FORMATION OF STABLE SILVER NANOPARTICLES USING GALLIC ACID

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Abstract

In this study, silver nanoparticles (AgNPs) were synthesized using gallic acid as both a reducing and stabilizing agent. The synthesis process was carried out under various pH conditions (ranging from 6.0 to 9.0) and at different ratios of gallic acid to silver nitrate (from 1:10 to 1:20). The obtained nanoparticles were analyzed using UV–Visible spectroscopy, Dynamic Light Scattering (DLS), Fourier-Transform Infrared Spectroscopy (FTIR), Atomic Force Microscopy (AFM), and X-ray Diffraction (XRD) techniques.

Experimental results indicated that the optimal conditions were achieved at pH 9 with a gallic acid/silver nitrate ratio of 1:15, using 0.01 M $AgNO_3$ and 1 g/L gallic acid solutions, with a reaction duration of 40 minutes at a temperature of 70 °C. Under these conditions, the synthesized nanoparticles had an average size of 28 nm, showing high monodispersity and stability. Scanning Electron Microscopy (SEM) results confirmed that gallic acid functions effectively not only as a reducing agent but also as a stabilizer for the silver nanoparticles. Microbiological studies demonstrated that the resulting silver nanosuspension possessed strong biocidal properties.

***Keywords*:** Gallic acid, silver nanoparticles, nanosuspension, dynamic light scattering, particle size, nanoparticle morphology, stabilizer, reducing agent.

Introduction

The synthesis of silver nanoparticles (AgNPs) has garnered significant attention due to their unique properties and broad applications in medicine, electronics, and environmental sciences. These nanoparticles possess remarkable antimicrobial, optical, and electrical characteristics, making them suitable for a wide range of applications [1–5]. Recent advances in nanotechnology have enabled the development of various methods for synthesizing AgNPs, each offering distinct advantages and addressing specific challenges [6–10]. The chemical, physical, and biological methods used for synthesizing silver nanoparticles each come with their own set of benefits and limitations, sparking extensive discussions in the field of modern nanotechnology.

In recent years, interest in "green" methods for the synthesis of AgNPs has grown significantly. This trend arises as an alternative to conventional chemical reduction methods, which are often associated with issues related to ecotoxicity and high costs [11–13]. Among the phytosynthesis approaches, the use of gallic acid (GA) has attracted particular interest. This

compound is considered highly promising for nanoparticle synthesis due to its dual functionality: it acts as a strong reducing agent (E^0 = +0.55 V) and serves as an effective stabilizer owing to the presence of functional groups in its structure [14].

Taking these factors into account, the aim of the present study is to determine the optimal conditions for the synthesis of silver nanoparticles using gallic acid.

Results and Discussion

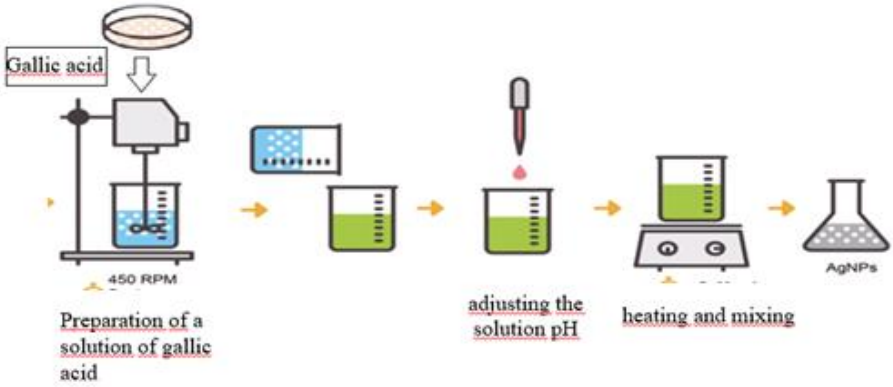

Figure 1. Process of silver nanosuspension synthesis in the presence of gallic acid

Figure 1 illustrates the sequential steps involved in the synthesis of silver nanoparticles using gallic acid. Initially, aqueous solutions of gallic acid and silver nitrate are prepared. The gallic acid solution is then added dropwise to the silver nitrate solution under continuous stirring using a magnetic stirrer. The pH of the reaction medium is gradually adjusted by adding 0.01 M NaOH solution. Once the desired pH level is reached, the mixture is heated and maintained under stirring for a specific period to facilitate the formation of silver nanoparticles.

In this process, the properties of the synthesized silver nanoparticles—such as particle size, size distribution, morphology, and stability—were investigated using various analytical techniques, including UV–Visible spectroscopy, Dynamic Light Scattering (DLS), X-ray Diffraction (XRD), Atomic Force Microscopy (AFM), and Scanning Electron Microscopy (SEM). The effects of different factors such as reagent concentrations, their ratios, temperature, and pH conditions on the particle size and distribution of the silver nanoparticles were also examined.

