

Green Synthesis of Silver Nanoparticles Using *Morus alba* Leaf Extract and Their Application in Enhancing Bioactive Peptides for Anti-Aging Cosmetics

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Abstract: Integrating nanotechnology within the formulation of bioactive peptides based on native plant stem cells holds significant potential for improving anti-aging cosmetics. This research involved the synthesis of silver nanoparticles which used the *Morus alba* (Vietnamese mulberry) leaves extract to reduce and stabilize silver nanoparticles in a reaction with silver nitrate solution... The properties of silver nanoparticles were analyzed by modern analytical methods including UV-Vis, HR-TEM, TGA/ DTA and FT-IR. The UV-Vis analysis results showed that the maximum absorption wavelength appeared at 430 nm, proving that silver ions were reduced to silver nanoparticles. Nanotechnology can greatly improve the bioavailability and effectiveness of these peptides in cosmetic products. This paper describes the synthesis of silver nanoparticles from mulberry leaf extract, their characterization, and their potential to further enhance the anti-aging properties of peptides.

Keywords: Nanotechnology in Cosmetics, *Morus alba*, Bioactive Peptides, Anti-Aging, AgPNs.

1. Introduction

In recent years, the green synthesis of silver nanoparticles (AgNPs) has gained considerable attention due to the increasing demand for environmentally friendly material synthesis technologies.

Nanotechnology is increasingly attracting not only scientific researchers but also businesses because of its high applicability in human life [1-4]. Because of their superior properties, nanomaterials have been applied in high-tech fields such as disease diagnosis, drug delivery in medicine, use as microprocessors in the electronics field, antibacterial products in the textile industry and many other fields. One of the most widely researched and applied nanomaterials is silver nanoparticles (AgNP) [2-4]. Silver nanoparticles with sizes in the range of 1-100 nm are used in many fields such as optoelectronics, photography, catalysis, cosmetics, textiles, medicine... thanks to their special chemical, physical and biological properties. Silver nanoparticles have antibacterial activity up to 50,000 times higher than silver in bulk form and are capable of killing more than 650 different species of bacteria. Moreover, silver nanoparticles have a large specific surface area, are chemically stable, can disperse stably in both polar and non-polar solvents, are non-toxic, nonirritating, and harmless to humans, animals, and the ecological environment [5-7]. To synthesize silver nanoparticles, we can use top-down methods such as electrospinning, high-energy beams, laser etching, mechanical grinding, chemical etching...; or bottom-up methods such as chemical vapor deposition, sol-gel, atomic condensation, supercritical fluid solutions, fiber spinning, green synthesis... [1-8]. Among them, chemical methods are still widely used because of their ability to synthesize quickly, easy to control particle size and relatively low cost. However, the by-products and chemical residues from this method can be toxic and have negative effects on the environment. Therefore, green synthesis methods, using active ingredients in extracts from algae, bacteria, fungi, yeast, plants... to synthesize silver nanoparticles is considered to be able to replace the above synthesis methods because of its low cost, environmental friendliness, no use of high energy sources, no use of toxic chemicals and can be synthesized on a large scale [2-4]. Active ingredients in plant extracts often contain polyphenol compounds, alkaloids, fatty acids, proteins... which act as reducing agents and stabilizers that coat silver nanoparticles to preventing them from aggregating to form large particles, and increase the efficiency of the fabrication process [3-7].

In the world and in the country, there have been many research projects using extracts from plant leaves to synthesize silver nanoparticles [3-7]. In Vietnam, research groups have used extracts from almond leaves, tea leaves, guava leaves, gotu kola leaves... to synthesize silver nanoparticles, but there have been no published works using mulberry leaves as research subjects [9, 11]. In this study, the author used an extract from Vietnamese mulberry leaves to synthesize silver nanoparticles.

