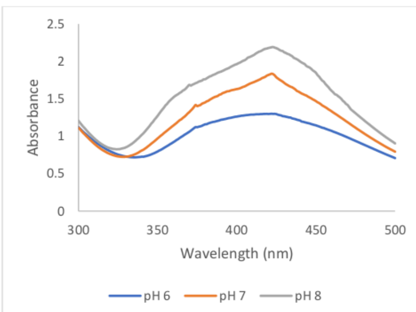



**Figure 3:** UV-visible absorption spectra of carrot extract-silver nanoparticle (CE-AgNPs) at different pH

*Particle Size of Carrot Extract Silver Nanoparticle (CE-AgNPs)*

Particle size analyzer (PSA) is used to measure size and distribution of particles. Table 1 shows the results of the particle size analysis of the CE-AgNPs at the different optimization conditions. The optimal condition that produced the smallest size of CE-AgNPs was silver nitrate concentration of 1 mM and pH 7. The carrot extract functions to reduce silver ions and also as a capping agent, reduce toxicity and increase the stability of nanoparticles.[4] Silver nanoparticles are generally 1-100 nm in size, but the results of the study showed that the size of the nanoparticles ranged from 105-238 nm. In another study, silver nanoparticles formed with mahkota dewa *(Phaleria macrocarpa)* leaves showed that at an extract concentration of 0.125% (w/v) produced nanoparticles with particle size range of 130 - 300 nm.[22] Research on the synthesis of silver nanoparticles using *Palmaria palmata* showed an average particle size of 185.5 nm.[23] In this study, the polydispersity index (PDI) measurement showed that the particle size was less than 100 nm, and was used to assess if there was aggregation of the nanoparticles. The results of the PDI test is presented in Figure 4.

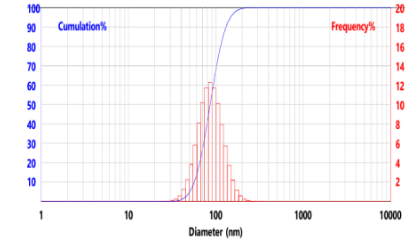



**Figure 4:** Polydisperse index (PDI) of carrot extract-silver nanoparticle (CE-AgNPs) at 1 mM $AgNO_3$ (pH 7).

**Table 1:** Carrot extract-silver nanoparticle (CE-AgNPs) size with various concentrations of $AgNO_3$ and pH

| Parameters | size (nm) | PDI |
|---|---|---|
| $C_{AgNO_3}$ (mM) | | |
| 1 | 105.73 | 0.1115 |
| 2 | 146.83 | 0.2267 |
| 3 | 238.62 | 0.2217 |
| pH | | |
| 6 | 106.13 | 0.1572 |
| 7 | 105.73 | 0.1115 |
| 8 | 119.63 | 0.1082 |

In this study, the aggregation of silver nanoparticles still resulted in particles at the nanoscale size range of 10-1000 nm.[24] The mechanisms of metal aggregation are cluster-particle aggregation and cluster-cluster aggregation. Cluster-particle aggregation occurs when nanoparticles are added when the cluster is growing, while cluster-cluster aggregation occurs when clusters merge to form a larger cluster size.[17] De-agglomeration of nanoparticles can be done by sonication, ultrasound, and heating.[25] The challenge in making silver nanoparticles using extracts is the occurrence of aggregation, which can affect the size and morphology of the resulting nanoparticles.

The results showed that pH 6 and 8 had larger particle sizes compared to pH 7. This also happened in previous studies that showed that *Avena sativa*-gold nanoparticles have large particle size at pH 2, while at pH 3 and 4, the nanoparticle size became smaller. This condition occurs because at pH 2 there was aggregation, while at pH 3 and 4, there were more functional groups of the extract, so the gold nanoparticles produced were more, and the particle size was smaller.[26] The study of silver nanoparticles of *Clitoria ternatea* extract and *Solanum nigrum* leaf extract also showed that at pH 4 larger nanoparticles were formed. At pH 7 nanoparticles of uniform particle size was formed, and at pH 9 nanoparticles formed were dispersed in large quantities and small in size.[5]

The polydispersity index (PDI) is used to determine the particle size distribution, and it is an important parameter used to characterize nanoparticles.[27] The PDI value ranges from 0 to 1. If the PDI value approaches 0, the particle size distribution is classified as monodisperse or homogeneous, while if the PDI value approaches 1, the particle size distribution is regarded as polydisperse or heterogeneous. Monodisperse silver nanoparticles have better application capabilities compared to polydisperse forms.[2] PDI value of less than 0.3 indicates that monodisperse nanoparticles are formed.[27] Based on the results of the study, it was shown that the size distribution of silver nanoparticles formed with the different $AgNO_3$ concentrations and pH values is monodisperse or homogeneous.

