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Impact of microwave synthesis time on the shape of silver nanostructures and their antibacterial activity

Nhat Hieu HOANG[1], Van Nghia NGUYEN[1], Thi Tho NGUYEN[1], and Thi Mong Diep NGUYEN[1,*]

[1] *Quy Nhon University, 170 An Duong Vuong, Quy Nhon City, Binh Dinh Province, 820000, Vietnam*

***Corresponding author e-mail:** nguyenthimongdiep@qnu.edu.vn

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Abstract

Silver is a well-known effective antibacterial and disinfectant material with relatively few side effects. Nanosilver derived from it, have strong antibacterial, antifungal and broad-spectrum antiviral properties. This study describes how the microwave synthesis durations of silver nanoparticles affect their shape, and the effect of the shapes of these nanoparticles on their antibacterial activity. The optical properties of the nanosilver were examined through UV-Vis absorption spectroscopy. The morphology of the grain was determined by transmission electron microscopy (TEM), and the crystallinity of the nanosilver was confirmed by X-ray diffraction (XRD). The antibacterial activities were assessed using bacterial pathogens *Bacillus cereus* and *Bacillus megaterium*, and were performed using the disk diffusion method. The obtained results show that (i) the shape and size of the nanosilver change when the microwave time is increased. They are of various sizes but almost all circular in shape when microwaved for 1.5 min, of larger sizes and different non-spherical geometric shapes after 3 min of microwave, and converted to nanowires after 5 min of microwave. (ii) *Bacillus cereus* and *Bacillus megaterium* were sensitive to all nanosilver but the antibacterial activity was more potent when the nanosilver possessed a defined shape than when they were silver nanowires.

1. Introduction

Bacillus cereus and *Bacillus megaterium* are members of the Bacillus species that have been extensively studied. Besides beneficial applications in the production of probiotics for animal feed, they pose a threat to the health of humans, animals, and plants. *Bacillus cereus* is known as causative agent of food poisoning, but this strain involvement has also recently been identified in ocular and systemic wound infections [1], while *Bacillus megaterium* is considered as a causative agent of lupine blight [2]. Therefore, the goal is to inhibit their growth without having to use a lot of antibiotics, and nanomaterials are one of the ways that scientists are studying to solve this problem.

Nanotechnology is a new field of research with various applications in science and technology, as well as in daily life. The unique properties of nanomaterials have made them interesting for materials science and biotechnology. The field encompasses various nanoparticles, but silver nanoparticles (AgNPs) are among the most studied [3]. Nanoparticles (NPs) have a small size from 1 nm to 100 nm, so their high surface-to-volume ratios exhibit unique properties against pathogens, like bacteria, fungi, and viruses [4,5]. AgNPs have been shown to be effective antibacterial agents against a wide variety of bacteria, including both gram-negative and gram-positive [6,7]. Hypotheses explaining the mechanism of the antibacterial action of AgNPs have been proposed, but much needs to be clarified yet. Silver ions can be continuously released from AgNPs and adhere to cell walls and cytoplasmic membranes, leading to enhanced permeability of the cytoplasmic membrane and breakdown of the bacterial envelope [8]. This action disrupts the production of adenosine triphosphate [9], as well as the cell membrane, transforms deoxyribonucleic acid (DNA), and inhibits protein synthesis by denaturing ribosomes in the cytoplasm [10].

Previous studies have shown that the antibacterial activity of AgNPs is highly dependent on characteristics such as particle size and shape, and the surrounding processing environment. Smaller AgNPs with a spherical or hemispherical shape are more antimicrobial, due to their larger surface area [11], and silver ions are released more rapidly in acidic solutions than in neutral solutions [12]. Therefore, the synthesis and processing factors play an important role in determining the activity of nanosilver.

Some of the popular methods for the synthesis of nanomaterials include chemical methods [13], photochemical methods [14], electrochemical methods [15], and biological methods [16]. Many of these methods involve the use of hazardous chemicals that lead to environmental pollution and require harsh reaction conditions. Therefore, many studies focus on the biosynthesis of nanoparticles because of its simplicity, its softer impact on the environment, and its cost-effectiveness, although the required synthesis time can be much longer than with chemical methods [6,17-19]. Microwave-assisted biosynthesis is a solution to this problem as it greatly reduces the synthesis time, while also providing low energy consumption but no reduction in product yield [20,21]. We used this method to generate silver nanomaterials. Variations in microwave duration will produce different shapes and antibacterial activity will be tested.

