



# Article Effect of Storage Conditions on the Stability of Colloidal Silver Solutions Prepared by Biological and Chemical Methods

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**Abstract:** The research aimed to observe the influence of the storage conditions of silver colloidal solutions prepared by biological (green) and chemical methods on their long-term stability. Green methods for reducing and stabilizing silver nanoparticles (AgNPs) use natural substances. The rosemary leaf extract was used for AgNPs synthesis, and prepared nanoparticles were spherical (average size of 12 nm). In the chemical method, commercial chemicals (NaBH<sub>4</sub>, TSC, PVP, and H<sub>2</sub>O<sub>2</sub>) were used, and two colloids were prepared; the first contained spherical nanoparticles with an average size of 8 nm, and the second triangular prisms with an average size of 35 nm. The prepared colloids were stored under four conditions: at room temperature in the light and the dark, and at a temperature of 5 °C (refrigerator) in the light and the dark. The results confirmed the influence of storage conditions on the stability of nanoparticles. Colloids stored at 5 °C in the dark show the best stability. However, differences in stability dependent on the shape of nanoparticles prepared by chemical method were also observed; triangular nanoparticles showed the least stability. Methods such as UV–vis spectrophotometry, TEM, and EDX were used to analyze the nanoparticles before and after storage.

Keywords: AgNPs; stability; green synthesis; chemical synthesis; TEM; UV-vis spectrophotometry

# 1. Introduction

One of the areas of science that is experiencing a rapid increase in interest is the area of nanotechnology. Nanoparticles have several unique properties that are used in various fields [1–3]. Their properties are determined by their size and shape [4,5]. Silver nanoparticles have gained attention mostly for their antimicrobial properties, which make them potentially useful for various applications, such as medicine and food packaging. For creating a food package with antibacterial properties, AgNPs have to be incorporated into packaging materials, for example polymers or biopolymers. Such materials can help inhibit the growth of bacteria, fungi, and other microorganisms. Silver nanoparticles are also irreplaceable in medicine, for example as drug delivery systems, diagnostic imaging, dental materials, and wound healing. AgNPs can be utilized as highly sensitive NP probes for targeting and imaging small molecules, DNA, proteins, cell tissue, and even tumors in vivo [6]. Diverse silver nanomaterials have been studied as components of nanocomposites due to their high dielectric constants in numerous systems. For example, silver nanowires can be used as conductive coatings in flexible electronics and transparent semiconductors [7].

In general, the two basic approaches to the preparation of nanoparticles are physical and chemical. The chemical approach is further divided into chemical methods [8,9] and



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**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). biological so-called green methods of synthesis of nanoparticles of metals [10,11]. Both chemical and biological synthesis methods have their advantages and disadvantages.

Among the main disadvantages of chemical methods is the use of toxic reducing and stabilizing agents; at the same time, the purchase of reagents requires financial costs. On the other hand, biological synthesis using plants is non-toxic, and if a plant growing in the vicinity is chosen for the synthesis, there is no need to invest in its purchase. A disadvantage of the chemical method can also be considered its sensitivity to the influence of various parameters (pH of solutions, concentrations, process temperature, mixing, light conditions, order, and speed of reactant addition). Even a small deviation in the process can make the repeatability of the process difficult. The common feature of both methods is that they are inexpensive; they do not require complex equipment or procedures. One of the fundamental advantages of the chemical method is that, by combining suitable reagents, it is possible to prepare nanoparticles of various shapes, which the biological method does not allow due to the complexity of the composition of plant extracts. The chemical method makes it possible to prepare triangles, cubes, sticks, tubes, flower shapes, etc. [12-14]. The shape of the particles can be influenced by several factors, for example by changing the range of concentrations, volumes, ratios of input substances, and other synthesis conditions. In addition, it is possible to use different reducing and stabilizing agents, as well as their combination. Commonly used reducing agents are glucose, hydrazine, ascorbate, sodium citrate, ethylene glycol, dimethylformamide, dextrose, and sodium hydrogen borate [13,15]. PVP, sodium citrate, or even ammonium citrate are usually used as stabilizing agents [14,16,17].

In the case of green methods, biological materials are used instead of chemical agents. Various microorganisms and plant extracts are used, which naturally contain both reducing and stabilizing components. The biological method has the greatest benefit in that the reactants replace toxic chemicals, which prevents the formation of potentially dangerous by-products, simplifies the process, and minimizes the cost of incoming materials [18–20].

