*materials proceedings*

MDPI

*Proceeding Paper*

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

**Ana Kesić *, Katarina Marković, Mirjana Grujović and Zoran Marković**

Institute for Information Technologies, University of Kragujevac, Jovana Cvijica bb, 34000 Kragujevac, Serbia;
* Correspondence: akesic@uni.kg.ac.rs

† Presented at the 4th International Online Conference on Nanomaterials, 5–19 May 2023; Available online: https://iocn2023.sciforum.net.

**Abstract:** In this research, silver nitrate and an aqueous extract of the plant *Agrimonia eupatoria* L. 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 maximum AgNPs yield, the best conditions were a 5 mM $AgNO_3$ 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

**Citation:** Kesić, A.; Marković, K.; Grujović, M.; Marković, Z. An Investigation of the Optimal Conditions for the Green Synthesis of Silver Nanoparticles Using an Aqueous Extract from the Plant Agrimonia eupatoria L. *Mater. Proc.* **2023**, *14*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: 5 May 2023



## 1. Introduction

Physical, chemical, and biological methods were used for the synthesis of metal nanoparticles (MtNP). Physical and chemical methods for MtNPs 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 MtNPs, the focus of which is the green synthesis of MtNPs 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 MtNP in different fields of medical science, environmental science, and agriculture, led to the accelerated development of different ways of synthesizing these compounds during the last years [1,5].

A silver nanoparticle (Ag NP), as stable, colloidal dispersion in water or organic solvents, is most commonly 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 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 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.

## 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 in 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 $HNO_3$. 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 s spectrophotometry were used to monitor the process of AgNPs 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, we used silver nitrate and an aqueous extract *A. eupatoria* L. for the synthesis of silver nanoparticles (AgNPs).

### *3.1. UV-Vis Spectral Analysis*

The generation of AgNPs in solution during their synthesis using extracts was monitored spectrophotometrically. The color change of solutions from light yellow to dark brown is a characteristic indicator r of the synthesis 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 AgNO3, 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 $AgNO_3$, 25 °C, and 1% extract concentration without adjusting pH values (pH ≈ 6).

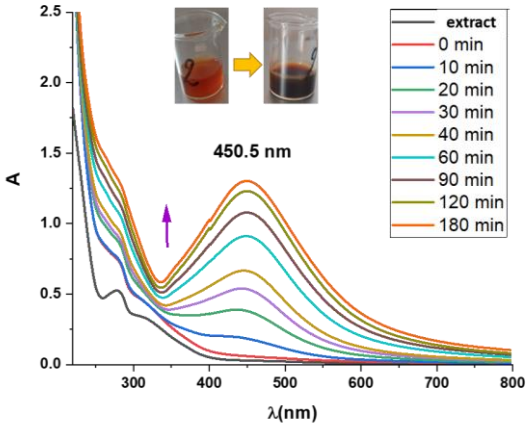


**Figure 1.** Solution color change and UV-Vis time dependence of AgNPs biosynthesis using aqueous extract *A. eupatoria.*

### *3.2. Influence of Temperature*

The starting point for testing the temperature sensitivity during the biosynthesis of nanoparticles was a concentration of 5 mM $AgNO_3$, 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 formation of nanoparticles increases significantly.

### *3.3. Influence of pH*

The initial conditions for testing pH sensitivity were a concentration of 5 mM $AgNO_3$, 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 $HNO_3$ solution were added to the mixture of extract solution and $AgNO_3$. The value of pH = 6–has a solution 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 f 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.

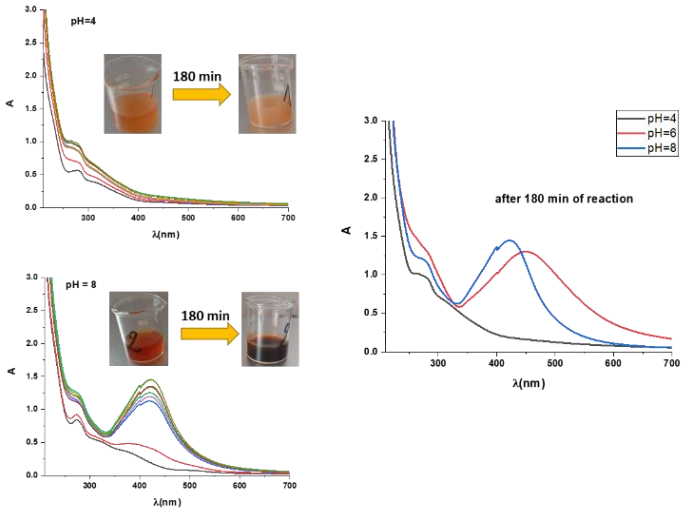


**Figure 2.** UV-Vis spectra of the time dependence of AgNP biosynthesis at different pH values.

### *3.4. Influence of Concentration*

Initially, 25 °C, 1% extract concentration, and pH = 6 conditions were used to examine the dependence on $AgNO_3$ concentration. Three extract solutions were prepared to which $AgNO_3$ was added in concentrations of 5 mm, 10 mm, and 20 mm. As seen in Figure 3, 5 mm $AgNO_3$ is the optimal concentration for AgNPs synthesis.

