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Effect of storage conditions on long-term stability of Ag nanoparticles formed via green synthesis

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Abstract: Spherical Ag nanoparticles (AgNPs) with a diameter of 20 nm or smaller were biologically synthesized using algae *Parachlorella kessleri*. The effect of storage conditions on the long-term stability of AgNPs was investigated. UV/Vis spectrophotometry, transmission electron microscopy, and dynamic light scattering measurements revealed that the long-term stability of AgNPs was influenced by light and temperature conditions. The most significant loss of stability was observed for the AgNPs stored in daylight at room temperature. The AgNPs stored under these conditions began to lose their stability after approximately 30 d; after 100 d, a substantial amount of agglomerated particles settled to the bottom of the Erlenmeyer flask. The AgNPs stored in the dark at room temperature exhibited better long-term stability. Weak particle agglomeration began at approximately the 100th day. The AgNPs stored in the dark at about 5°C exhibited the best long-term stability; the AgNPs stored under such conditions remained spherical, with a narrow size distribution, and stable (no agglomeration) even after 6 months. Zeta-potential measurements confirmed better dispersity and stability of AgNPs stored under these conditions.

Keywords: silver nanoparticles; stability; storage conditions; green synthesis; *Parachlorella kessleri*

1. Introduction

Substantial attention has been devoted to silver nanoparticles (AgNPs) because of their potential applications in various fields (e.g., water filtration, bandages, sensors, commercial products, catalysis, and antimicrobial surface coatings) [1–3]. The preparation of uniform NPs with a narrow size distribution is critical for their utilization [4–5]. Therefore, numerous physical, chemical, and biological methods focused on the formation of AgNPs have been developed [6–9]. Physical methods (e.g., chemical vapor deposition and molecular beam epitaxy) are energy efficient but require expensive apparatus. Chemical methods are more affordable but are not environmentally friendly.

Given the promise demonstrated by green synthesis involving various plant extracts, researchers have used bacteria, fungi, and algae as biological materials for the synthesis of NPs [10–17]. Reducing sugars, ketones/aldehydes, amine groups, water-soluble heterocyclic compounds, and proteins naturally present in such materials have been proposed to play a key role not only in the reduction of silver ions but also in stabilizing the resultant AgNPs. Furthermore, because overall material and energy consumptions in biological methods are substantially lower than those in conventional syntheses, biological methods offer a low-cost, green alternative [9]. The biochemical pathways responsible for the production of metal NPs using biological materials have been well researched. However, the lifecycle or stability of AgNPs remains an active research topic. Some research has been conducted in the field of environmental health and safety to better understand the entire lifecycle of AgNPs [18–19]. However, the long-term stability of AgNPs prepared via green synthesis routes has not been researched in detail; thus, numerous questions remain unanswered.

In the present study, the extract of *Parachlorella kessleri* algae was used to synthesize spherical AgNPs. The aim was to investigate the influence of storage conditions (exposure to light or dark and different temperatures) on the long-term

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stability of AgNPs. Because the knowledge of AgNPs' stability is lacking, the results reported in the present study will shed light on the entire lifecycle of AgNPs.

2. Experimental

The algae (*Parachlorella kessleri*) were cultivated in Petri dishes for 3 weeks at ambient temperature. The nutrient medium consisted of 2wt% agar and Milieu Bristol nutrient solution. After cultivation, the algae were collected and boiled in a water bath for 15 min. After being heat treated, the algae were centrifuged at 3000 r/min for 15 min. The liquid phase (extract) was removed and transferred into Erlenmeyer flasks containing 0.92 mmol/L $AgNO_3$ solution (Ag concentration of 100 mg/L). The solution was allowed to stand for 4 d at room temperature. Subsequently, the AgNP solution was divided into three Erlenmeyer flasks and stored under different conditions: at room temperature in daylight, at room temperature in the dark, and at 5°C (in refrigerator) in the dark.

The AgNPs' stability was monitored by collecting the UV/Vis spectra of the solutions in 10-mm-optical-path-length quartz semi-micro cuvettes (UNICAM UV/Vis Spectrometer UV4). The size and morphology of the NPs were studied by transmission electron microscopy (TEM; JEOL model JEM-2000FX microscope operated at an accelerating voltage of 200 kV). Dynamic light scattering (DLS) was used to obtain the zeta-potential of the solutions.

3. Results and discussion

The addition of the green algae extract to the $AgNO_3$ solution led to a change in the color of the solution to yellow-brown, indicating AgNP formation. This color change is the result of the radiation absorption in the visible region of the electromagnetic spectrum (380–450 nm) due to the localized surface plasmon of AgNPs [19–20].