Several authors are currently working on synthesizing silver nanoparticles using the green method. For example, A.M. Fayaz et al. [21] used the microscopic fungus *Trichoderma viride* to research the extracellular biosynthesis of AgNPs from silver nitrate solution. An example of the application of a plant extract is the work of M. Gomathi and co-authors [22], where they used an extract from the leaves of *Gymnema sylvestre* as a reducing agent. They obtained spherical particles with a size of 20–30 nm. One of the ecological methods of preparing AgNPs was verified by X. Ma and his team [12]. In this case, the authors prepared silver nanoparticles in the form of nanoflowers using L-cysteine and confirmed that L-cysteine functions as both a reducing agent and a capping agent at the same time and can ensure the long-term stability of the prepared AgNPs.

While in the case of biological synthesis it is complicated to separate the biomass components responsible for reduction from those responsible for stabilization, in chemical synthesis the combination of reactants, their amount, and concentration is relatively simple. Therefore, in some cases, it is more appropriate to use a chemical method. In their work, M. Ledwith and colleagues [13] verified the possibility of combining reducing agents in the synthesis of AgNPs. In the first step of the synthesis, they used NaBH<sub>4</sub>, and in the second step they added a weaker reducing agent, L-ascorbic acid. Using this procedure, they prepared triangular-shaped nanoparticles. The team of researchers led by Y. Wang pioneered the method of preparing nanocubes using polyol synthesis. They used silver trifluoroacetate (CF<sub>3</sub>COOAg) as a metal precursor, which made it possible to obtain nanocubes with a size of  $\sim 30$  nm [15]. A similar procedure for the preparation of cubic AgNPs was chosen by B. Wiley et al., with the difference that they used chloride ions from NaCl to protect the particles from aggregation. The synthesized particles had a size between 20–80 nm [23]. C.J. Murphy et al. published a procedure for the preparation of nanoparticles by reduction with sodium borohydride, citrate, and cetyltrimethylammonium bromide. They obtained particles in the form of nanorods (with a length of ~250 nm and a diameter of 10–20 nm) and nanowires (with a length of  $\sim$ 2–4 µm and a diameter of 10–20 nm) [14]. The given examples confirm the ability of chemical agents to influence the shape of the resulting particles.

The problem with the resulting nanoparticles is their stability. Particles tend to form agglomerates and undergo surface oxidation. Aggregation can be minimized by several methods. Steric repulsion uses polymeric substances such as polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), polymethacrylic acid (PMAA), polymethyl methacrylate (PMMA) and dodecanethiol [24]. The second option is electrostatic repulsion.

Miesen et al. [24] published a study in which they stabilized nanoparticles by creating a surface layer consisting of hybrid lipids and hydrophobic thiols. The thiols used had different hydrocarbon chain lengths. Stability in the strong oxidizing agent KCN was also investigated. The study showed that nanoparticles modified in this way are not subject to surface oxidation or shape conversion. They are also stable against the action of oxidizing agents. The determining factor was the length of the hydrocarbon chain of the thiol used. Longer chain thiols provided higher stability. This stability was observed for 21 days, during which time no changes in shape or aggregation of nanoparticles were observed. Oliveira M. and colleagues confirmed the effectiveness of steric stabilization and used dodecanethiol as a stabilizing and capping agent [25]. In this way, they prepared spherical nanoparticles in a narrow size range. Subsequently, their stability was investigated. The resulting particles had stability from 60 days to several months, depending on the ratio of concentrations of dodecanethiol to silver cation in the metal precursor.

An example of electrostatic repulsion in ensuring the stability of nanoparticles is the work of Mendis et al. [26]. The authors prepared spherical nanoparticles of different sizes. The metal precursor was AgNO<sub>3</sub> reduced with sodium tetrahydroborate. Trisodium citrate and hydrazine sulfate were used as stabilizing agents. They then monitored their stability depending on the temperature and pH of the solution. The results showed the best stability of the particles at a temperature of 40 °C in the dark at a pH of 7–8 for up to 90 days.

Ensuring long-term stability is a very important factor that has a direct impact on synthesis costs and time spent. Therefore, it is important to analyze the conditions affecting the stability of colloidal solutions of metal nanoparticles.

This work aimed to assess the effect of storage conditions on the stability of nanoparticles prepared by biological and chemical methods. At the same time, the effect of stabilizing agents used in biological and chemical synthesis was analyzed. Nanoparticles were stored under four conditions: cold, dark; cold, light; room temperature, dark; room temperature, light. We characterize prepared silver nanoparticles by standard analyzing techniques such as FTIR spectroscopy, UV–vis spectrophotometry, EDX, and TEM analysis. The size and shape of nanoparticles were characterized by electron microscopy and the image size analyzer ImageJ.