Based on the color of the mulberry fruit, mulberry trees are divided into three main types including *Morus alba* L. (white mulberry), *Morus nigra* L. (black mulberry) and *Morus rubra* L. (red mulberry) [12]. White mulberry trees grow mainly in Asia, especially Southeast Asia, with white fruit or reddish edges, and leaves used to feed silkworms. Black mulberry trees grow mainly in Europe, with black fruit, and red mulberry trees grow in North America, purple fruit. Mulberry leaves contain flavones, flavone glycosides, steroids, alkaloids, carotenoids, amino acids, proteins... [6, 12, 13]. These organic compounds can act as reducing agents

and stabilizers in the synthesis of AgNPs from a silver nitrate. This study focused on the spectral characteristics, structure, shape, size, size distribution, and overall structure of AgNPs in order to define the best conditions for their synthesis.

The benefits of nanotechnology have permeated almost all domains, especially the cosmetics industry, which enjoys novel benefits from the use of nanoparticles. The industry is also capitalizing on the profound natural therapeutic potentials of skin care products. The growing trend in the industry is natural, plantbased ingredients. Among the targeted ingredients in the industry are plant stem cells with strong antiaging benefits. In terms of biodiversity, Vietnam is rich and diverse in unique plants which could have promising sources of bioactive peptides with anti aging cosmetic potentials. Among such plants, *Morus alba* is cited in the literature to be important in stem cell extraction and skin health benefits in cosmetic products. This study seeks to combine the benefits of nanotechnology and the use of bioactive peptides from the plants, demonstrating their potential effectiveness for antiaging use. It also analyzes the use AgNPs to improve the bioactive peptides efficiency in the cosmetic formulation.

Peptides, those short sequences of amino acids, are very important in the preservation and rejuvenation of skin. In skincare, peptides boost collagen production, improve skin elasticity, and diminish fine line and wrinkle cut. In cosmetic formulations, there are many different kinds of peptides.

Signal peptides: These peptides stimulate collagen synthesis and promote skin regeneration.

- **Carrier peptides:** These peptides aid in the delivery of essential trace elements to skin cells.
- **Neurotransmitter-inhibiting peptides:** Peptides like acetyl hexapeptide-3 mimic botulinum toxin, reducing muscle contractions and preventing wrinkle formation.
- **Enzyme-inhibiting peptides:** Peptides that inhibit the breakdown of collagen and elastin, thus preventing skin aging.

Nanotechnology in Cosmetics

Nanotechnology has emerged as a powerful tool for improving the efficacy of cosmetic ingredients. Nanoparticles, due to their small size, offer enhanced absorption rates and better penetration of active ingredients into the skin. In particular, silver nanoparticles (AgNPs) are widely used for their antimicrobial properties and their ability to stabilize and improve the delivery of other active ingredients, including peptides.

The combination of peptides and nanotechnology offers a promising solution for the development of more effective anti-aging products, particularly those that address collagen degradation and skin elasticity.

The integration of the AgNPs – peptide complex opens up a potential cosmeceutical platform thanks to the synergy between the antibacterial-stabilizing capacity of nanosilver and the extracellular matrix (ECM) regulating activity of peptides. At the molecular level, the Ag/Ag⁰ surface of the nanoparticle acts as a “carrier” for the peptide to anchor through coordination bonds (donor N/O/S at –NH₂, –COO[–], –SH, imidazole) and electrostatic interactions, which helps to reduce hydrolysis/oxidation, limit the loss of activity due to skin protease, and control the release according to pH/ion (the off-loading mechanism in the epidermal microenvironment). The nanosize and large surface area support increased diffusion through the stratum corneum and local residence, thereby allowing the use of lower peptide doses while still achieving effectiveness (increased collagen/elastin synthesis, improved elasticity). Silver provides a broad-spectrum antimicrobial barrier, reducing the microbial load in formulations and on the skin—particularly useful in situations of low-level inflammatory stress associated with aging. Technologically, green synthesis of AgNPs (e.g., with *Morus alba*) combined with seafood by-product-sourced peptides enables a circular model—sustainable, scalable, and suitable for “clean beauty.”