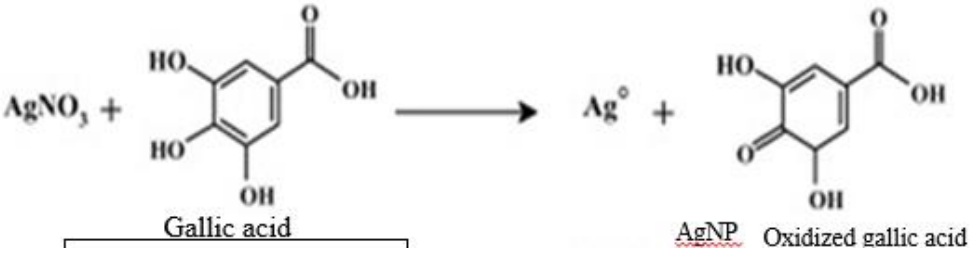

Figure 2. Reduction reaction equation of silver ions by gallic acid

Gallic acid exhibits strong reducing properties due to its ability to donate electrons, which results from the dissociation of hydrogen atoms from the three hydroxyl groups and one carboxyl group attached to its benzene ring. The released electrons reduce silver ions (Ag^+) to elemental silver atoms (Ag^0). These silver atoms subsequently aggregate to form silver nanoparticles.

Preventing the agglomeration of the newly formed silver nanoparticles is of crucial importance, as it ensures the formation of stable nanoparticles. Without proper stabilization, agglomeration can lead to the growth of larger microparticles, resulting in an unstable suspension. The use of gallic acid, therefore, not only facilitates the reduction process but also plays a key role in stabilizing the nanoparticles and preventing their aggregation.

The influence of synthesis conditions on the size and size distribution of silver nanoparticles in nanosuspensions synthesized under various conditions. The relationship between synthesis parameters and the size and size distribution of silver nanoparticles in nanosuspensions synthesized under different conditions was investigated. In particular, the effect of pH on the formation of silver nanoparticles was studied. Ultraviolet–visible (UV–Vis) spectra of the samples synthesized at pH values ranging from 6 to 9 were compared. In all samples, a characteristic absorption peak appeared at a wavelength of around 520 nm, indicating the presence of silver nanoparticles. As the pH increased toward more alkaline conditions, a gradual increase in the absorption intensity was observed.

This indicates that gallic acid possesses reducing properties under all tested pH conditions, and that its reducing capability increases in alkaline media. This behavior can be explained by the fact that during the reduction of silver ions by gallic acid, protons are released. In an alkaline medium, these protons are neutralized, thereby shifting the equilibrium of the reaction to the right, according to Le Chatelier's principle. As a result, a greater proportion of silver ions are reduced under mildly alkaline conditions, leading to enhanced formation of silver nanoparticles and, consequently, an increase in the intensity of light absorption.

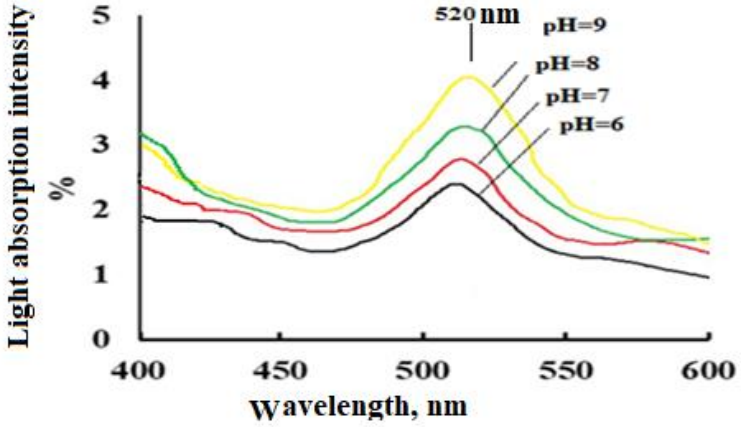

Figure 3. UV–Visible Spectroscopy Analysis

When the synthesis process was carried out at pH values higher than 9, a noticeable darkening of the solution was observed. This phenomenon can be explained by the hydrolysis of silver nitrate in highly alkaline media, leading to the formation of silver oxide.

The influence of pH on the formation of silver nanoparticles was also studied using Dynamic Light Scattering (DLS) to analyze the particle size and size distribution. The results obtained from DLS measurements correspond well with the UV–Visible spectroscopic data, confirming the formation of nanoparticles. It was found that increasing the pH from mildly acidic (pH = 6) to mildly alkaline (pH = 9) led to a higher yield of nanoparticle formation, along with a decrease in average particle size and a narrower size distribution.