## 2. Materials and Methods

Silver nitrate (>98%) purchased from Mikrochem Ltd., Pezinok, Slovakia was used as a silver precursor. The leaves of *Rosmarinus officinalis* were collected in summer in the Botanical Garden of Košice, Slovakia. Dried Rosemary leaves were used for the extract preparation. Sodium borohydride (NaBH<sub>4</sub>,  $\geq$ 98%), sodium citrate (TSC,  $\geq$ 99%), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%), polyvinylpyrrolidone (PVP, M.W. approx. 360,000) were also purchased from Mikrochem Ltd., Pezinok, Slovakia, and used as received. De-ionized water was used for preparing all solutions. For the preparation of solutions, their dilution and the preparation of colloids, laboratory glassware was used, as needed: beakers of different volumes, Erlenmeyer flasks, funnels, and pipettes.

### 2.1. Biological (Green) Synthesis of AgNPs

Procedure for extract preparation from dry Rosemary leaves: a beaker with volume 100 mL was used to leach of 1.3 g of dry rosemary leaves in 50 mL of distilled H<sub>2</sub>O; this mixture was heated to 75 °C for 15 min. The extract was filtered through Whatman filter paper. The pH of the prepared extract was 5.43.

Nanoparticle preparation procedure:

- 1. Precursor of Ag<sup>+</sup> ions—200 mL of AgNO<sub>3</sub> solution with a concentration of 50 mg/L Ag was used. The pH of the solution was 5.78;
- 2. AgNO<sub>3</sub> solution was heated to  $\sim$ 70 °C;
- 3. 20 mL of the prepared extract was added at constant stirring to the heated AgNO<sub>3</sub> solution. The pH of the prepared colloidal solution was 4.87;
- 4. Prepared 220 mL of silver colloidal solution (AgNPs) was divided into four Erlenmeyer flasks at 50 mL. Each flask was stored under different conditions, flasks were labeled as follows:
  - AgNPs prepared by Rosmarinus extract stored at room temperature in daylight— R—RT, light;
  - AgNPs prepared by Rosmarinus extract stored at room temperature in the dark— R—RT, dark;
  - AgNPs prepared by Rosmarinus extract stored in the refrigerator (~5 °C) in daylight—R—cold, light;
  - AgNPs prepared by Rosmarinus extract stored in the refrigerator (~5 °C) in the dark—R—cold, dark;
- 5. Lighting conditions: in the case of storage at room temperature, daylight was used, and in the case of storage in the refrigerator, artificial lighting imitating daylight was used. In the refrigerator, the light was turned off in accordance with the daylight;
- 6. The UV–vis spectra of the colloids were measured half an hour after synthesis (D0) and on the first (D1), the fourth day (D4), and each 7th day for two months (D7, D14, D21, D28, D35, D42, D49, D56, and D60);
- 7. All experiments were repeated three times, and the results were comparable, almost identical.

## 2.2. Chemical Synthesis of AgNPs

Two colloidal solutions were prepared by chemical methods (yellow—Y and blue—B); the difference between both solutions was in the content of reactants. The concentrations and amounts of reactants are given in Table 1.

Labeling of Solutions	AgNO <sub>3</sub>	TSC	PVP	$H_2O_2$	NaBH <sub>4</sub>
Yellow solution—Y	0.11 mM 172 mL	30 mM 14.72 mL	-	-	100 mM 0.8 mL
Blue solution—B	0.11 mM 172 mL	30 mM 14.72 mL	2% <i>w/w</i> 14.72 mL	30% <i>w/w</i> 0.48 mL	100 mM 0.8 mL

Table 1. Combination of reagents.

The procedure of AgNP colloid preparation was as follows:

- 1. To prepare the yellow colloid (Y), TSC and NaBH<sub>4</sub> solution was added during constant stirring to AgNO<sub>3</sub> stock solution, Table 1;
- 2. To prepare the blue colloid (B), TSC, PVP, H<sub>2</sub>O<sub>2</sub>, and NaBH<sub>4</sub> solutions (in this order) were added during constant stirring to AgNO<sub>3</sub> stock solution, Table 1;
- 3. All solutions were used as freshly prepared and NaBH<sub>4</sub> solution must also be ice-cold;
- 4. The prepared colloidal solution was each divided into four Erlenmeyer flasks at 50 mL. Flasks were stored under different conditions (as in the case of biologically prepared AgNPs); flasks were labeled as follows:
  - yellow and blue solutions of AgNPs stored at room temperature in daylight were labeled—Y—RT, light, and B—RT, light, respectively;
  - yellow and blue solutions of AgNPs stored at room temperature in the dark were labeled—Y—RT, dark, and B—RT, dark, respectively;

- yellow and blue solutions of AgNPs stored in the refrigerator (~5 °C) in daylight were labeled—Y—cold, light, and B—cold, light, respectively;
- yellow and blue solutions of AgNPs stored in the refrigerator (~5 °C) in the dark were labeled—Y—cold, dark, and B—cold, dark, respectively;
- 5. The UV–vis spectra of the colloids were measured in the same intervals as in the case of biologically prepared colloids. All experiments were repeated three times, and the results were comparable, almost identical.