The surface plasmon resonance (SPR) bands measured on the 1st, 2nd, and 4th days are shown in Fig. 1. AgNPs are expected to display different SPR bands depending on their size and shape anisotropy: the spectra of small spherical NPs exhibit a single, symmetrical SPR band, whereas those of large and/or differently shaped particles contain two or three peaks [13,21]. The UV/Vis spectra in Fig. 1 confirm the presence of AgNPs. The symmetric SPR band with a maximum at approximately 415 nm indicates the presence of AgNPs with a narrow size distribution and a uniform spherical shape.

The TEM image of the AgNPs collected on the 4th day (Fig. 1) clearly confirms the formation of spherical AgNPs surrounded by a thin layer of organic material, which is characteristic of AgNPs prepared in plant extracts. The particle size histogram constructed from the TEM images of the NPs removed from the solution on the 4th day of the experiment reveals that more than 90% of the AgNPs are 20 nm in diameter or smaller. These NPs were used in experiments to assess the influence of the storage conditions on their stability. The 4th day was considered as 0 day in subsequent experiments.

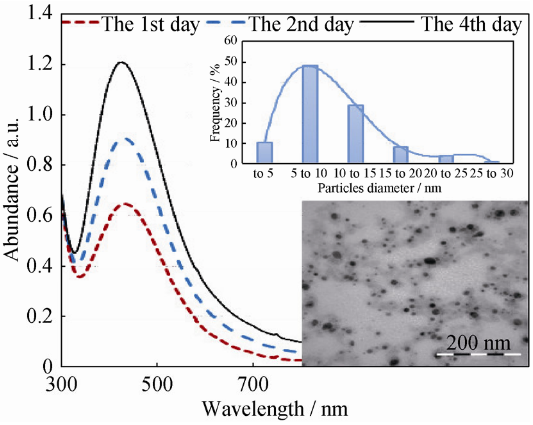

Fig. 1. UV/Vis absorption spectra of the AgNPs (the insets show the size distribution histogram and a TEM image of AgNPs on the 4th day of the experiment).

The information gathered through analysis of the UV/Vis spectra for all three experimental solutions stored under different conditions is presented in Fig. 2.

The storage of AgNPs at room temperature in daylight strongly affected their long-term stability (Fig. 2(a)). On the 20th day, the SPR band was symmetrical, indicating that the particles were spherical and well dispersed. After two months, the SPR band lost symmetry, a shoulder appeared, and the SPR-band peak (428 nm on the day 0) noticeably shifted to a longer wavelength (458 nm on the 60th day); the $a_{\max}$ (maximum of absorbance) had already increased from 1.206 on the day 0 to 2.463 on the 20th day (Fig. 3).

Further losses of symmetry of the SRP bands were observed on the 100th and 200th days. We assumed that NPs with various shapes and/or sizes were present, as previously reported [13,22–24]. However, differently shaped AgNPs were not observed by TEM (Fig. 4). Such deviations in SPR bands can also be caused by NPs dissolving or agglomerating [25–26]. Agglomeration was confirmed by the substantial amount of AgNP sediment that settled to the bottom of the Erlenmeyer flask. Only small, well dispersed particles (with diameters of 10 nm or less) remained in solution. The TEM image of AgNPs and their size distribution on the 200th day clearly indicated the presence of such particles (Fig. 4).