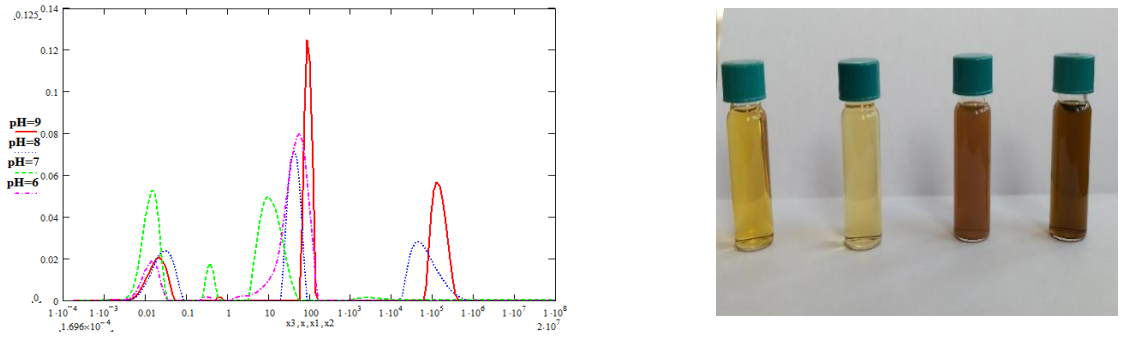

Figure 4. Particle size dependence on ph in nanosuspension analyzed by dynamic light scattering (DLS**)**

Dynamic Light Scattering (DLS) analysis of the synthesized nanosuspensions revealed the presence of three distinct particle size populations. The first group consisted of unreduced silver ions with particle sizes ranging from approximately 0.01 to 0.1 nm. The second group included well-formed silver nanoparticles with sizes between 10 and 100 nm. The third group comprised larger microclusters, with particle sizes ranging from 104 to 106 nm, which were formed due to the agglomeration of individual nanoparticles (Figure 4).

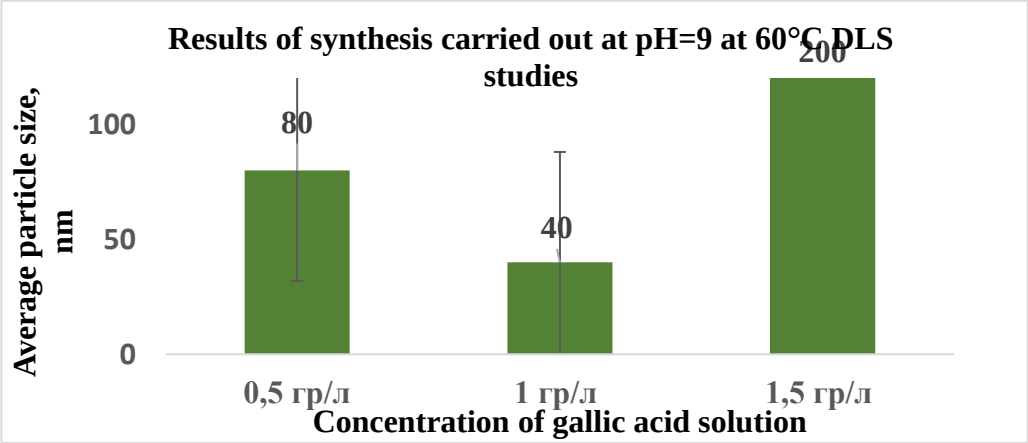

Figure 5. Diagram of the relationship between gallic acid solution concentration and average particle size of nanoparticles

In a 0.5 g/L solution of gallic acid, the average particle size of the nanoparticles was found to be 80 nm. When the concentration of gallic acid was increased to 1 g/L, the average particle size decreased to 40 nm, and in 1.5 g/L solutions, nanoparticles with an average size of 200 nm were formed. These observations indicate that the concentration of gallic acid plays a crucial role in controlling the size of silver nanoparticles during the reduction process.

Based on these findings, the optimal concentrations of gallic acid and silver nitrate for the reduction process were determined to be 1 g/L for gallic acid and 0.01 M for $AgNO_3$. These concentrations were selected as optimal for the synthesis of nanoparticles, and further studies were conducted to investigate the impact of various synthesis parameters on particle size and size distribution.

Subsequent investigations focused on the formation of nanoparticles at different ratios of gallic acid and silver nitrate solutions. The relationship between nanoparticle size, size distribution, and particle morphology was studied for these solution concentrations.