# 2.3. Methods

Synthesized AgNPs were monitored by UV–vis Spectrometer (UNICAM UV–vis Spectrophotometer UV4, Waltham, MA, USA). The size and morphology of the nanoparticles were studied using TEM (JEOL model JEM-2000FX, Tokyo, Japan, with an accelerating voltage of 200 kV). EDX analysis was used to determine the chemical composition of nanoparticles. The image analysis (ImageJ 1.54g software) was used for the analysis of Ag nanoparticles' size distribution. The number of measured nanoparticles ranged from 300–500 nanoparticles. Spherical nanoparticles were measured in two mutually perpendicular directions and averaged; for triangles, the height of the triangle was measured and averaged.

The infrared spectra were recorded on a Bruker Tensor 27 FTIR spectrometer equipped with DTGS KBr detector (Munich, Germany). For each sample, 64 scans were measured in the 4000–400 cm<sup>-1</sup> spectral range in the abs mode with a resolution of 4 cm<sup>-1</sup>. The KBr pressed-disc technique was used for preparing a solid sample for routine scanning of the spectra. Samples (dried) of approximately 0.1 mg were dispersed in 150 mg of KBr to record optimal spectra in the regions 4000–400 cm<sup>-1</sup>. The diameter of the pellets, pressed from samples, was 13 mm.

## 3. Results and Discussion

#### 3.1. AgNP Synthesis—Influence of Reagents

The first sign of AgNP synthesis is a change in the color of the solution. The color change is the result of Local Surface Plasmon Resonance (LSPR), which is characteristic of precious metal nanoparticles, and its color manifestation is dependent on the shape and size of the nanoparticles. The addition of rosemary leaf extract to AgNO<sub>3</sub> stock solution caused the solution to turn a honey-brown color, Figure 1a. Such typical behavior was observed by other authors [27,28].



Figure 1. AgNP colloids prepared by biological method (a), and chemical methods Y (b) and B (c).

In the case of the synthesis of silver nanoparticles by chemical method, depending on the reactants used, two solutions were created, yellow, Figure 1b and blue, Figure 1c. Biologically synthesized nanoparticles were formed within 10 min of the addition of the extract; the yellow solution became colored after 10 min and blue after 30 min.

*R. officinalis* is a good source of natural antioxidants, essential oils, flavonoids, polyphenols, and phytochemicals, which functional groups and organic compounds can provide

effective metal-reducing, capping/stabilizing effects [29–33]. Comparing the IR peaks before and after AgNP synthesis can help to determine functional groups responsible for the reduction and stabilization of AgNPs.

# 3.1.1. Biological Reagents

The extract from the rosemary leaves was analyzed using FTIR analysis, to find out its approximate composition and also the changes that occur after the synthesis of silver nanoparticles. Based on these changes, we could predict which phytochemicals participated in the nanoparticle synthesis. The IR spectrum, Figure 2, shows strong, broad hydrogenbonded peaks at 3353 cm<sup>-1</sup> which are assigned to O–H stretch in phenols and alcohol compounds. Hydroxyl groups and C–H stretches (alkyl groups—present in most organic molecules) were observed at 2929 cm<sup>-1</sup> [33,34].



Figure 2. IR spectra of *R. officinalis* plant extracts before and after AgNPs synthesis.

The IR spectrum of *R. offcinalis* also shows a strong peak at 1600 cm<sup>-1</sup>, which is assigned to C=O carbonyl groups of proteins. C-C stretch (in the aromatic ring) is evident at 1526 cm<sup>-1</sup>. Peaks at 1720 and 1264 cm<sup>-1</sup> correspond to C-O-C stretching vibration band of unsaturated esters carbonyl group and ethers, respectively. Alcohols have characteristic IR absorptions associated with both the O–H and the C–O stretching vibrations, C=O stretching from alcohol, ester, and ether can be identified at 1260–1100 cm<sup>-1</sup> [33,34]. The free amino acid has a peak at 1400 cm<sup>-1</sup> (CH<sub>2</sub> bend). The IR spectra for *R. offcinalis* show the main peak positions of carbonyl groups, amino acid, and aromatic C–C–C stretch.