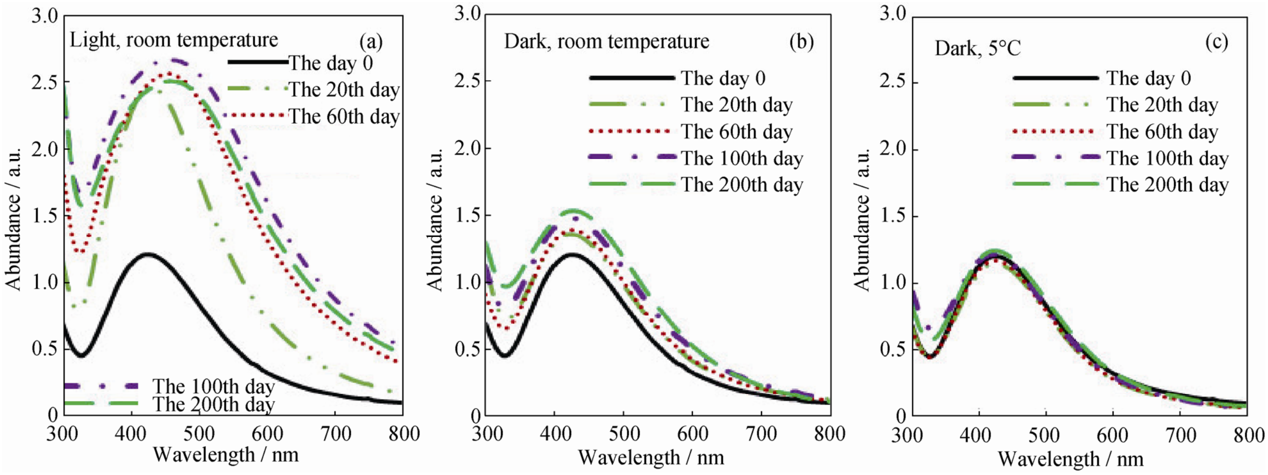

Fig. 2. The UV/Vis spectra of AgNPs for different storage conditions and days of experiment.

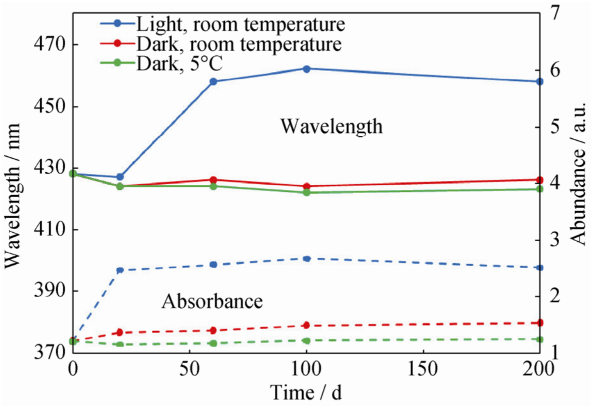

Fig. 3. Changes in absorbance and wavelength as functions of time.

Izak-Nau *et al.* [19] speculated that such changes in AgNPs' stability under similar storage conditions are attributable to the elevated temperature of the dispersions exposed to daylight, which can increase the NPs' collision rate and thereby induce faster agglomeration. Additionally, daylight can cause photo-reduction of already dissolved Ag^+ ions, which may in turn lead to the production of new NPs, increasing the overall sample polydispersity.

Fig. 5 shows the size distribution of AgNPs stored at room temperature in the dark. Almost 90% of the AgNPs were 20 nm or smaller, and their average size did not substantially change with increasing experiment time. The SPR bands were symmetrical (Fig. 2(b)). The $\lambda_{\max}$ values negligibly changed from 424 to 426 nm on the 20th and 200th days (Fig. 3), respectively, for the sample stored at room temperature in the dark; the position of the peaks remained stable over time, indicating that AgNPs were stable, small, and spherical. However, a steady increase in absorbance with increasing time of the experiment (from 1.206 on the day 0 to 1.533 on the 200th day) (Fig. 3) and a slight increase in the width of SPR bands with increasing time indicate possible changes in the solution. This behavior can be explained by the formation of large aggregated NPs in the solution. A visual inspection of the stored solution confirmed the slight sedimentation of AgNPs on the bottom of Erlenmeyer flask.

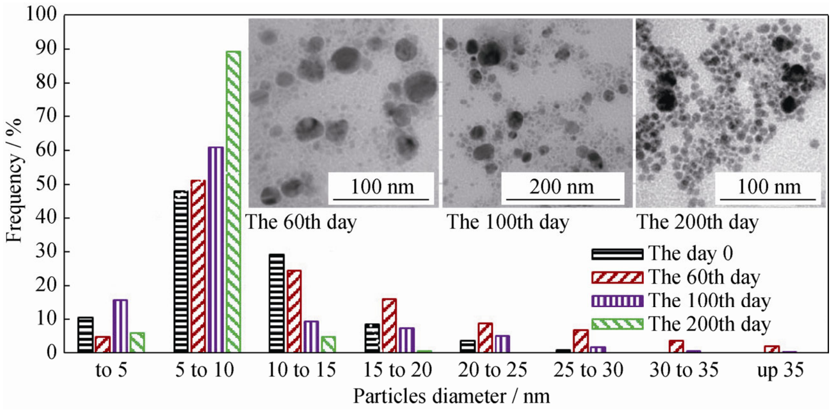

Fig. 4. Size distribution of AgNPs stored at room temperature in daylight (the insets show the TEM images of AgNPs on the 60th, 100th, and 200th days of the experiment).

