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Size Distribution of Gold Nanoparticles Synthesized by *Artemisia annua* L. Hairy Root Extracts

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"Green" synthesis is a novel and effective method of obtaining metal nanoparticles. Using plant extracts to synthesize nanoparticles is safe for the environment and does not require expensive and complex equipment. Based on their particular characteristics, such nanomaterials have potential applications in various fields of industry and medicine. Gold nanoparticles (AuNPs) are used in different areas where chemical inertia, nontoxicity, and good biocompatibility are required. That is why optimization of methods of AuNP obtaining besides theoretical has a practical interest. In this work, the features of gold nanoparticles obtained using extracts from the hairy roots of *Artemisia annua* L. during storage were investigated. AuNPs were obtained by adding ethanol (70%) extracts from the roots to a solution of $HAuCl_4$. Nanoparticles were studied using transmission electron microscopy (TEM) on the first and 14th days after initiation. TEM analysis revealed significant differences in the sizes of nanoparticles and changes during their storage. After two weeks post-initiation, AuNPs increased in size, and conglomerates formed, leading to nanoparticle precipitation. Such changes were noted for samples of AuNPs produced using extracts from transgenic roots and, to a lesser extent, those samples obtained when extracts from the roots of the control plants were added to the $HAuCl_4$ solution. This data indicates the necessity for additional research to explain the mechanisms of this effect and to test more hairy root lines to find extracts that will promote the formation of more stable AuNPs.

Keywords: *Artemisia annua*, hairy roots, gold nanoparticles, "green" synthesis, nanotechnology

Introduction

Metal nanoparticles (NPs) are a modern material with a wide range of applications. The nanosize of such objects determines the specificity of their application. The importance of nanomaterials, including gold nanoparticles (AuNPs), lies in their potential utilization in industry, such as in biosensors. The economic significance and necessity of the so-called "green" synthesis of metal NPs is related to the rather simple technology of their creation. Such synthesis can be conducted without expensive materials, reagents, and specific equipment.

Nowadays, a significant quantity of studies aimed at the synthesis of nanosized inorganic materials are being performed all over the world. This interest of

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researchers is connected with a striking difference in nanomaterials in physical, chemical, and optical characteristics (Lloyd et al., 2011). The small size provides a relatively easy penetration of these particles through the cell membrane. The nanosize of NPs contributes to their homogeneity of distribution in cells in the process of implication. Based on their special characteristics, such metal nanoparticles have potential applications in various fields of industry and medicine.

Various methods of synthesis, including chemical, electrochemical, photochemical, and biological, can be used to obtain AuNPs. Natural organic compounds from plant extracts can also be used for the so-called "green" synthesis of NPs (Begum et al., 2009; Patil and Kim, 2017). This synthesis has some advantages over chemical one due to the lack of hazardous components and high energy requirements (Kharissova et al., 2013).

In general, plant extracts usually have alkaloids, flavonoids, proteins, polysaccharides, cellulose, polyphenolic compounds, and their metabolites, which can initiate the synthesis of NPs. During the "green" synthesis of NPs, the components of the extract act not only as a reducing agent but also as a stabilizer, making it possible to obtain a stable solution of nanoparticles without the inclusion of additional components. This feature makes the "green" synthesis of NPs a fairly simple, effective, and cheap way for valuable material production. The composition and concentration of reducing agents in plant extracts greatly influence the size, shape (e.g., ellipsoidal, spherical, stars, triangles, etc.), morphology, and stability of the NPs obtained during the synthesis (Dhand et al., 2016).

Gold nanoparticles are used in different areas (Rajeshkumar, 2016; Asiya et al., 2020; Muddapur et al., 2022). They can be used in industry as conductors that link electronic elements in chips. In photodynamic therapy, they absorb near-infrared light from 700 to 800 nm and produce heat that allows nanoparticles to destroy target tumors. AuNPs deliver therapeutic agents that can be applied to the surface of gold nanoparticles (the advantage is a large surface area). They may be used in sensors (a colorimetric sensor based on AuNPs can determine whether food products are fit for consumption). Wide usage of AuNPs noted in diagnostics: for the detection of biomarkers in the diagnosis of heart disease, cancer, and infectious agents and lateral flow immunoassays where a typical example of home use is a home pregnancy test. The chemical industry utilizes AuNPs in catalysis of numerous chemical reactions.