Comparison of the IR spectra of the *R. offcinalis* extracts before and after AgNP synthesis confirmed the presence of the groups that form the bonds to silver. Noticeable changes were recorded for peaks around 1400, 1264, 1117–1075, and 817–668 cm<sup>-1</sup>. Blue shift of the free amino acid peak from 1400 to 1350 cm<sup>-1</sup> together with a decrease in intensity and red shift of the band at 1264 and 1117–1075 cm<sup>-1</sup> corroborate the formation of bonds with AgNPs. The decrease in organic compound peaks reveals the removal of the organic compounds from the extract. Such changes coordinate with AgNPs and form the protective layer, favoring their stability in water solution [34]. It is very difficult to determine exactly which phytochemicals participate in the reduction and which in the stabilization of nanoparticles. However, based on the change of the spectrum and the study of other articles with similar issues, we assume that terpenes and polyphenols are more responsible for the reduction and proteins and carbohydrates for stabilization.

## 3.1.2. Chemical Reagents

The stability, shape, and size of the prepared particles depend on the reaction conditions (temperature, time, mixing, light/darkness, pH) and the reagents used (chemical structure, molecular weight of polymers, dose, ionic strength, etc.) [35–39]. Control of nucleation kinetics during synthesis by mutual ratio, combination, and order of addition of stabilizing/capping agents can influence the shape, size, and aggregation of particles [40,41]. Under the stated conditions (Table 1, solution Y), the reduction of AgNO<sub>3</sub> with TSC and NaBH<sub>4</sub> lasted 1 min, and after 5 min, thermodynamically stable round AgNPs were prepared. The reduction proceeded according to the reaction:

$$4AgNO_{3}(aq) + Na_{3}C_{6}H_{5}O_{7}.2H_{2}O(aq) \rightarrow 4Ag(s) + C_{6}H_{5}O_{7}H_{3}(aq) + O_{2}(g) + 3NaNO_{3}(aq) + HNO_{3}(aq)$$
(1)

$$AgNO_{3}(aq) + NaBH_{4}(aq) + 3H_{2}O(\ell) \rightarrow Ag(s) + H_{3}BO_{3}(aq) + 7/2 H_{2}(g) + NaNO_{3}(aq)$$
(2)

 $NaBH_4$  acts as a strong reducing agent and citrate is a secondary reducing agent that synergistically supports the reducing effect of  $NaBH_4$  in the conversion of silver ions  $Ag^+$  to  $Ag^0$  and at the same time ensures the stabilization of the formed AgNPs before aggregation.

The transformation from round to triangular required the addition of other substances such as PVP and  $H_2O_2$ . Explaining the effect of each ingredient requires more comprehensive research. According to research by Zhang et al. and Zannotti et al.,  $H_2O_2$  plays the most important role in the transformation of spherical nanoparticles into triangular prisms [39,42].  $H_2O_2$  has the oxidizing ability of etching the AgNPs and modifying (changing) the shapes and dimensions of the nanoparticles. The proportion of nanoplates increases with increasing concentration of  $H_2O_2$  [39,42].  $H_2O_2$  in the presence of TSC and PVP stabilizers is the most suitable combination leading to the formation of triangles [42]. According to Zhang et al., PVP is not essential for prism formation, but the presence of hydrogen peroxide is most important [39]. PVP, in addition to the stabilizing/capping effect like TSC, also has an effect as a surfactant on the narrowing of the particle size distribution and the production of smaller, more uniform nanoparticles [39,40]. Besides the size and shape of nanoparticles, the most important characteristic of nanoparticles is their stability.

To compare the long-term stability of the nanoparticles, biologically (Rosmarinus (R)) and chemically prepared AgNP colloidal solutions (yellow (Y) and blue (B)) were stored under different conditions: at room temperature at light and dark; in the refrigerator ( $\sim$ 5 °C) at light and dark.

#### 3.2. Analysis of AgNP Colloids on D0

UV–vis spectrophotometry is the first analysis used to analyze colloidal solutions. The positions of the peaks, the number of peaks, and the symmetry of the absorption spectrum (ABS) can provide a lot of important information about the nanoparticles in the colloids. The slim and tall spectrum indicates nanoparticles in a narrow size interval. The ABS spectrum of spherical AgNPs usually shows ABS<sub>max</sub> at a wavelength near 400 nm. For example, a blue shift of ABS<sub>max</sub> indicates a decrease in mean nanoparticle diameter, and a red shift an increase. Several peaks indicate the presence of rod-shaped, triangular, or a mixture of nanoparticles of different shapes; it depends on the position and shape of the spectrum.