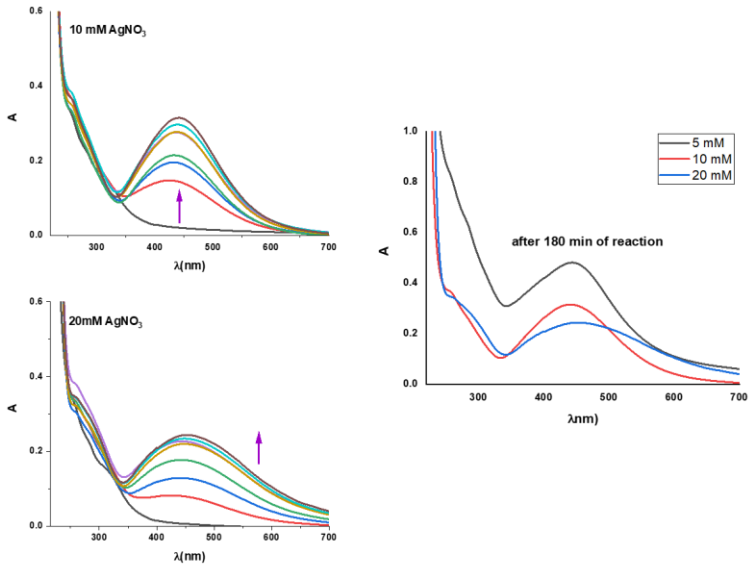


**Figure 3.** UV-Vis spectra of the time dependence of AgNPs biosynthesis at different concentrations of $AgNO_3$.

## 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. 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 the 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 of 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 $AgNO_3$ salt, it was concluded that the best concentration of $AgNO_3$ is 5 mM. And finally, it can be concluded that the best conditions for obtaining the highest yield of AgNPs are the $AgNO_3$ initial salt concentration of 5 mM, the temperature of the reaction mixture of 25 °C, the pH = 6, and the duration of the nanoparticle biosynthesis reaction of 3 h.

**Author Contributions:** Conceptualization, Z.M. and A.K.; methodology, K.M.; software, M.G.; validation, Z.M., A.K. and K.M.; formal analysis, M.G.; investigation, A.K.; resources, K.M.; data curation, A.K.; writing—original draft preparation, A.K.; writing—review and editing, M.G.; visualization, A.K.; supervision, Z.M.; project administration, K.M.; funding acquisition, Z.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Minister of Science, Technological Development and Innovation of the Republic of Serbia (Agreement No. 451-03-47/2023-01/200378).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors would like to thank the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-47/2023-01/200378), the Institute for Information Technologies and the University of Kragujevac for their support during the research.

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

## References

1. Gahlawat, G.; Choudhury, A.R. A review on the biosynthesis of metal and metal salt nanoparticles by microbes. *RSC Adv.* **2019**, *9*, 12944–12967.
2. Soni, M.; Mehta, P.; Soni, A.; Goswami, G.K. Green nanoparticles: Synthesis and applications. *J. Biotechnol. Biochem.* **2018**, *4*, 78–83.
3. Pal, G.; Rai, P.; Pandey, A. Green synthesis of nanoparticles: A greener approach for a cleaner future. In *Green Synthesis, Characterization and Applications of Nanoparticles*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 1–26.
4. Roychoudhury, A. Yeast-mediated green synthesis of nanoparticles for biological applications. *Indian J. Pharm. Biol. Res.* **2020**, *8*, 26–31.
5. Bahrulolum, H.; Nooraei, S.; Javanshir, N.; Tarrahimofrad, H.; Mirbagheri, V.S.; Easton, A.J.; Ahmadian, G. Green synthesis of metal nanoparticles using microorganisms and their application in the agrifood sector. *J. Nanobiotechnology* **2021**, *19*, 86–126.
6. Sharma, V.K.; Yngard, R.A.; Lin, Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. *Adv. Colloid Interface* **2009**, *45*, 83–96.
7. Srikar, S.K; Giri1, D.D; Pal1, D.B; Mishra1, P.K; Upadhyay, S.N. Green and Sustainable Chemistry. *Green Sustain. Chem.* **2016**, *6*, 34–56.
8. Muruzović, M.Ž.; Mladenović, G.K.; Stefanović, O.D.; Vasić, S.M.; Čomić, L.J.R. Extracts of Agrimonia eupatoria L. as sources of biologically active compounds and evaluation of their antioxidant, antimicrobial, and antibiofilm activities. *J. Food Drug Anal.* **2016**, *24*, 539–547.
9. Marković, G.K.; Grujović, Ž.M.; Kesić, S.A.; Markovic, S.Z. Green Synthesis of Silver Nanoparticles Using *A. eupatoria* extract in certain conditions. In Proceedings of the 8th International Electronic Conferences on Medicinal Chemistry, (ECMC 2022)), Online, 1–30 November 2022.
10. Srećković, N.Z.; Nedić, Z.P.; Liberti, D.; Monti, D.M.; Mihailović, N.R.; Katanić Stanković, J.S.; Dimitrijević, S.; Mihailović, V.B. Application potential of biogenically synthesized silver nanoparticles using *Lythrum salicaria* L. extracts as pharmaceuticals and catalysts for organic pollutant degradation. *RSC Adv.* **2021**, *11*, 35585–35599.