Previous studies have shown that microwaves are a simple method to fabricate nanomaterials. They also showed that the size of AgNPs was strongly dependent on the concentration of $AgNO_3$ used [22] and the catalyst added to the $AgNO_3$ solution [22-25], and tested for antimicrobial activity based on the size or shape of the nanoparticle. However, the difference and interest of our study is that we showed for the first time that variation of microwave durations produced many different shapes of silver nanomaterials, and that we systematically compared the antibacterial activity of these structures against *Bacillus cereus* and *Bacillus megaterium*.

2. Experimental

2.1 Materials

All reagents and chemicals were purchased from Merck (China). Bacterial culture media, Luria Bertani Agar and Macconkey Agar, are products of HiMedia, India. Cultures of *Bacillus cereus* and *Bacillus megaterium* were obtained from the Department of Microbiology, Vietnam National University, Hanoi. Double distilled water was used in the experiments.

2.2 Synthesis of nanosilver

A total of 0.222 g of poly (vinylpyrrolidone) (PVP) was dissolved in 80 mL of Ethylene glycol with a magnetic stirrer for 10 min to obtain 80 mL of 25 mM PVP/EG solution. Then 60 mM $AgNO_3$ and 1.3 mM NaCl were dissolved in 80 mL of 25 mM PVP/EG solution with a magnetic stirrer for 2 min to obtain a mixture of 80 mL of $AgNO_3$/NaCl/PVP/EG solution. Each sample of 20 mL of $AgNO_3$/ NaCl/ PVP/EG solution was put into the microwave oven (the microwave oven is set to a power of 800 W and a frequency of 2,450 MHz). The solution was then microwaved for 1.5 min, 3 min, and 5 min.

2.3 Nanosilver characterization

The synthesized nanosilver were examined by obtaining UV-Vis spectra of the reaction medium at wavelengths ranging from 350 nm to 700 nm by using UV-1800 spectrometer (Shimadzu, Tokyo, Japan).

The size and morphology of nanosilver were determined by a transmission electron microscope (model JEM-100 CX). AgNPs were placed on a carbon-coated TEM grid and dried at room temperature, the extra solution was removed using blotting paper. The AgNPs size was calculated from the TEM image by measuring the diameters of about 50 nanoparticles. The formation of silver nanoparticles was measured by X-ray diffraction (XRD) by an X-ray (Bruker D2 diffractometer equipped with a Cu Kα radiation source).

2.4 Determination of antimicrobial activity

The antibacterial activity of nanosilver was examined using the disk diffusion method against the pathogenic microorganisms *Bacillus cereus* and *Bacillus megaterium*. Pure cultures of organisms were subcultured on sterilized Luria Bertani Agar and Macconkey Agar. A cork drill was used to puncture gel and make 6 mm diameter wells. Then, 20 µL of nanosilver was added to each well with a micropipette. After 24 h of incubation at 35°C, the zone of inhibition was calculated by measuring its diameter around each well to the nearest mm.

2.5 Statistical analysis

Testing the significance of antimicrobial activity of nanosilver was carried out by standard analysis of variance (ANOVA) (GraphPad Software 5.0, San Diego CA). The determinations were done in triplicate and the mean values ± SD were presented.

3. Results

3.1 Synthesis of nanosilver

The nanosilver formation was monitored by recording the absorption spectrum of the reaction mixture at wavelengths from 350 nm to 700 nm by UV-Vis spectroscopic analysis. Nanosilver are known to exhibit absorption peaks in the 400 nm to 500 nm range [26]. The obtained data is presented in Figure 1 and show a surface plasmon resonance (SPR) peak at the wavelength of 443 nm after 1.5 min and 3 min of microwave, and a peak of SPR at the wavelength of 421 nm when the microwave time was increased to 5 min.

In addition, the formation of nanosilver from Ag^+ ions reduction can be monitored through the color change of the reaction mixture [26]. The color of the reaction mixture gradually changed from colorless to yellow after 1.5 min, to brownish yellow after 3 min, and to milky white when the microwave time was increased to 5 min (Figure 2). These colour changes indicate changes in the shape and size of the nanostructures during the synthesis process.

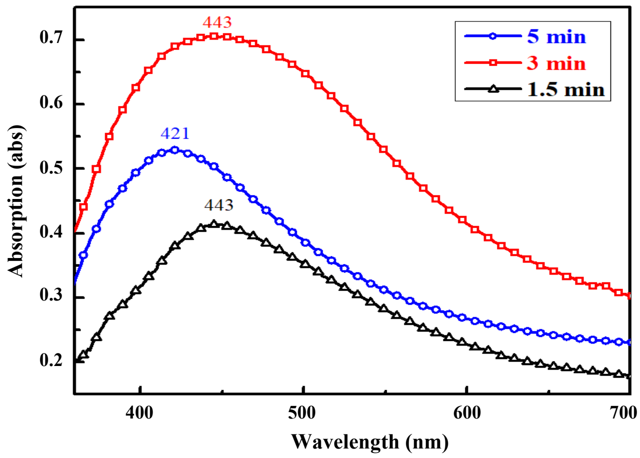

Figure 1. UV-Vis spectrum of nanosilver produced by microwave.