Figure 3a shows the UV–vis spectra of the prepared colloids. The yellow colloid prepared by the chemical method has an ABS<sub>max</sub> at a wavelength of 388 nm; the spectrum is symmetrical and slim, indicating that the prepared nanoparticles are uniform and in a narrow size interval. The spectrum of AgNPs prepared by the biological method shows a red shift of ABS<sub>max</sub> to 436 nm compared to the yellow solution. The red shift indicates an increase in the size of the nanoparticles and the mean width of the spectrum is higher than in the case of the yellow colloid, which supports the assumption that the nanoparticles will be larger, and the size interval wider. The UV–vis spectrum of the blue solution has three visible peaks and the ABS<sub>max</sub> is around 800 nm. Similar UV–vis spectra were observed also by Miesen et al. and Wu et al., both of which confirmed the presence of triangular nanoparticles will be in the blue solution.



**Figure 3.** UV–vis spectra of the colloidal silver solution prepared using the extract from *R. officinalis*, by chemical method—yellow, and blue solution on D0: (**a**), TEM micrographs of the colloidal silver solutions prepared by the *R. officinalis* extract (**b**), by chemical method—yellow (**c**), and by chemical method—blue solution (**d**), on D0.

TEM analysis confirmed the assumptions that resulted from the UV–vis spectra. Nanoparticles prepared using rosemary extract are spherical with an average size of 12 nm (size interval 2–28 nm), Figure 3b. Figure 3c (AgNPs from yellow solution) confirms the presence of spherical nanoparticles with an average size of 9 nm, in a narrower size interval of 2–18 nm. Figure 3d confirmed the presence of triangular prisms with an average size of 35 nm and a thickness of ~3–5 nm (size interval 20–65 nm). Xu et al. and Bahlol et al. in their works studied the synthesis of triangular nanoprisms and AgNPs of different shapes and proved that the AgNP colloids that contained triangular prisms have a blue color [44,45]. The histogram of size distribution is shown in Figure S1.

The synthesis of silver nanoparticles was also confirmed by EDX analysis, Figure 4. The analysis of biologically synthesized nanoparticles showed the presence of accompanying elements such as C, Cl, and S whose signals could have come from the extract that was used in the process of silver nanoparticle synthesis. The cause of accompanying signals is probably insufficient removal of biological components during sample preparation. Signals of elements such as Si and Cu present in chemically prepared nanoparticles may be due to impurities from carbon film preparation or grid.



**Figure 4.** EDX analysis of AgNP colloids prepared by biological method (**a**), and chemical method Y (**b**) and B (**c**).

# 3.3. Analysis of AgNPs' Stability under Different Conditions

The stability of three AgNP colloidal solutions (prepared using rosemary extract—R; chemically prepared yellow—Y; and blue solution—B) was observed for 60 days. The solutions were stored under different conditions:

- room temperature in the dark—RT, dark;
- room temperature in the light—RT, light;
- in the refrigerator in the dark—cold, dark;
- in the refrigerator in the light—cold, light.

We consider colloidal solutions to be stable if there is no or very small change in the shape or location of the spectra during the monitored time (60 days). Such spectra, without



change, are visible in colloids prepared using rosemary extract (R) and in chemically prepared yellow colloids (Y) but only in the case of storage in the dark, Figure 5a,d and Figure 6a,d.



**Figure 5.** UV–vis spectra of the colloidal silver solution prepared using the extract from *Rosmarinus officinalis*, stored: at room temperature in the dark (**a**), at room temperature in the light (**b**), in the refrigerator in the light (**c**), and in the refrigerator in the dark (**d**).



**Figure 6.** UV–vis spectra of the colloidal silver solution prepared by chemical method (yellow solution—Y), stored: at room temperature in the dark (**a**), at room temperature in the light (**b**), in the refrigerator in the light (**c**), and in the refrigerator in the dark (**d**).

A significant change can be observed in the spectra of the samples stored in the light at room temperature and in the refrigerator, Figure 5b,c and Figure 6b,c. The significant lifting of the shoulder at higher wavelengths and the loss of symmetry of the spectra, Figure 5b,c indicate that after 60 days there was an increase in the mean size of the nanoparticles, or their agglomeration. Since agglomeration was not observed, we assume that the loss of symmetry was caused by an increase in the size of the nanoparticles.