2. Material and Methods

2.1. Chemicals and Materials

Morus alba leaves of the Ha Bac variety were harvested in Hung Yen province in August 2024. Silver nitrate (AgNO₃) was provided by T & T Medical Equipment and Scientific Materials Chemical Company, Hanoi. The solvent used in the experiments was double-distilled water. The experiments and analyses were carried out at the Textile and Footwear Materials Testing Center, Rubber Science and Technology Center, Advanced Institute of Science and Technology, Laboratory of Petrochemical Refinery and Catalysis Technology, Hanoi University of Science and Technology, and Vietnam Academy of Science and Technology.

2.2. Method for Extracting Active Ingredients from *Morus alba* Leaves

The extraction method was used to extract the active ingredients from leaves. *Morus alba* leaves were washed, dried at 60°C, cut into small samples of about 5×5 mm, and stored in sealed plastic bags. Mulberry leaf samples were extracted in distilled water at a ratio of 1:20 (g dry leaves/ml distilled water) at boiling temperature for 10 minutes. After cooling, the extract was filtered through

a mesh to collect the extract. The extract was then filtered twice using Whatman No. 1 filter paper to remove all suspended solids in the extract. A portion of the extract was dried for FT-IR analysis. The remaining portion was used to synthesize silver nanoparticles.

2.3. Method for Synthesizing Silver Nanoparticles

To synthesize silver nanoparticles, the mulberry leaf extract solution was diluted 10 times before reacting with the silver nitrate solution, according to the experimental plans.

- **Option 1: Fixed reaction time.** Take 10 mL of diluted extract and react with 5, 25, 50, and 75 mL of 1M AgNO₃ at room temperature (25°C) for 6 hours.
- **Option 2: Fix the concentration of AgNO₃.** Take 10 mL of diluted extract and react with 50 mL of 1M AgNO₃ at room temperature for 1, 2, 4, 6, and 24 hours.

After the reaction time, the samples were immediately centrifuged and sonicated twice to remove the remaining reactants and to obtain AgNPs. The centrifugation conditions included a speed of 15,000 rpm for 30 minutes at 5°C on a Tomy MX-305, Japan (R112805) centrifuge. The ultrasonic conditions were at a frequency of 37 kHz, at room temperature, for 30 minutes on a Sharp UT-106H ultrasonicator, Japan.



Figure 1. Flowchart of the extraction process of compounds in leaves and synthesis of silver nanoparticles

2.4. Analytical Methods

Suitable conditions for synthesizing silver nanoparticles were determined using a Shimadzu UV-1800 molecular absorption spectrophotometer.

High resolution transmission electron microscopy (HR-TEM) was used to analyze the technical properties of silver nanoparticles including shape, size and particle size distribution.

Thermogravimetric analysis (TGA) method was used to determine the efficiency of silver nano synthesis process according to formula

$$H = \frac{(\%m_{TG} \times m_{AgNP})}{m_{Ag}} \times 100\%$$

$$m_{AgNP} = m_s - m_t$$

$$m_{Ag} = 10^3 \times C_M \times V \times M_{Ag} \times n_{Ag}$$

$$\%m_{TG} = \frac{m_{AgNP}}{m_{Ag}} \times 100$$

in where:

m_{TG} is the mass of nano silver according to TGA (%);

m_{AgNP} is the amount of synthesized silver nanoparticles (mg);

m_t is the mass of centrifuge tube (mg);

m_s is the mass of centrifuge tube containing AgNPs (mg);

m_{Ag} is the amount of silver in solution before reaction (mg);

M_{Ag} is the molecular mass of silver ($\text{g}\cdot\text{mol}^{-1}$);

C_M is the concentration of AgNO_3 solution ($\text{mol}\cdot\text{L}^{-1}$);

n_{Ag} is the number of moles of silver in the synthetic solution (mol);

V is the volume of the reaction solution (L).

The FT-IR analysis method was used on a Thermo Scientific Nicolet 6700 FT-IR spectrometer to determine the functional groups in the leaf extract.

3. Results

3.1. Color Changes of The Solution during Silver Nanoparticle Synthesis

The color of the solution clearly changed during each step of the AgNP synthesis, as shown in Figure 2.

The pale yellow leaf extract solution reacted with colorless silver nitrate solution and turned a brownish-yellow color after a reaction period. After the reaction period is over, centrifuge and ultrasonicate several times to remove the substances.