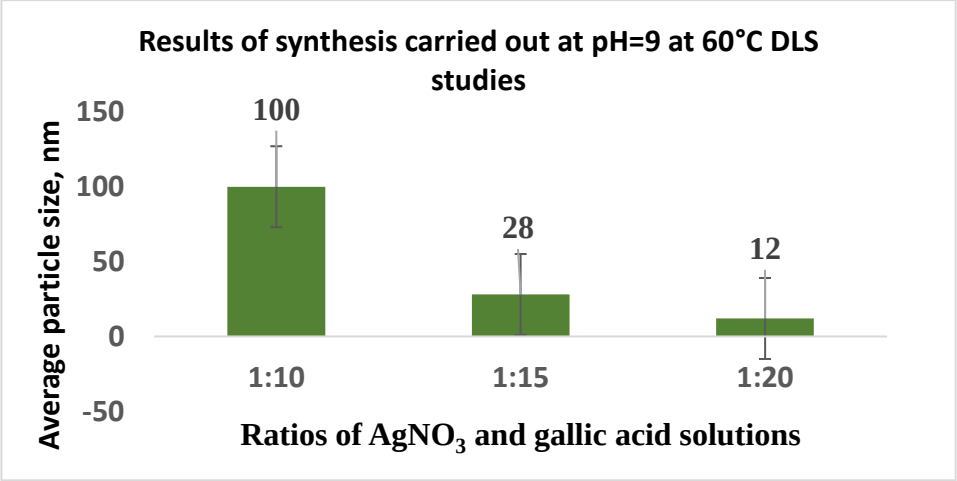

Figure 6. diagram of the relationship between $AgNO_3$ and gallic acid solution ratios and average particle size

The synthesis process was carried out under optimal conditions at pH = 9 and a temperature of 60°C. The average particle size was determined using the Dynamic Light Scattering (DLS) method. In the experiments, $AgNO_3$ at 0.001 M and gallic acid solutions at 1 g/L were used in volumetric ratios of 1:10, 1:15, and 1:20 and compared.

The results showed that as the concentration of gallic acid increased, the average particle size of the nanoparticles decreased. This can be explained by the stabilizing properties of gallic acid, which prevent agglomeration as its concentration increases, leading to the formation of smaller nanoparticles. At a 1:15 ratio of $AgNO_3$ to gallic acid, silver nanoparticles with an average size of 28 nm were obtained, resulting in a stable nanosuspension.

Additionally, the effect of $AgNO_3$ and gallic acid solution ratios on the size distribution of nanoparticles in the resulting nanosuspensions was investigated. The size distribution of the particles was analyzed using Dynamic Light Scattering (DLS), and the relative contributions of the main particles forming the nanoparticles were calculated based on the surface area under the absorption curves, as shown in Figure 6.

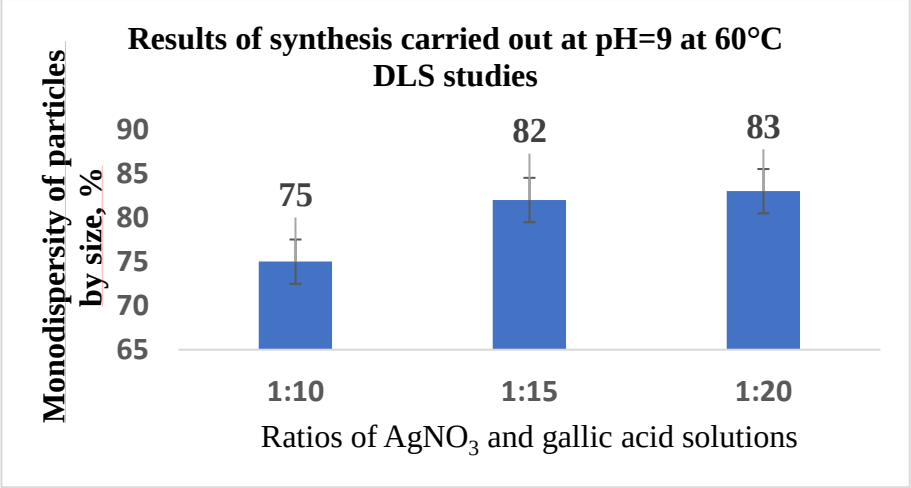

Figure 7. Relationship between $AgNO_3$ and gallic acid solution ratios and the proportion of uniformly sized nanoparticles

When studying the relationship between $AgNO_3$ and gallic acid solution ratios and the average particle sizes, it was found that in a nanosuspension prepared with a 1:10 ratio of $AgNO_3$ to gallic acid, 75% of the nanoparticles had a size of 100 nm. Additionally, in a nanosuspension prepared with a 1:15 ratio, 82% of the nanoparticles were 28 nm in size, and in a nanosuspension prepared with a 1:20 ratio, 83% of the nanoparticles were 12 nm in size (Figure 7). These results indicate that the ratio of $AgNO_3$ to gallic acid not only influences the particle size of the nanosuspension but also has a significant effect on the size distribution of the particles. Increasing the concentration of gallic acid facilitates the reduction of silver ions to silver atoms and helps prevent the agglomeration of nanoparticles simultaneously.