In the case of the yellow solution, Figure 6b,c, the formation of a second peak, or rather a prominent shoulder at a higher wavelength, indicates a change in the shape of the nanoparticles. Since the original peak remained and another one appeared, it can be assumed that other shapes will be present in the colloid in addition to the original spherical nanoparticles.

It follows that the most significant factor affecting the stability of nanoparticles is light.

TEM analysis, Figures 7 and 8, confirmed the conclusions from the UV–vis spectra. Nanoparticles prepared using rosemary extract and nanoparticles in a yellow solution stored in the dark did not change their size or shape at all, Figures 7a,d and 8a,d. The average size of nanoparticles stored in the dark, prepared using rosemary extract and in the yellow solution, were 14 and 9 nm, respectively. The histograms of size distribution are shown in Figures S1 and S2. It is clear that rosemary extract contains effective stabilizing agents and even spherical nanoparticles prepared by a chemical method (TSC is the stabilizing agent) can maintain long-term stability when stored in the dark.



**Figure 7.** TEM micrographs of the colloidal silver solution prepared using the extract from *Rosmarinus officinalis*, stored: at room temperature in the dark (**a**), at room temperature in the light (**b**), in the refrigerator in the light (**c**), and in the refrigerator in the dark (**d**) on D60.



**Figure 8.** TEM micrographs of the colloidal silver solution prepared by chemical method (yellow solution—Y), stored: at room temperature in the dark (**a**), at room temperature in the light (**b**), in the refrigerator in the light (**c**), and in the refrigerator in the dark (**d**) on D60.

On the other hand, nanoparticles stored in the light, regardless of whether they were in the cold or at room temperature, experienced significant changes. In all samples, Figures 7b,c and 8b,c, there was a change in size and shape, and other than spherical nanoparticles were also observed. The average size of light-stored nanoparticles prepared using rosemary extract increased to 18 nm and almost tripled to 35 nm in the yellow solution. In both cases, but mainly with nanoparticles prepared by the chemical method, hexagonal and triangular prisms, but also other shapes, were observed, Figures 7b,c and 8b,c.

The analysis of the results shows that, in addition to the increase in nanoparticles, light also promotes a change in their shape [46].

The biggest changes and at the same time the worst instability were shown by nanoparticles in the blue solution, which was confirmed by both UV–vis and TEM analysis, Figures 9 and 10. Regardless of the storage conditions, there was a significant shift of all  $ABS_{max}$  to smaller wavelength values. In addition to the significant shift of all  $ABS_{max}$ , there was also a change in the shape of the UV–vis spectra; the shoulder at wavelengths around 500–580 nm was lost, Figure 9. These changes occurred in all samples but at different times depending on the storage conditions:

- cold, dark—for 21 days;
- RT, darkness—for 7 days;
- cold, light, and RT, light—provided stability for only 1 day.

The explanation for such behavior of the spectra is a change in the shape of all present nanoparticles. It is possible to assume that truncated triangles will appear or that the original triangles will turn into cylindrical prisms. A similar change in nanoparticle shape was also observed by the authors Li et al. [46]. It is clear that the stabilizing effect of TSC and PVP is not permanent. They prevent the degradation of nanoparticles only for a short



time, which depends on the storage conditions. Nanoparticles stored under cold, dark conditions seem to be the most stable.

**Figure 9.** UV–vis spectra of the colloidal silver solution prepared by chemical method (blue solution— B), stored under different conditions: at room temperature in the dark (**a**), at room temperature in the light (**b**), in the refrigerator in the light (**c**), and in the refrigerator in the dark (**d**).

TEM images, Figure 10, show significant changes in the shape of nanoparticles (solution B) after 60 days. The triangles are more rounded (they have lost the sharpness of the corners) and some prisms have become cylindrical.



**Figure 10.** TEM micrographs of the colloidal silver solution prepared by chemical method (blue solution—B), stored: at room temperature in the dark (**a**), at room temperature in the light (**b**), in the refrigerator in the light (**c**), and in the refrigerator in the dark (**d**) on D60.

Figure 10c,d show that the mean size of the nanoparticles stored in the refrigerator did not significantly change, but the shape of the nanoparticles changed (the nanoparticles become more rounded). The mean size of the nanoparticles stored at room temperature increased from ~35 nm to ~40 nm. The histogram of nanoparticle size distribution is shown in Figure S4. Temperature promotes an increase in the mean diameter of the nanoparticles. However, when stored in the refrigerator, this growth-promoting factor is absent, so the mean radius stays unchanged. Table 2 compares the mean size and interval of the size distribution for all samples.