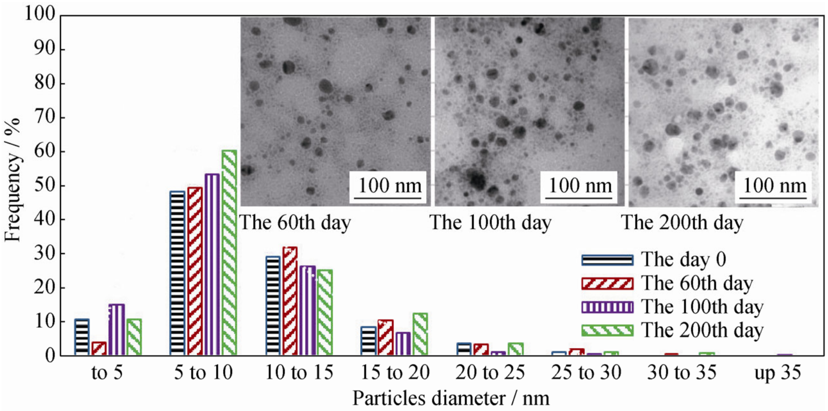

Fig. 5. Size distribution of AgNPs stored at room temperature in the dark (the insets show the TEM images of AgNPs on the 60th, 100th, and 200th days of the experiment).

The aforementioned experimental results show that the presence or absence of light critically influences the AgNP stability. However, a combination of darkness and low temperature can ensure even better long-term stability of AgNPs. The UV/Vis spectra did not change (Fig. 2(c)), and no agglomeration or significant increase in particle frequency was observed over time (Fig. 6).

Important information about the surface charge of AgNPs and the stability of their colloidal solutions was obtained from the surface charge measurements as a function of zeta-potential. According to the research of Izak-Nau *et al.* [19], the stability of AgNPs mainly depends on their surface charge. They reported that, in general, neutral particles exhibited no changes, positively charged particles were the most sensitive (even in the case of a solution stored in the dark at 4°C), and the sensitivity of negatively charged particles strongly depended on the storage conditions, which is in good agreement with our own observations. The measurement of zeta-potentials on the day 0 revealed a negative charge (−18.8 mV), which confirmed the presence of free negatively charged functional groups on AgNPs prepared via green synthesis. The zeta-potential on the 200th day for AgNPs stored at room temperature in daylight and in the dark decreased slightly to −13.6 and −15.4 mV, respectively, which indicates a loss of stability. AgNPs stored at 5°C in the dark showed a slight increase in zeta-potential (−19.8 mV). Although this increase was not substantial, it nonetheless confirms that the presence of more negatively charged groups ensures better stability of AgNPs in an aqueous medium.

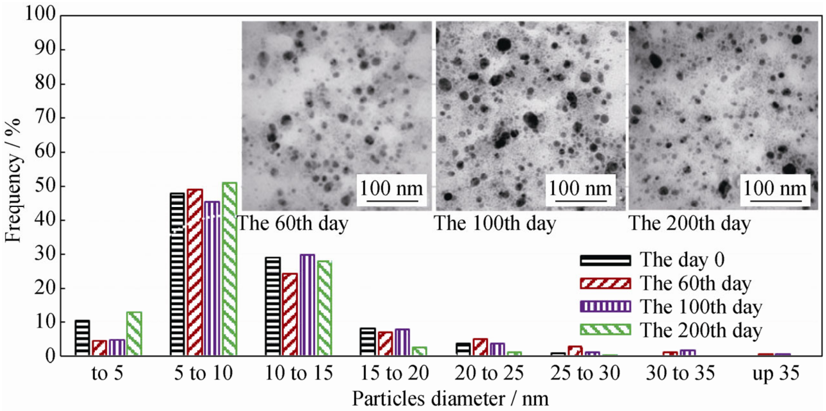

Fig. 6. Size distribution of AgNPs stored at 5°C in the dark (the insets show the TEM images of AgNPs on the 60th, 100th, and 200th days of the experiment).

4. Conclusions

AgNPs with a particle size as low as 20 nm were successfully prepared using algae *Parachlorella kessleri*. The experimental results are summarized as follows:

(1) The long-term stability of AgNPs is influenced by their storage conditions.

(2) The strongest transformations occur in the samples

stored at room temperature and exposed to daylight.

(3) Storage in dark conditions positively influences the AgNPs' stability.

(4) The combination of darkness and about 5°C has a critical positive influence on AgNPs' stability. AgNPs stored in this manner are stable when dispersed in solution, and no phase separation or agglomeration is observed even after months of storage.

(5) Zeta-potential measurements confirm better dispersity and stability of AgNP solutions stored at 5°C in the dark.

Acknowledgements

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