Gold nanoparticles can be obtained by the method of "green" synthesis (Singh et al., 2016; Muddapur et al., 2022). In particular, plants such as *Rosa hybrida* (Ser.) Posp., *Artemisia vulgaris* L., *Theobroma cacao* L., *Curcuma longa* L., *Panax ginseng* C.A.Mey., and others were used to obtain AuNPs (Leonard et al., 2011; Noruzi et al., 2011; Fazal et al., 2014; Sundararajan and Ranjitha Kumari, 2017; Bharadwaj et al., 2021; Rodríguez-León et al., 2019).

PubMed search finds 48,982 results on request "gold nanoparticles", 15,246 results on request "gold nanoparticles application", and 1,674 results on request “gold nanoparticles "green" synthesis. Therefore, recently, much attention has been paid to searching for new methods of obtaining gold nanoparticles, as well as their study. Among numerous publications, we found only a few that used extracts from plants of the *Artemisia* genus including *A. dracunculus*, *A. vulgaris*, *A. capillaris* (Lim et al., 2016; Sundararajan and Ranjitha Kumari, 2017; Wacławek et al., 2018; Sundararajan et al., 2022;) and only one article on the usage of *A. annua* extracts for "green" synthesis of gold nanoparticles (Basavegowda et al., 2014). Our research was aimed to determine the features of the synthesis of gold nanoparticles using extracts from *A. annua* roots and hairy roots, as well as comparing the features of changes in these nanoparticles during their storage.

The utilization of extracts produced from hairy roots is attributed to potential wide variability in their efficacy. It is widely acknowledged that the *rol* genes of *Agrobacterium rhizogenes*, transferred to the plant genome during genetic transformation, serve as inducers of secondary plant metabolism. Transformed hairy roots with these genes can differ significantly from the original plants in terms of chemical composition and bioactivity of cellular components (Hussain et al., 2012). The indeterminate placement of transferred genes in the plant genome increases the variability of the chemical content of extracts obtained from lines of hair roots. Extracts from transgenic roots may exhibit greater bioactivity than extracts from control plants. In particular, the reducing power may be greater. Thus, hairy roots have the potential to be a valuable resource for compounds with reducing activity for their use in the "green" synthesis of metal nanoparticles.

Materials and methodology

Plant material

Artemisia annua L. plants and hairy roots from the collection of the Laboratory of Adaptational Biotechnology of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine were used in the experiments.

Extracts preparation

The plant material was grown in the liquid Murashige and Skoog (Duchefa) (MS) medium. In 3 weeks, the roots were separated from the medium, lyophilized, and extracted with 70% ethanol. The obtained extracts were lyophilized and then dissolved in 70% ethanol up to the concentration of 1 mg/mL in rutin equivalent (RE).

AuNPs preparation

For gold NP synthesis, the extracts were added in the ratio of 0.08 mL to 1 mL of aqueous solution of $HAuCl_4$ (0.7 mg/mL, Sigma). The mixture was heated at 50 °C for 30 min until the color of the mixture changed from yellowish to violet-pink.

TEM analysis of AuNPs

Transmission electron microscopy (TEM) was used to examine the size and morphology of synthesized AuNPs. The images were done on a JEM-1400 (Jeol, Japan) microscope with an accelerating voltage of 80 kV. The samples were prepared by applying a suspension of AuNPs to a copper grid with a formvar coating, reinforced by carbon sputtering.

Statistical analysis

The sizes of NPs were measured by ImageJ software using TEM photos. All calculations and graphing were performed using the R (version 4.4.1) and RStudio (build 2024.04.2+764) software.

Results and discussions

Why was chosen *Artemisia annua*? The formation of nanoparticles was observed after heating the solution of $HAuCl_4$ (0.7 mg/mL) with extracts from the roots of the nontransformed plants or hairy root lines (Figure 1a). The colorless $HAuCl_4$ solution became violet-pink that is typical for solutions of gold nanoparticles (Teimuri-Mofrad et al., 2017; Abdulwahed et al., 2021; Alhumaydhi, 2022). Significant changes in the solutions were found after two weeks of storage at 25 °C. In particular, the intensity of the color decreased, and some sediment formation was observed (Figure 1b).