*FE-SEM Images of Carrot Extract Silver Nanoparticle (CE-AgNPs)*

FE-SEM was used to determine the morphology of the carrot extract-silver nanoparticles. The results of FE-SEM are shown in Figure 5. The shape of CE-AgNPs formed with a 1 mM concentration of $AgNO_3$ and pH 7 is spherical. FE-SEM results also showed the presence of small

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and large particle sizes, which indicates some agglomeration of particles. In a previous study, the SEM results of silver nanoparticle synthesized using *Parinari curatelli* showed that the nanoparticles undergo agglomeration to form large aggregates.[28]
Silver nanoparticles with aqueous extract of carrot showed spherical nanoparticle morphology.[3] Similar studies of silver nanoparticles with carrot juice and silver nanoparticles with *β*-carotene from carrots also showed the formation of spherical nanoparticles.[1,17]

*X-Ray Diffractogram of Carrot Extract Silver Nanoparticle (CE-AgNPs)*
X-ray diffraction was used to confirm the formation of carrot extract silver nanoparticles. The test was carried out at an angle of 2θ with a range of 5-90°. The result of X-ray diffraction of CE-AgNPs as shown in Figure 6 showed that there were peaks at [illegible].80°, 32.38°, 38.27°, 46.21°, and 77.38°, which correspond to the planes (111), (200), and (311).
The XRD results of silver nanoparticles using carrot juice showed four diffraction peaks at 37.9° (111), 44.1°(200), 64.3°(220) and 77.2°(311).[17] The peaks produced by carrot extract silver nanoparticles are almost the same as the XRD of silver reference, but there was no peak at 64.3° which is related to the (220) plane. Similar findings was observed in the XRD of carrot β-carotene silver nanoparticles which gave absorption peak at 37.8° and 43.9° which are related to the (200) and (220) planes due to the presence of anthocyanins on the surface of AgNPs.[1]

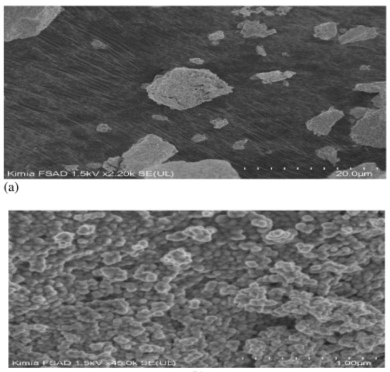


**Figure 5:** FE-SEM image of carrot extract-silver nanoparticle (CE-AgNPs) at 1 mM $AgNO_3$ and pH 7 (a) 2.200x (b) 45.000x

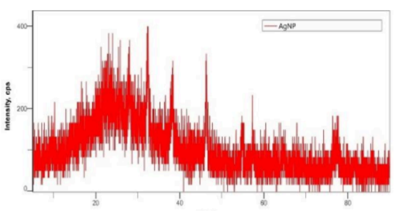


**Figure 6:** X-ray diffractogram of carrot extract-silver nanoparticle (CE-AgNPs)

## Conclusion

In this study, carrot ethanol extract was used as a bioreductor and capping agent in the production of silver nanoparticles (AgNP). Based on the optimization results, the smallest size of carrot extract silver nanoparticles (CE-AgNPs) was 105.73 nm and PDI 0.1115. Increasing $AgNO_3$ concentration formed silver nanoparticle with larger size, and different pH levels affected the size of the nanoparticles. The results of FE-SEM analysis showed that carrot extract silver nanoparticles were spherical, and XRD results showed that the formed nanoparticles contained silver components. It can be concluded that the condition under which CE-AgNPs is synthesized can affect the wavelength of maximum absorption, particle size, and morphology of nanoparticles. The challenge in the green synthesis of nanoparticle is nanoparticle aggregation, which affects the size and morphology of nanoparticles. Further research is needed to ensure the stability of nanoparticles, and prevent aggregation from occuring.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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