Upon centrifugation, the silver nanoparticles, having a high specific gravity, settled at the bottom of the centrifuge tube, and the supernatant solution became light yellow. This solution was then discarded, and a corresponding amount of distilled water was added. Ultrasonic vibration was used to disperse the solid containing the agglomerated AgNPs, resulting in a dark brown solution. The centrifugation and ultrasonic vibration process was repeated until the supernatant solution became clear and colorless. In this study, the centrifugation and ultrasonic vibration process twice was satisfactory. To prove that the solution contained AgNP and did not contain excess reactants, molecular absorption spectroscopy was performed.

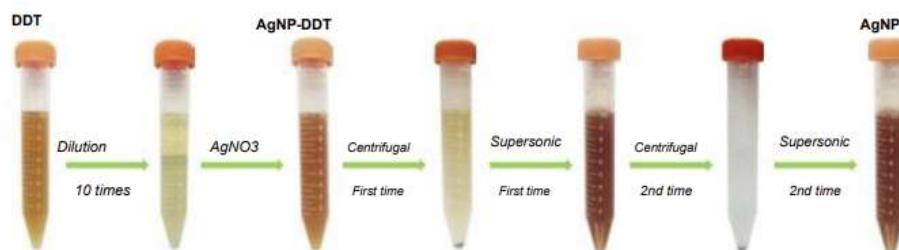


Figure 2. Color change of the solution during the synthesis of silver nanoparticles.

3.2. UV-Vis Molecular Absorption Spectra

The UV-Vis molecular absorption spectra of the mulberry leaf extract (DDT), the unpurified reaction solution with an initial AgNO_3 concentration of 5.0 mM and a reaction time of 6 h (AgNP-DDT), and the purified nanosilver solution (AgNP) are shown in Figure 3.

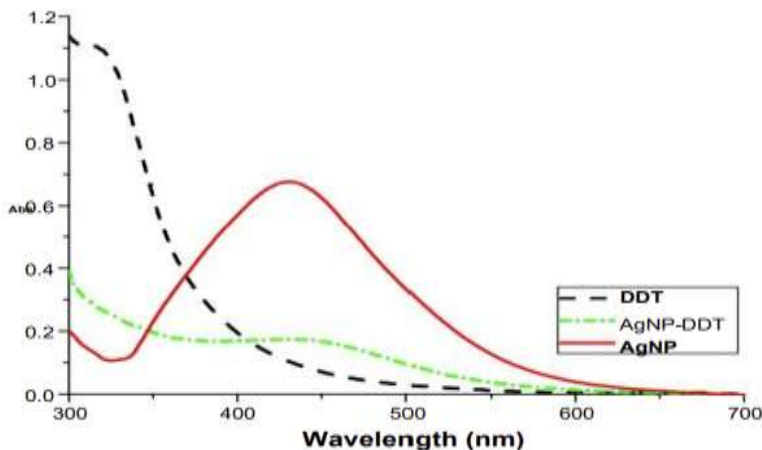


Figure 3. UV-Vis spectra of mulberry leaf extract (DDT), unpurified reaction solution (AgNP-DDT) and purified silver nano solution (AgNP).

The UV-Vis molecular absorption spectra of the mulberry leaf extract did not show any peak in the wavelength range of 400–700 nm, while the AgNP-DDT solution showed a peak at 430 nm. After removing the residual reactants from the AgNP-DDT solution by centrifugation and ultrasonic vibration, the peak of the purified AgNP solution became clearer and sharper. According to published literature on AgNP synthesis, the peak appearing at a wavelength of 430 nm corresponds to the surface plasmon resonance.

