

# An Investigation of the Optimal Conditions for the Green Synthesis of Silver Nanoparticles Using an Aqueous Extract from the *Agrimonia eupatoria* L. Plant <sup>†</sup>

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**Abstract:** In this research study, silver nitrate and an aqueous extract of the *Agrimonia eupatoria* L. plant were used for the synthesis of silver nanoparticles (AgNPs). The optimal conditions for this green synthesis were examined: the concentration of starting substances, pH value, and temperature. For the maximum AgNP yield, the best conditions were a 5 mM AgNO<sub>3</sub> concentration, 1% extract concentration, a temperature of 25 °C, pH = 6, and a 3 h reaction time.

**Keywords:** green synthesis; *A. eupatoria* L.; silver nanoparticles

## 1. Introduction

Physical, chemical, and biological methods were used for the synthesis of metal nanoparticles (MnNP). Physical and chemical methods for MnNP synthesis have many drawbacks, including the use of expensive equipment, high energy consumption, and the use of toxic chemicals, which pose an environmental problem [1–3]. There has been a need for an environmentally friendly alternative to synthesizing MnNPs, the focus of which is the green synthesis of MnNPs using plants, microorganisms, enzymes, polysaccharides, and biodegradable polymers [3,4]. In the synthesis of nanoparticles, organism extracts can act as reducing agents as well as stabilizers. Innovative and diverse applications of MnNP in different fields of medical science, environmental science, and agriculture led to the accelerated development of different methods of synthesizing these compounds during recent years [1,5].

A silver nanoparticle (Ag NP), a stable, colloidal dispersion in water or organic solvents, is most prepared by chemical reduction in organic solvents or water. A plant extract can be used as a reducing agent and as a stabilizer (to prevent unwanted agglomeration of colloids) during nanoparticle synthesis [6]. Nanoparticles of silver have unique physical, chemical, and biological properties. There is significant catalytic and antibacterial activity in these nanoparticles, as well as good potential for nanobiotechnological applications [7].

*Agrimonia eupatoria* L. (common name: *agrimony*) belongs to the family *Rosaceae* (Tribe: *Sanguisorbeae*). The plant is known for being used as a raw material for the extraction of medicinal ingredients or the production of medicines in the pharmaceutical industry. The plant not only has antioxidant and antibacterial properties but also anti-inflammatory, neuroprotective, antidiabetic, hepatoprotective, and anticancer properties [8]. As part of our earlier research, silver nitrate, and the acetone extract of *A. eupatoria* L. were used for the synthesis of silver nanoparticles [9]. Our study examined the best conditions for the synthesis of silver nanoparticles from *A. eupatoria* L. aqueous extracts.



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## 2. Materials and Methods

Silver nitrate, sodium hydroxide, and nitric acid used were from Sigma Aldrich, USA. All solutions were prepared in distilled water. The aqueous extract of the plant was prepared according to a previously published procedure [8]. Dried, crushed plant material was extracted in distilled water by maceration. In brief, 60 g of the plant material was soaked in 800 mL of the solvent. The plant material was macerated three times at room temperature using a fresh solvent every 24 h. After every 24 h, the samples were filtered through a filter paper, and the filtrates were collected and evaporated to dryness using a rotary evaporator (DLAB, RE 100 S) at 40 °C. All nanoparticle synthesis reactions were performed on a magnetic stirrer (MAGE 12/17) under controlled conditions. Monitoring the synthesis of AgNPs within the wavelength range of 200–800 nm was carried out using a Perkin Elmer Lambda365 spectrophotometer. A microcentrifuge DM0412 from Scilogek | Laboratory was used to centrifuge the suspension for 20 min at 4500 rpm. The data were analyzed using OriginPro 2019b-64bit software.