This comparison confirmed that spherical nanoparticles are more stable in the dark, regardless of the temperature. However, in the case of triangular nanoparticles, better stability was observed by storing them in the refrigerator with a small influence of light. However, it is important to remember that a significant change in the shape of the triangular nanoparticles occurred.

There are several works in which the authors observed the shape change of nanoparticles with time, but none of them monitored and compared the stability of nanoparticles under defined conditions. For example, the authors Lee et al. describe the ability of silver nanoparticles prepared by a chemical method to reversibly transform from triangular prisms to disks [46]. These authors also observed the loss of the peak at wavelengths

around 500–600 nm when converting triangular prisms to discs. However, they initiated this transformation on purpose and proved that it is possible to reversibly control the shape change of nanoparticles by changing the light conditions.

	D	Y	В		
	K	I	Triangular	Cylinder	
D0	12 nm (size interval 2–30 nm)	9 nm (size interval 2–20 nm)	35 (size interval 20–65 nm)	-	
D60	14 nm	8 nm	40 nm	30 nm	
RT—dark	(size interval 4–40 nm)	(size interval 2–25 nm)	(size interval 25–80 nm)	(size interval 15–45 nm)	
D60	18 nm	20 nm	40 nm	20 nm	
RT—light	(size interval 3–55 nm)	(size interval 2–50 nm)	(size interval 15–45 nm)	(size interval 5–50 nm)	
D60	16 nm	35 nm	35 nm	25 nm	
refrigerator—light	(size interval 4–50 nm)	(size interval 5–100 nm)	(size interval 30–45 nm)	(size interval 10–50 nm)	
D60	14 nm	9 nm	35 nm	15 nm	
refrigerator—dark	(size interval 3–30 nm)	(size interval 2–30 nm)	(size interval 25–45 nm)	(size interval 2–45 nm)	

Table 2. Comparison of the mean size and interval of the size distribution for all samples.

They also proved that during the cycles other prismatic shapes such as truncated prisms, polygon disks, and polygon prisms were created. According to the authors, the observed photomorphic effect was the result of a complex process of chemical equilibrium influenced by subtle changes in the initial concentrations of the components. We assume that when the particles are exposed to darkness, a shift in chemical equilibrium allows the more easily oxidized corner and tip regions of the particles to complex and dissolve. A competitive reaction occurs between the photochemical reduction of Ag<sup>+</sup> on the surface of AgNPs and the slower oxidation of AgNPs, which ultimately determines the final shape of the particle [46]. In our case, only the change of triangular prisms to prismatic discs was observed, Figure 10.

Even a minimal intervention in the chemical balance of the system can cause fundamental changes. In addition to lighting conditions, reagent concentrations and amounts are very important. In work [47], the team of authors led by Ngoc N. observed the influence of TSC, PVP, and  $H_2O_2$  on the formation of AgNPs at different amounts. They proved that even a small change in the amount of added reagents leads to a change in the shape of the nanoparticles. They also observed similar shapes of UV–vis spectra during the synthesis of triangular and cylindrical prisms as we observed. This confirms the correctness of our measurements when changing the shape of nanoparticles. However, in our case, there was no change through the change in concentration and amount of input reagents, but the main factor was the time and the influence of the light.

## 4. Conclusions

In the article, we compared the effect of storage conditions on the stability of silver nanoparticles prepared by biological and chemical synthesis. Spherical nanoparticles with an average size of 12 nm were successfully prepared by biological synthesis using rosemary extract. For comparison, spherical nanoparticles (average size 9 nm) and triangular prisms (average size 38 nm) were prepared using a chemical method.

The results of the experiments showed that the storage method of colloidal silver solutions has a fundamental influence on the stability of nanoparticles. In addition, it was confirmed that rosemary extract contains effective stabilizing agents that ensure the perfect stability of nanoparticles stored in the cold ( $\sim$ 5 °C) or even at room temperature in the dark. Changes were observed during storage in the light.

The stability of chemically prepared nanoparticles depends not only on the storage method but also on the shape (which is determined by the reagents used). Spherical nanoparticles are more stable than triangular prisms. At the same time, in both cases, the destructive effect of light on the stability of nanoparticles was observed.

In general, all nanoparticles were more stable when stored in the dark, regardless of whether they were in the cold or at room temperature.

**Supplementary Materials:** The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/met14050513/s1. Figure S1: The histogram of size distribution of nanoparticles prepared on D0; Figure S2: The histogram of size distribution of nanoparticles prepared on D60 by extract of *R. officinalis;* Figure S3: The histogram of size distribution of chemically prepared nanoparticles on D60—sample Y; Figure S4: The histogram of size distribution of chemically prepared nanoparticles on D60—sample B.

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