3.3. HR-TEM Analysis

The HR-TEM results for the purified AgNP samples synthesized with a 5.0 mM AgNO₃ concentration and a 6-hour reaction time are shown in Figure 4. The AgNP particles were all spherical in shape, with diameters ranging from a relatively narrow 20–35 nm. This result is consistent with the findings from the UV-Vis analysis and with previous publications. Thus, the organic compounds in the mulberry leaf extract not only act as reducing agents in the AgNP synthesis reaction but also act as stabilizers, preventing the agglomeration of AgNP particles. Without stabilizers, high-energy AgNP particles tend to agglomerate together to form large-sized, low-energy, more stable particles. Observe the image at high magnification of 400,000x (Figure 4b) and 800,000x (Figure 4c) clearly shows the crystal lattice layers of the metal. In a single AgNP particle, these crystal lattice layers have different orientations, proving that the synthesized AgNP has a polycrystalline structure.

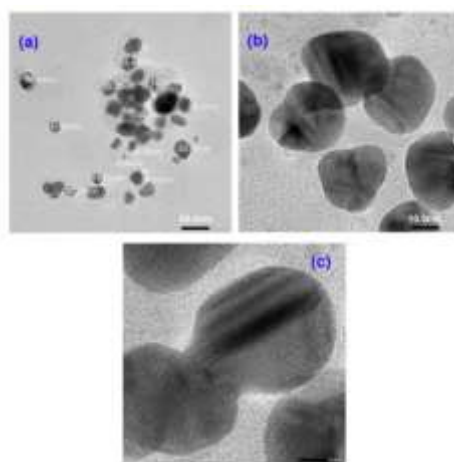


Figure 4. HR-TEM images of AgNPs at magnifications of (a) 80,000x, (b) 400,000x, and (c) 800,000x.

3.4. Influence of Reaction Conditions on AgNP Synthesis

The influence of the initial AgNO₃ salt concentration and reaction time on the AgNP synthesis process was studied by analyzing the UV-Vis absorption spectra of the purified AgNP solution. The results are presented in Figure 5.

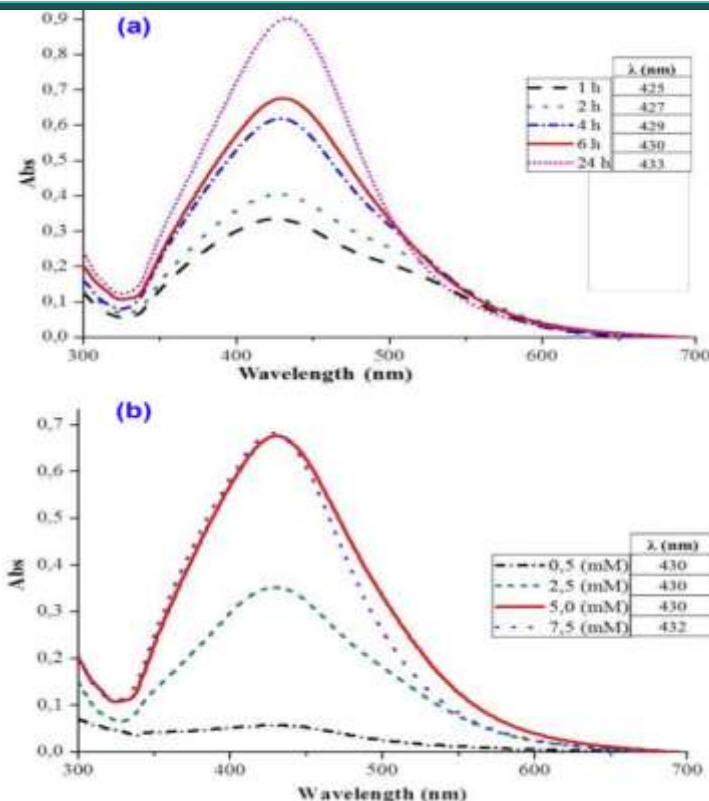


Figure 5. (a) UV-Vis spectra of AgNPs obtained after different reaction times;
(b) UV-Vis spectra of AgNPs obtained with different concentrations of AgNO₃

The UV-Vis spectra of AgNPs synthesized with a 5.0 mM AgNO₃ concentration and reaction times of 1, 2, 4, 6, and 24 hours (Figure 5a) show that the maximum absorption value increases gradually with the extension of the reaction time, indicating that the amount of AgNPs formed gradually increases. However, after 4 hours of reaction, the increase in the maximum absorption value tended to slow down. On the other hand, a longer the reaction time also gradually increased the wavelength at the maximum absorption peak (λ_{max}). The increase in wavelength is explained by the increase in AgNP particle size. This result is undesirable when using AgNPs as antibacterial agents. Experiments show that the AgNP solution synthesized over 24 hours, after purification, exhibited a sedimentation phenomenon when stored for several days. This phenomenon can be explained by the fact that the larger AgNP particle size reduces the stability of the dispersion system, causing the AgNP particles to settle more easily.