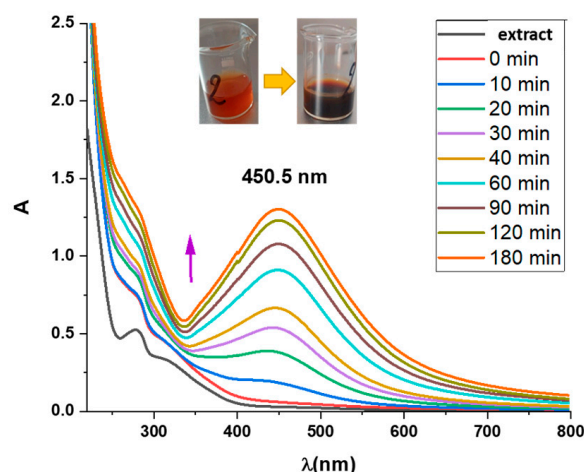
The optimal conditions for this green synthesis were examined: the concentration of starting substances, pH value, and temperature [10]. Silver nitrate was dissolved in concentrations of 5 mM, 10 mM, and 20 mM. The pH of the reaction mixtures was adjusted to 4, 6, and 8 using solutions of 0.1 M NaOH and 0.1 M HNO<sub>3</sub>. The reaction mixture was heated to 25 °C and 50 °C on a magnetic stirrer under controlled conditions. Visual color change (from light yellow to dark brown) and UV-Vis spectrophotometry were used to monitor the process of AgNP formation. The suspensions were centrifuged for 20 min at 4500 rpm after AgNPs synthesis. After centrifugation, the residue was resuspended in demineralized water and centrifuged again. Precipitated nanoparticles were then dried in a hot air oven (40 °C) and stored at 4 °C in the fridge.

## 3. Results

In this research study, we used silver nitrate and an aqueous *A. eupatoria* L. extract for the synthesis of silver nanoparticles (AgNPs).

### 3.1. UV-Vis Spectral Analysis

The generation of AgNPs in a solution during their synthesis using extracts was monitored spectrophotometrically. The color change in solutions from light yellow to dark brown is a characteristic indicator of the synthesized AgNPs. The color change is caused by surface plasmon resonance (SPR). We recorded the UV-Vis absorption spectra of formed nanoparticles at 200 to 800 nm. There were peaks within 425–475 nm (typical peak for AgNPs), indicating that AgNPs were formed. (Figure 1). At 3 h, the maximum absorption values were obtained, and thereafter, there was no increase in absorption, indicating the end of the synthesis process. The effects of AgNO<sub>3</sub>, temperature, and pH on the biosynthesis of nanoparticles using *A. eupatoria* aqueous extracts were evaluated. Initially, both types of nanoparticles were synthesized at 5 mM AgNO<sub>3</sub>, 25 °C and 1% extract concentration without adjusting pH values (pH ≈ 6).



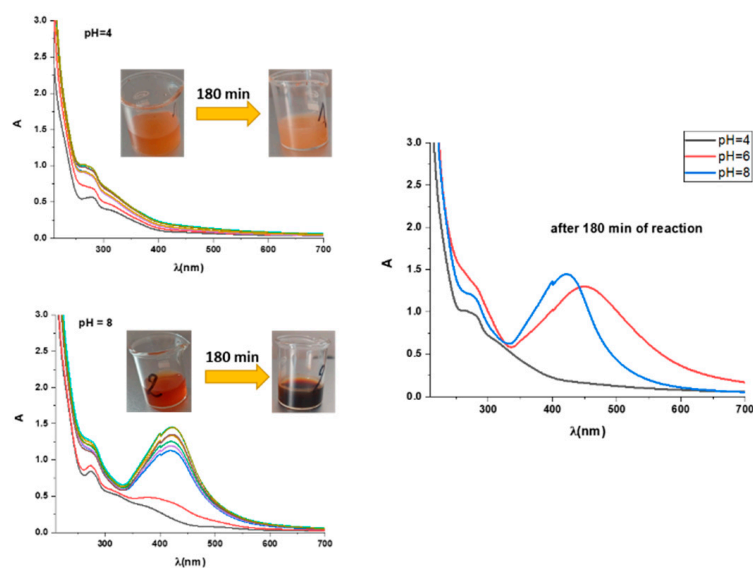
**Figure 1.** Solution color change and the UV-Vis time dependence of AgNPs biosynthesis using an aqueous *A. eupatoria* extract.

### 3.2. Influence of Temperature

The starting point for testing the temperature sensitivity during the biosynthesis of nanoparticles was a concentration of 5 mM  $\text{AgNO}_3$ , 1% of the concentrated plant extract without additional adjustment of the pH value. The reaction mixture was heated to 25 °C and 50 °C on a magnetic stirrer under controlled conditions. When the temperature increases to 50 °C, the rate of the formation of nanoparticles increases significantly.