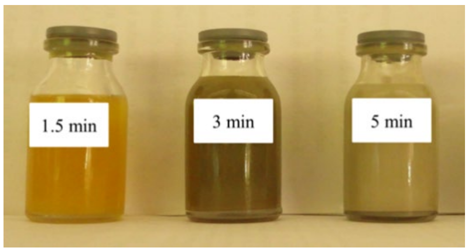

Figure 2. Different durations of microwave give different colors of nanosilver synthesized in NaCl/PVP/EG solution with $AgNO_3$.

3.2 TEM analysis

We measured the size and shape of the synthesized nanosilver using transmission electron microscopy (TEM). The TEM images of nanosilver synthesized using the microwave method are shown in Figure 3.

Nanosilver synthesized from $AgNO_3$/NaCl/PVP/EG with a 1.5 min microwave time have various sizes but are almost all of spherical shape. The diameters of these nanoparticles ranged from 3 nm to 30 nm (Figure 3(a)). The TEM image in Figure 3(b) shows that, when increasing the microwave duration to 3 min (Figure 3(b)), AgNPs changed from being spherical to being triangular, tetragonal, or hexagonal in shape, and are interlaced with silver nanowires (AgNWs). When the microwave time was further increased to 5 min, the AgNWs appeared even more while the small AgNPs disappeared (Figure 3(c)). The average size of AgNPs created by a 3 min time of microwave, as well as the average diameter of the AgNWs created by a microwave time of 5 min, is about 50 nm.

3.3 X-Ray diffraction (XRD) analysis

We also examined the crystal structure of nanosilver by X-ray diffraction (XRD). X-ray diffraction pattern of nanosilver synthesized by microwave is shown in Figure 4.

XRD spectra from the sample microwaved for 5 min showed that the number of Bragg diffraction peaks is observed at 2θ values 38.1° and 44.3° which corresponds to (111) and (200) planes of pure silver based on the face-centered cubic structure (JCPDS file No. 04-0783). With a shorter microwave time (3 min), a weak peak, corresponding to the (200) facet of AgCl, was observed partially overlapping with the strong (111) peak of Ag. With a 1.5 min microwave time, many weak peaks corresponding to the (220) facet of AgCl, (023), (302) and (311) facet of $AgNO_3$ were observed.

3.4 Antimicrobial activity of nanosilver

The antibacterial activity of nanosilver synthesized by microwave was tested against *Bacillus cereus* and *Bacillus megaterium*. Antimicrobial studies were performed using the disk diffusion method. $AgNO_3$ solution was used as a control. The results of antibacterial activity and the values of inhibition zone observed around the wells are shown in Figure 5.

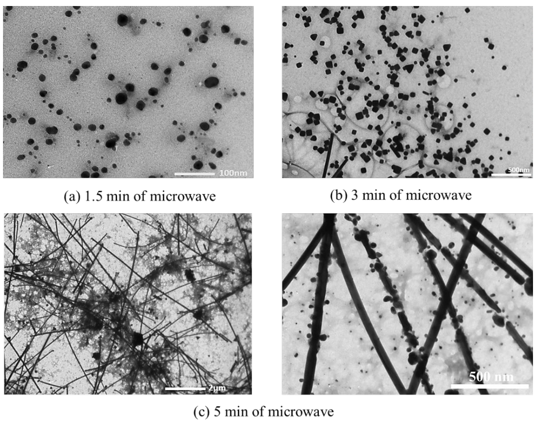

Figure 3. TEM images of nanosilver produced by microwave.

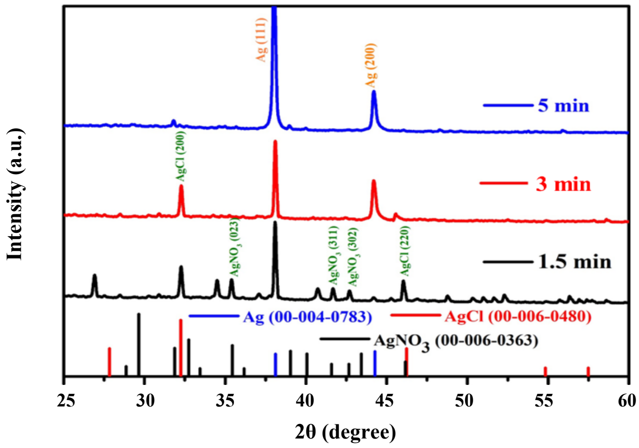

Figure 4. XRD pattern of nanosilver synthesized by microwave.