The silver ion reduction process in all methods is temperature-dependent and follows the Van't Hoff rule. In the case of the gallic acid reduction method, both the reduction of silver ions via electron attachment and the hydrolysis process occur simultaneously. The efficiency of the reduction reaction and the low occurrence of hydrolysis are crucial factors in the synthesis of stable nanosuspensions. Taking these factors into account, further investigations were conducted to determine the influence of reaction temperature on the average size of nanoparticles during the formation of silver nanoparticles using gallic acid.

Atomic force microscopy studies

The particle sizes of the silver nanosuspensions synthesized under the selected optimal conditions were investigated using Atomic Force Microscopy (AFM). The surface morphology of the nanosuspensions was analyzed by drying the suspension on a glass surface. The topography of the surface was examined in both contact and non-contact modes using a cantilever.

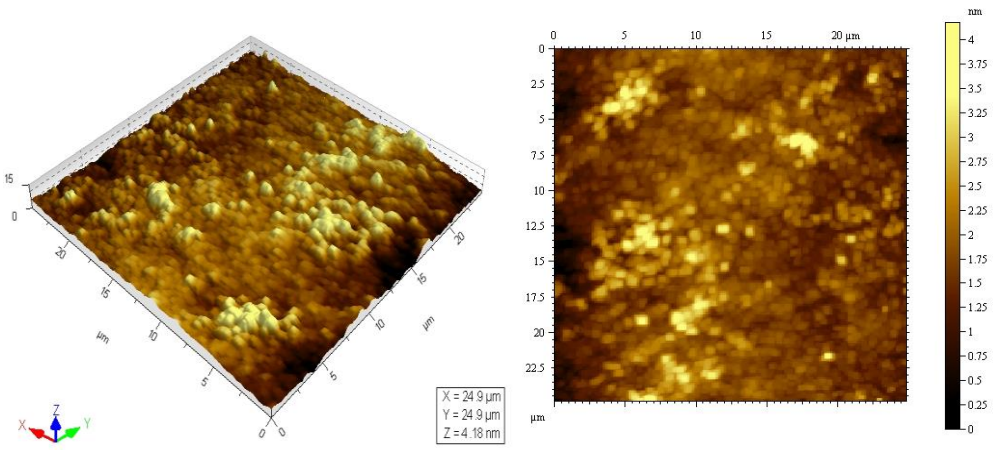

Figure 8. Atomic force microscopy images of surface morphology of silver nanoparticles

Atomic Force Microscopy (AFM) studies of the surface morphology of silver nanoparticles in the nanosuspension dried on a glass surface confirmed the presence of nanoparticles with sizes ranging from 50 to 150 nm.

X-ray diffraction studies of silver nanoparticles synthesized based on gallic acid in different ph environments. X-ray diffraction (XRD) studies were conducted to investigate the

amorphous-crystalline structure of silver nanoparticles obtained from gallic acid, by drying the nanosuspension on a glass surface in different pH environments.

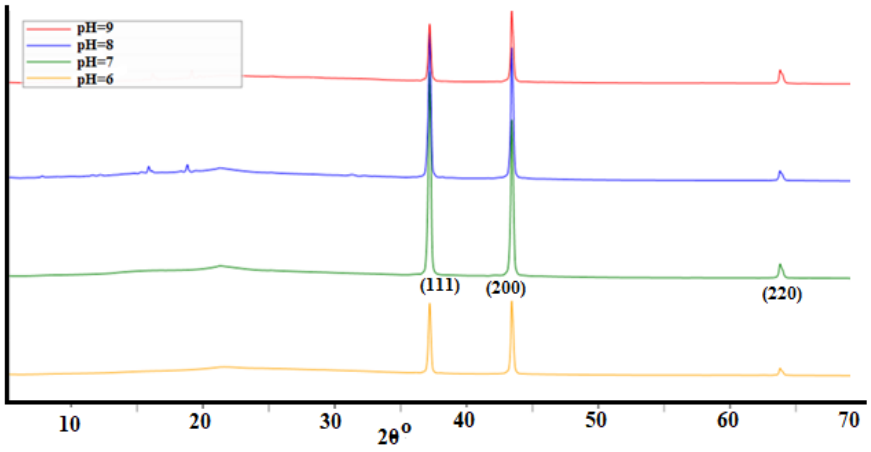

Figure 9. X-ray diffraction pattern of silver nanoparticles

The results of X-ray diffraction studies showed that characteristic peaks of silver nanoparticles were observed at the 38.53°, 44.53°, and 64.83° 2θ angles, corresponding to the (111), (200), and (220) crystal planes. The diffraction patterns of the samples matched with the reported peaks of crystalline silver nanoparticles (111), (200), and (220) from the literature [116]. By using the half-width of the diffraction peaks, the crystal size was determined to be 3-4 nm. Moreover, by varying the pH from 6 to 9, the crystallinity of the samples was evaluated using the Debye-Scherrer equation, which indicated an increase in crystallinity from 80% to 88%.