Figure 5b shows the UV-Vis spectra of AgNPs synthesized over 6 hours with initial AgNO₃ concentrations of 0.5, 2.5, 5.0, and 7.5 mM, respectively. The maximum absorption values gradually increased with the increase in the concentration of the AgNO₃ reaction solution, indicating that the amount of AgNPs formed increased. However, the maximum absorption value increased insignificantly when the AgNO₃ concentration increased from 5.0 mM to 7.5 mM. This indicates that the amount of reducing agent in the reaction solution was only enough to react with an AgNO₃ concentration of 5 mM. Steroid and carotenoid compounds... act as AgNP stabilizers due to their surfactantlike structure. FTAR analysis method with mulberry leaf extract samples shows the functional groups of substances in the solution. FT-IR amount of reducing agent in the reaction solution was only enough to react with AgNO₃ concentration of 5mM. In addition, when increasing the concentration of AgNO₃. Increasing the concentration to 7.5 mM also increased the AgNP particle size, as shown by the increase in the maximum absorption wavelength (λ_{max}).

Through the above analysis, the author chose the optimal conditions for AgNP synthesis using mulberry leaf extract: a 5.0 mM AgNO₃ concentration and a 6-hour reaction time at room temperature.

3.5. TGA/DTA Analysis and Nanosilver Synthesis Yield

To determine the efficiency of the AgNP synthesis process at the selected reaction conditions, the author used thermogravimetric analysis (TGA) and differential thermal analysis (DTA), as shown in Figure 6. Observing the mass loss of the sample on the TGA curve (Figure 6a), it is evident that the mass loss below 200°C is due to water vaporization. In the range of 200-500°C, the loss may be due to the decomposition of residual reactants, while above 500°C, it may

be due to the evaporation of AgNPs. According to published documents, AgNP particles have a melting point of about 200°C and start to evaporate at about 600°C [14]. The DTA curve (Figure 6b) shows a large change in mass loss at 570.7°C, which corresponds to a TGA value of 88%. Therefore, the efficiency of AgNP synthesis calculated by formula (1) was 78.22%.

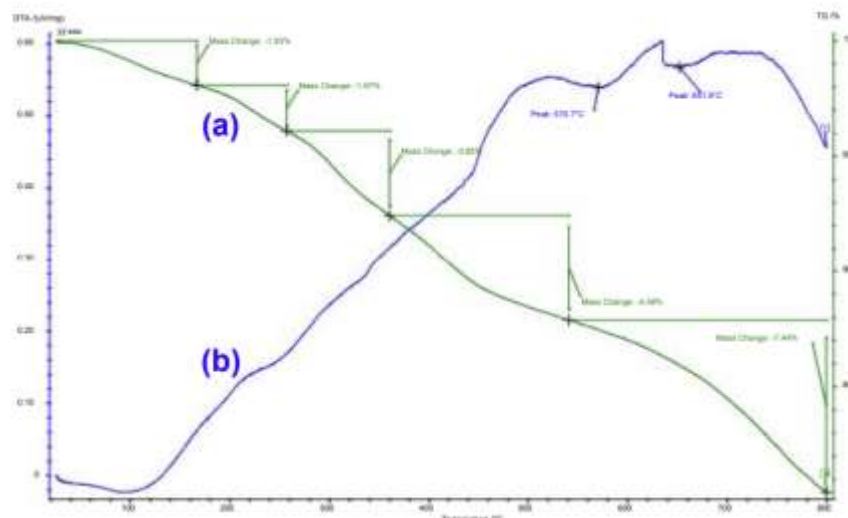


Figure 6. TGA/DTA thermal analysis of AgNPs: (a) TGA and (b) DTA lines

3.6. FT-IR Analysis and Proposed Reaction Mechanism

As presented in the overview, mulberry leaves contain many organic compounds that can act as reducing agents and stabilizers in synthesis reaction of AgNPs from silver nitrate salt. Organic compounds containing amine, carboxyl, carbonyl, and hydroxyl functional groups can act as reducing agents for silver ions. The FT-IR spectrum of the mulberry leaf extract in this study is presented in Figure 7.