The study conducted using transmission electron microscopy confirmed the presence of nanoparticles (Figure 2). They were formed both when using an extract from the roots of the control plants and when using extracts from different lines of hairy roots.

In the process of reducing the $HAuCl_4$ to metallic gold, nanoparticles with a diameter of 1–100 nm were formed (Figure 3). Most of them (up to 95%) had a diameter of up to 40 nm on the day of formation by an extract from control plants. After 14 days, this indicator increased to 60 nm. On the other hand, most of the AuNPs formed by adding hairy root extracts were up to 25 and 35 nm (for lines 2-3 and 2-14, respectively) and had magnified up to 70 nm after storage. In addition, partial aggregation and sedimentation were observed.

According to TEM data in the variant where the extract of the control roots was used for AuNPs initiation, small nanoparticles (<10 nm) accounted for 35% on the day of obtaining and 32% after two weeks. At the same time, small nanoparticles accounted for 71–80% on the day of initiation and 14–27% after two weeks in the case of extracts from transgenic roots used in the reaction with $HAuCl_4$ solution. After 14 days of storage, the part of big nanoparticles (more than 40 nm) had significantly increased from 0.5–4% to 35–36% in "transgenic" variants and from 5 to 19% in the control one.

a 2-14 2-3 C b 2-14 2-3 C

Figure 1 Gold nanoparticles obtained by *Artemisia annua* L. root extracts: a – on the first day after initiation; b – in 14 days (2-14 and 2-3 – extracts from the hairy roots; C – extract from the control plant roots were used)

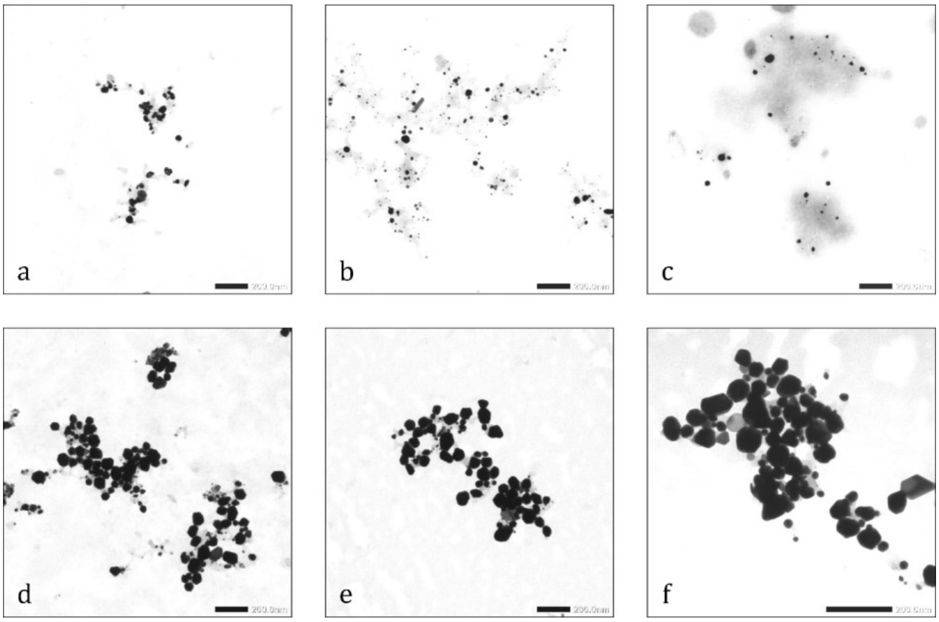

Figure 2 Transmission electron microscopy of gold nanoparticles obtained by *Artemisia annua* L. root extracts: a, b, c – on the first day after initiation; d, e, f – in 14 days. Extracts from hairy root line 2-14 (a and d), hairy root line 2-3 (b and e), the roots of the control plants (c and f) were used. Mark size is 200 nm