The obtained X-ray diffraction patterns did not show peaks corresponding to Ag_2O crystals, confirming the absence of Ag_2O.

Importance of maintaining nanoparticle stability during silver nanoparticle synthesis In the process of synthesizing silver nanoparticles, it is crucial to maintain their stability in the nanoparticle form. As highlighted in the literature review section of the research report, one of the major drawbacks of silver nanoparticles synthesized by physical and chemical methods is their inability to remain stable in nanoparticle form, requiring the use of stabilizing agents. While stabilizers incur additional economic costs, the use of chemical reagents for stabilization imposes limitations on the applications of the obtained nanosuspensions due to their specific properties.

For silver nanoparticles synthesized by biological methods, stabilizing agents are not required. This is because the substances and extracts used in biological synthesis contain various polar functional groups that enable stabilization. These groups interact with the positively charged silver nanoparticle surface and form a protective layer around the nanoparticles, stabilizing them. The positive surface charge of the silver nanoparticles leads to the formation of a negatively charged shell by the extract, increasing the Zeta potential, which in turn reduces nanoparticle agglomeration and improves stability.

However, the composition of the extracts used in biological synthesis can vary due to differences in the biological source (plants, microorganisms), environmental conditions, and geographical regions, which leads to variations in the chemical composition of the extracts. These differences can result in varying effects when forming nanoparticles using a specific plant or microorganism extract.

Molecular mechanism of stabilization of silver nanoparticles using gallic acid. The molecular mechanism behind the stabilization of silver nanoparticles synthesized using gallic acid is explained in the study. Gallic acid plays a significant role in the stabilization of silver nanoparticles by reducing silver ions and simultaneously preventing nanoparticle aggregation. The interaction between the gallic acid molecules and the silver nanoparticles ensures their stability, preventing their agglomeration and maintaining their nanoparticle form throughout the synthesis process.

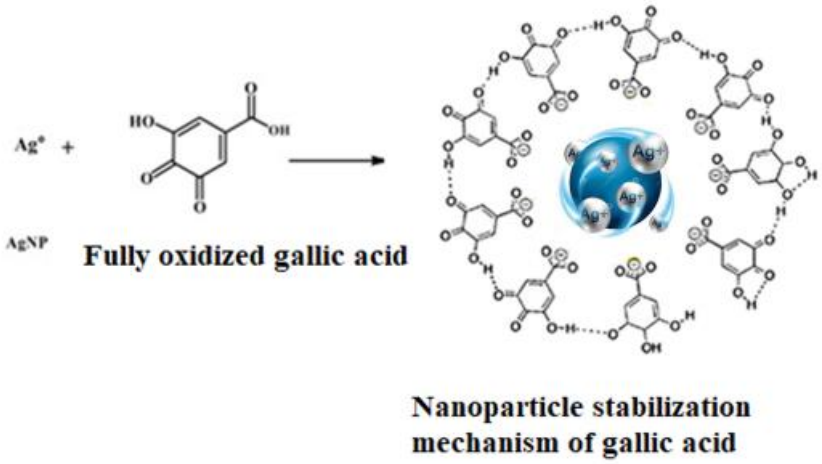

Figure 10. Molecular mechanism of stabilization of silver nanoparticles by oxidized gallic acid

In the synthesis of silver nanoparticles using gallic acid, the reaction results in the reduction of silver nitrate to silver atoms, while gallic acid itself gets oxidized, forming oxidized gallic acid. Several silver atoms then aggregate to form silver nanoparticles. Simultaneously, the fully oxidized gallic acid participates in stabilizing the formed silver nanoparticles. The stabilizing function of oxidized gallic acid prevents nanoparticle agglomeration.

The three hydroxyl groups in the benzene ring of gallic acid and the delocalized electrons in the ring cause a strong attraction to the relatively positive oxidation state of the carbon in the carboxyl group. This indicates that even the non-oxidized form of gallic acid has the ability to stabilize nanoparticles. As a result of the reduction of silver ions, the hydroxyl groups in oxidized gallic acid are oxidized to carbonyl groups, and the electron cloud in the ring is strongly directed towards the oxygen atom in the carbonyl group. This results in an increase in the positive oxidation state of the carbon in the carboxyl group and a simultaneous increase in the negative charge of the oxygen in the carbonyl group.

The strongly negative charge accumulated at the polar atom leads to the wrapping of the silver nanoparticle surface with the stabilizing structure of oxidized gallic acid. The carbonyl groups formed as a result of oxidation completely surround the nanoparticle surface, which increases the Zeta potential of the nanoparticles and prevents agglomeration, thus stabilizing the nanoparticles.

The results suggest that gallic acid is not only an effective reducing and stabilizing agent but also expands the possibilities for the biomedical applications of silver nanoparticles.