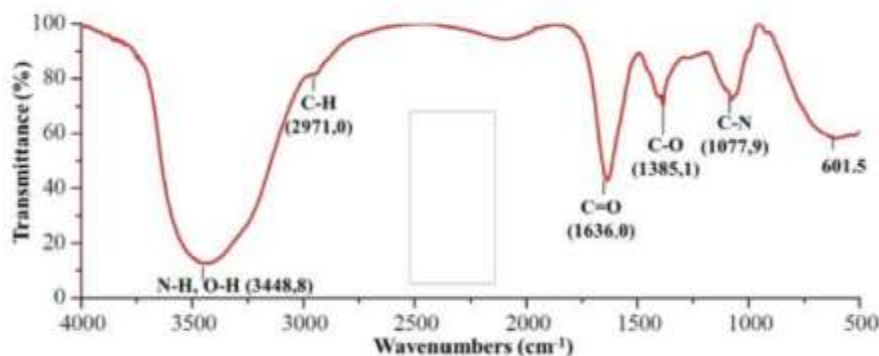


Figure 7. FT-IR spectrum of leaf extract

The spectrum shows the appearance of a peak at wave number 3448.8 cm^{-1} , which is characteristic of hydroxyl (OH) and amine (NH) functional groups. A peak at 2971.0 cm^{-1} is characteristic of the alkyl group (CH) in the hydrocarbon chain, while a peak at 1636.0 cm^{-1} is characteristic of the carbonyl bond (C=O) in the ester or ketone functional group. The peaks at 1385 cm^{-1} and 1077.9 cm^{-1} correspond to C-O and C-N bonds in peptide groups, amino acids, and so on. This is consistent with published literature on the FT-IR analysis of mulberry leaf extract.

Thus, the extract of mulberry leaves of the Hebei variety used in this study contains compounds with functional groups that can act as reducing agents and stabilizers in the AgNP synthesis reaction. Based on published studies, the author proposed the reaction mechanism for AgNP synthesis using mulberry leaf extract, as shown in Figure 8.

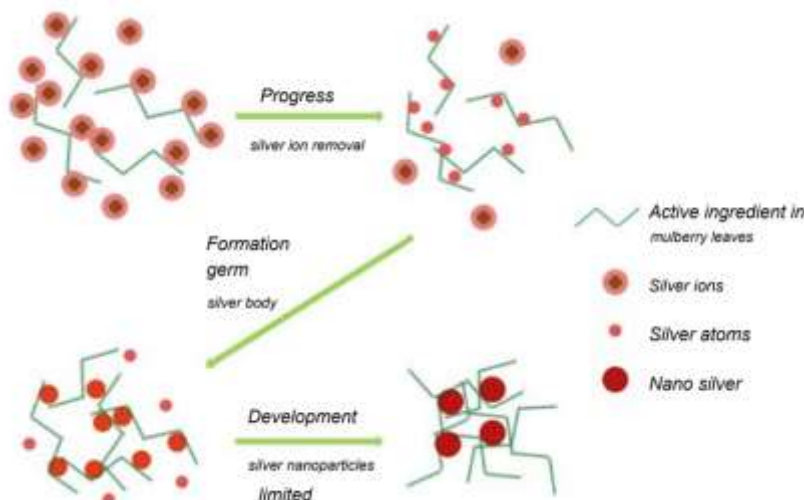


Figure 8. Reaction mechanism of silver nano synthesis

The AgNP synthesis mechanism is a redox reaction between silver ions in a silver nitrate solution (acting as an oxidizing agent) and compounds in the mulberry leaf extract (acting as a reducing agent). The mechanism of this reaction includes the process of receiving electrons of silver ions to become silver atoms and the process of donating electrons of reducing agents in the extract.