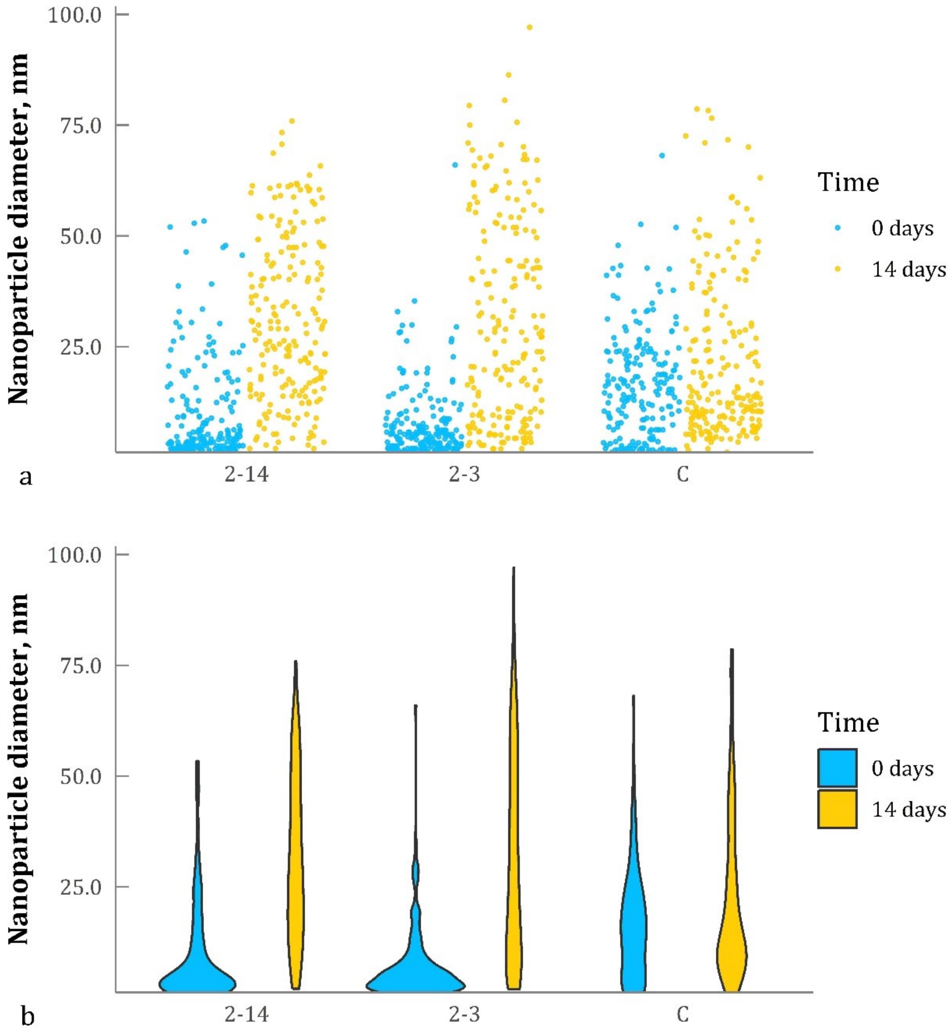

Figure 3 Gold nanoparticle size distribution: a – dot plot, b – violin diagram; extracts from the control roots (C), hairy root lines No2-3 (2-3) and No2-14 (2-14) were used for nanoparticles production

As can be seen, in the case of AuNPs, the extracts of untransformed plants had a higher stabilizing effect on the nanoparticles. More studies on the composition of the extracts are needed to explain the mechanisms of this effect and to test more hairy root lines to find extracts that will promote the formation of more stable AuNPs. On the other hand, individual projects require small AuNPs of more or less the same size for application immediately after obtaining. In this case, extracts from demonstrated transgenic roots could be used as of today.

Numerous studies have been conducted on the preparation of AuNPs through "green" synthesis (Timoszyk, 2018; Hano and Abbasi, 2021; Dash et al., 2022). The authors determine different sizes of the obtained nanoparticles, for example, in the range of 5–53 nm, 75–150 nm, 5–25 nm, 13–52 nm, and 10–500 nm (Ghodake et al., 2010; Gan et al., 2012; Rimal Isaac et al., 2013; Fouda et al., 2022). Unfortunately, the authors do not provide data on possible changes in the size of nanoparticles during their storage.

Conclusion

Thus, the possibility of obtaining gold nanoparticles using extracts from *Artemisia annua* plant roots as well as extracts from transgenic roots cultivated in vitro has been established. The obtained nanoparticles had different distributions in size. During the storage of nanoparticles, their size increased, especially in the samples obtained by extracts of transgenic roots. Such features may be related to differences in the chemical composition of the extracts used.

Conflict of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

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