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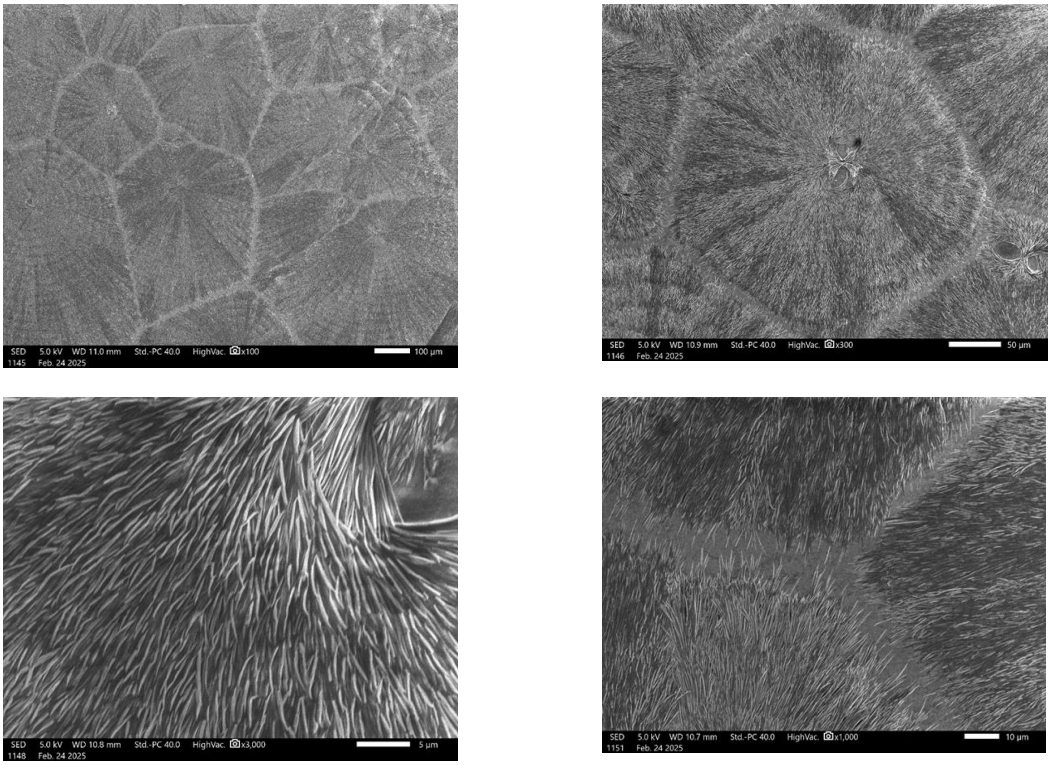

Figure 10. Scanning electron microscope (SEM) images of silver nanoparticles synthesized with the aid of gallic acid

The possibilities of stabilizing silver nanoparticles synthesized under the selected conditions using oxidized gallic acid were investigated by obtaining scanning electron microscope (SEM) images of the nanoparticles obtained with the participation of gallic acid. The positive surface charge of the silver nanoparticles and the negatively charged polar groups of the oxidized form of gallic acid interact through electrostatic forces, leading to the accumulation of both oxidized and non-oxidized gallic acid around the silver nanoparticles, which results in the stabilization of the silver nanoparticles.

From the scanning electron microscope images, it can be observed that needle-like crystal formations of oxidized gallic acid accumulate concentrically around the spherical silver nanoparticles. The image shows that the needle-like aggregations of gallic acid are distinctively organized around the center of a silver nanoparticle, and this aggregation is separate from other silver nanoparticles. This confirms that gallic acid not only plays a role as a reducing agent during the synthesis process but also functions as a stabilizer for the formed silver nanoparticles.

Considering the biocidal properties of silver nanosuspension, its use in medicine, pharmaceuticals, and as a disinfectant has been studied. In this context, the bactericidal activity of silver nanosuspension against pathogenic microorganisms was investigated by researchers at the Institute of Microbiology, using appropriate methods.

According to microbiological studies (Table 1), the silver nanosuspension synthesized using gallic acid as a stabilizing and reducing agent demonstrated high bactericidal properties. The optimal synthesis conditions included a 1:15 volume ratio of 0.01 M $AgNO_3$ to 1 g/L gallic acid solutions, carried out at pH 9, for a duration of 40 minutes, and at a reaction temperature of 70°C.

Table 1

Assessment of antimicrobial activity of silver nanosuspension

Test cultures	Zone of inhibition, mm
Bacillus subtilis	12
Escherichia coli	26
Candida albicans	26
Staphylococcus aureus	22
Pseudomonas aeruginosa	15

For this purpose, test cultures of pathogenic microorganisms such as *Escherichia coli* (E. coli), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Bacillus subtilis* from the collection of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan were used (Figure 11).