### 3.3. Influence of pH

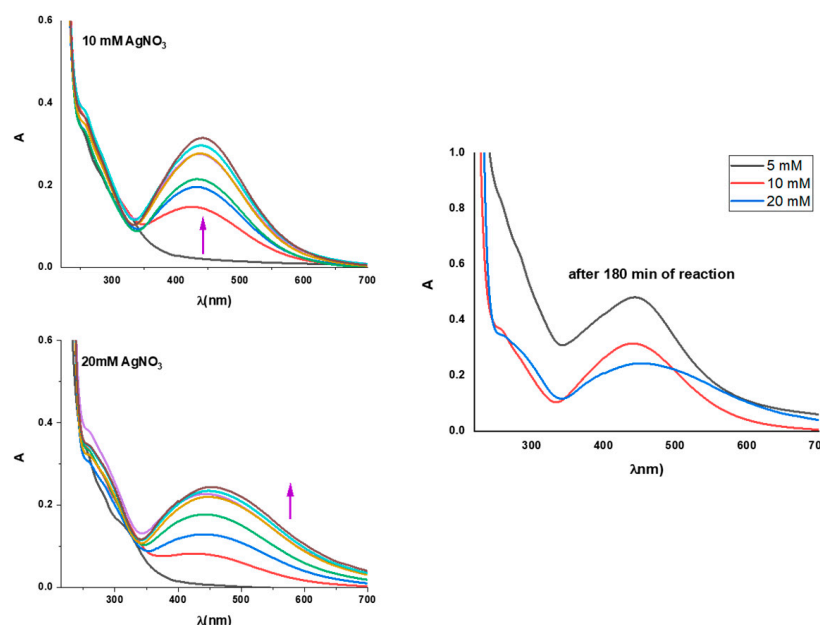
The initial conditions for testing pH sensitivity were a concentration of 5 mM  $\text{AgNO}_3$ , a temperature of the reaction mixture of 25 °C, and a 1% concentration of the aqueous extract of the plant. To adjust the pH = 4, a few drops of 0.1 M  $\text{HNO}_3$  solution were added to the mixture of extract solution and  $\text{AgNO}_3$ . The solution's pH = 6 value was obtained by mixing a solution of extract and silver nitrate. To adjust pH = 8, a few drops of 0.1M NaOH solution were added. Figure 2 shows the UV-Vis absorption spectra of AgNP biosynthesis depending on the change in the pH value. Based on the obtained results, it can be concluded that pH = 6 is the most optimal value for the synthesis of AgNPs with the help of this plant.



**Figure 2.** UV-Vis spectra of the time dependence of AgNP biosynthesis at different pH values.

### 3.4. Influence of Concentration

Initially, 25 °C, 1% extract concentration, and pH = 6 conditions were used to examine the dependence on the AgNO<sub>3</sub> concentration. Three extract solutions were prepared, to which AgNO<sub>3</sub> was added in concentrations of 5 mM, 10 mM, and 20 mM. As observed in Figure 3, 5 mM AgNO<sub>3</sub> is the optimal concentration for AgNP synthesis.



**Figure 3.** UV-Vis spectra of the time dependence of AgNPs biosynthesis at different concentrations of AgNO<sub>3</sub>.

### 4. Conclusions

According to the results of this research work, *A. eupatoria* is a good reducing agent and therefore a suitable plant for the green synthesis of silver nanoparticles. The plant's aqueous extract is an effective reducer and stabilizer of nanoparticles. Silver nanoparticles are gradually synthesized, which is confirmed by the change in the color of the reaction solution from light yellow to dark brown and the change in the appearance of UV-Vis absorption spectra over time. When the temperature of the reaction mixture increases, the rate of biosynthesis of silver nanoparticles increases drastically. Based on the research on optimal pH values for this biosynthesis, it could be concluded that an acidic environment is more suitable, and the most optimal pH value is 6, which is achieved by simply mixing the starting substances. Based on the examination of this biosynthesis at different concentrations of the starting AgNO<sub>3</sub> salt, it was concluded that the best concentration of AgNO<sub>3</sub> is 5 mM. Finally, it can be concluded that the best conditions for obtaining the highest yield of AgNPs are as follows: AgNO<sub>3</sub> initial salt concentration of 5 mM, a temperature of the reaction mixture of 25 °C, pH = 6, and duration of the nanoparticle biosynthesis reaction of 3 h.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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