The silver atoms produced bond together to form nuclei, or crystallization centers, which develop into silver nanoparticles. Because of the large number of reducing agents in the reaction mixture and their flexible association with silver crystal seeds, the process leads to isotropic growth and the formation of stable, spherical AgNP particles.

Thus, the compounds in mulberry leaf extract act as both reducing agents and stabilizers, limiting the growth of silver nanoparticles and preventing their aggregation into large particles. This helps to maintain the stability of the dispersion system and the antibacterial ability of the AgNPs.

In the coming period, we will research and develop the AgNP–peptide complex system according to three main axes: (i) optimizing the material platform by experimental design (QbD/DoE) to control the size–morphology of green synthesized AgNPs from *Morus alba*, adjusting the surface chemistry (pH/ionic strength, Ag:peptide ratio, adding light linker $-\text{COO}^-/-\text{SH}$) to increase peptide loading, binding strength and targeted release profile; (ii) establishing a safety-performance profile at the preclinical level: cytotoxicity (keratinocyte/fibroblast), irritation/phototoxicity on 3D skin tissue, quantification of Ag^+ leakage, broad-spectrum antibacterial ability, along with anti-aging markers (procollagen I/III, elastin, MMPs, ROS) and skin penetration survey (Franz cell, 3D skin model) to standardize safe-effective concentrations; (iii) developing formulations and clinical evidence: transferring to serum gel/emulsion/mask formats with accelerated stability assessment (heat-cold, centrifugation, light), then performing small-scale clinical trials with instrument measurements (cutometer, corneometer, 3D wrinkle imaging) to demonstrate improvement in elasticity-moisture-wrinkle depth. In parallel, the team will standardize the process according to good cosmetic manufacturing practices, evaluate packaging compatibility, establish intellectual property protection (conjugation process and key operating parameters), and expand the target peptide portfolio (Pal-KTTKS, GHK-Cu, rice/soybean peptides) to form a new generation of anti-aging product platforms that are both green and sustainable, effective and safe.

4. Discussion

This study shows how *Morus alba* leaf extract can act as an effective mediator for green synthesis of silver nanoparticles (AgNPs) study with properties that can be used for cosmetic purposes. The AgNPs synthesized were mostly spherical and within 20-35 nm as seen in the (TEM) and had even distribution in size which correlates with reports on AgNPs of mulberry leaf extract which can mean the phytochemicals in the extract can function as effective reducing and capping agents. The synthesized AgNPs were confirmed by UV-Vis with a surface plasmon resonance peak at ~ 430 nm and functional groups with the leaf extract were bonded to the surface of the nanoparticles as shown in the FT-IR that confirmed plant extract compounds. The green approach to synthesis does not have toxic reagents and does not need much energy which correlates to the principles of the production of eco-friendly nanoparticles. The AgNPs synthesized had a yield of around 78 % which is similarly reported to other green synthesis and thus is highly efficient as well. Stretching the reaction to mild conditions shows the process is not only eco-friendly as mentioned earlier but can also be scaled up for the production of the cosmetics.

5. Conclusion

To conclude, this study points to the successful green synthesis of silver nanoparticles using *Morus alba* leaf extract and their application in anti-aging cosmetics to boost the activity of peptides within the formulation. The AgNPs generated were of uniform structure (sized in a few tens of nanometers) and synthesized efficiently in a green manner. Such eco-friendly synthesized nanomaterials provide several functions in a topical formulation: they could serve as protective carriers to boost the anti-wrinkle peptide's stability, penetration, and skin delivery while providing antimicrobial properties to the formulation. The synergistic effect of AgNPs and collagen-stimulating peptides is bound to have a significant, incomparable, and cutting-edge impact in the field of anti-aging. In summary, the AgNP-peptide system studied herein is undoubtedly forward thinking, sustainable, and innovative in skin rejuvenation. This concept clearly offers scope for enhanced research and development in the field of cosmetic science.

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