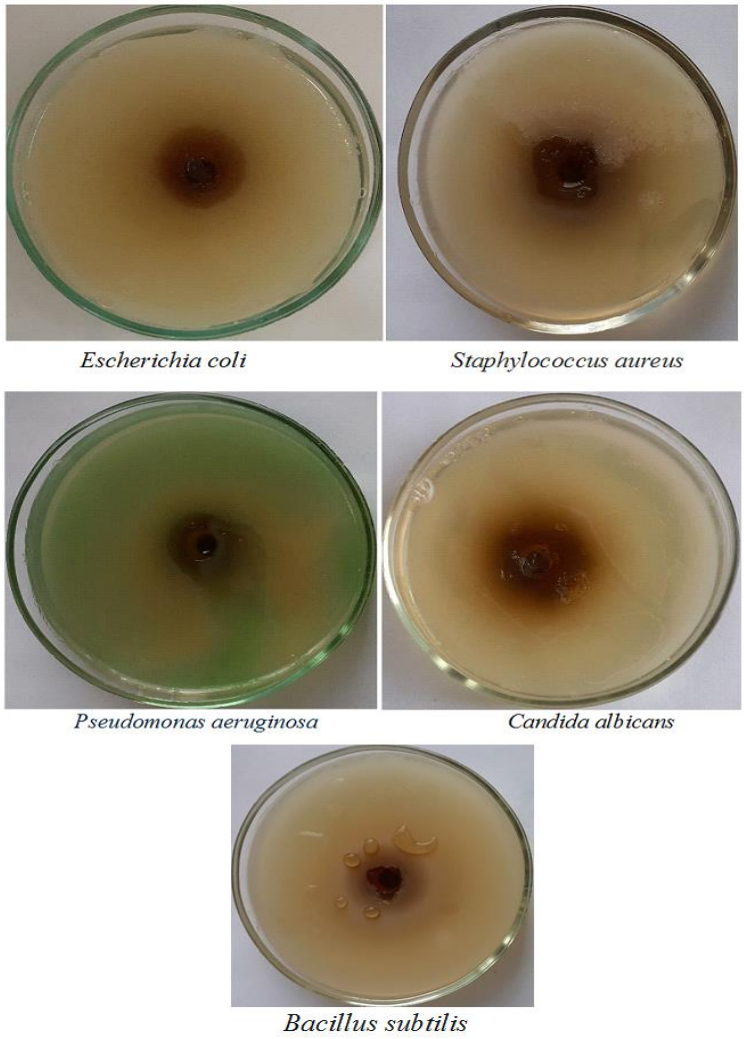

Figure 11. Determination of the antagonistic properties of silver nanosuspension synthesized using gallic acid

Conclusion

Nearly 120 scientific articles, patents, and academic literature were gathered and discussed on the synthesis, applications, and prospects of silver nanoparticles (AgNPs) using physical, chemical, and biological methods. Among the various synthesis methods, the approach utilizing gallic acid (GA) was identified as the most optimal, considering its stability in nanoparticle suspensions, broader applicability, and enhanced properties for use in medicine

and pharmacy. Based on the collected literature, a review article was submitted to *Chemical Review and Letters* journal.

The formation of silver nanoparticles, their size, and size distribution (monodispersity) in the presence of gallic acid were studied as a function of the pH of the solution. It was found that pH 9 provided the most optimal environment for the formation of silver nanoparticles.

The influence of the concentrations and ratios of $AgNO_3$ and GA solutions on the size and distribution of nanoparticles was investigated. Through various concentration and ratio experiments, it was determined that 0.01M $AgNO_3$ and 1 g/L GA solutions, in a 1:15 volume ratio, were optimal for nanoparticle formation.

The effect of reaction time and temperature on the size and distribution of nanoparticles was explored. It was found that to obtain monodispersed and stable nanoparticle suspensions, an optimal reaction time of 40 minutes and a temperature of 70°C were required.

During the synthesis of silver nanoparticle suspensions with gallic acid, the reduction of silver ions to form nanoparticles was accompanied by the oxidation of gallic acid. The oxidized form of gallic acid acted as a stabilizing agent, ensuring the stability of the nanoparticle suspension. This was confirmed through UV-Vis spectroscopy, Atomic Force Microscopy (AFM), and Scanning Electron Microscopy (SEM) analyses.

Silver nanoparticle suspensions synthesized under optimal conditions (0.01M $AgNO_3$, 1 g/L GA, 1:15 ratio, pH 9, 40 minutes at 70°C) exhibited significant biocidal properties. These properties were confirmed through microbiological testing, indicating the potential for applications in antimicrobial treatments.

Based on the optimal synthesis conditions, experimental batches of silver nanoparticle suspensions were produced on an industrial scale. The results led to the development of a technological regulation for producing biocidal silver nanoparticle suspensions using gallic acid, which was subsequently approved for larger-scale production.

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