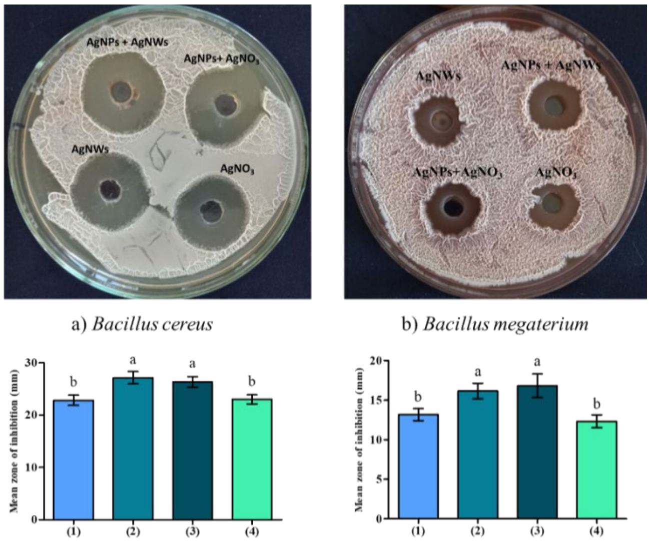

Figure 5. Antibacterial activity assay against (a) *Bacillus cereus* and (b) *Bacillus megaterium* by the disk diffusion method. (1) Ctrl: 0 min of microwave, $AgNO_3$; (2) 1.5 min of microwave, AgNPs + $AgNO_3$; (3) 3 min of microwave, AgNPs + AgNWs; (4) 5 min of microwave, AgNWs.

Our result shows that the nanosilver produced by microwave were effective against *Bacillus cereus* and *Bacillus megaterium*. The inhibition zones caused by nanosilver synthesized after 3 min and 5 min of microwave were greater than that of the nanosilver synthesized after 1.5 min and that of the control ($AgNO_3$).

4. Discussion

Our study has shown that (1) nanosilver produced in the $AgNO_3$/NaCl/PVP/EG mixture using the microwave method has shape and size characteristics that depend on microwave time. (2) The nanosilver thus produced retains its antibacterial activity since it was effective against pathogenic bacteria, *Bacillus cereus* and *Bacillus megaterium*.

We recorded the Ag^+ ions reduction to nanosilver by UV-Vis spectroscopic analysis. Our results show that the surface plasmon resonance (SPR) peaks of the nanosilver synthesized by microwave are in the wavelength range of 400 nm to 500 nm. This is a range of UV-Vis absorption wavelength of nanosilver about which we recently published [26]. This indicates the reduction of Ag^+ ions to Ag^0 and the formation of nanosilver. The formation of nanosilver can also be perceived through the color change of the reaction solution: we found that the transparent solution of the original silver nitrate gradually turned yellow-brown during the 1.5 min of microwaving. As the microwave time was increased, the color of the solution continued to change and the absorbance increased as well.

Previous studies have demonstrated that the shape and size of the nanosilver strongly influence the SPR bands formed by the general oscillation of the conduction electrons of the nanoparticles in the presence of visible light [20,21,26,27]. The increases in particle size is accompanied by a shift towards a longer wavelength of the absorption peak [28]. In our study, the microwave time changed the value of SPR peaks of the nanosilver, leading to a change in the shape of nanosilver. A short microwaving time means that the reaction of Ag^+ ions to AgNPs takes place quickly, which leads to AgNPs with a spherical shape and small sizes. In a previous study, Joseph and Mathew (2014) [29] also shown that the Ag^+ ions reduction to AgPNs and the simultaneous formation of nanoparticles can occur as quickly as 90 sec. The main asset of microwave synthesis is that it produces small, circular-shaped nanoparticles in a short time and environmentally friendly manner.

Our result shows that a short microwave time reduces fewer Ag^+ ions to AgPNs, and that nucleation, grain formation and crystal growth occur less often than with a long microwave time. In this case, NaCl is decomposed into Na^+ and Cl^-. Then, Ag^+ ions pair with Cl^- but there is still a large excess of $AgNO_3$ in the solution. Therefore, there are many weak peaks corresponding to the crystal faces of $AgNO_3$ and AgCl. Thus, we found that the microwave time strongly affects the final shape of the silver nanomaterials synthesized with this method and the addition of NaCl, PVP with a [NaCl]/[$AgNO_3$] ratio of about 0.02 [30]. Short microwave time (less than 3 min) is beneficial for the formation of spheres, squares and bipyramids, while a longer microwave time up to about 5 min is the best for nanowires formation. This is also shown in the XRD spectrum at 1.5 min and 3 min of microwave times, as peaks of AgCl appeared. In this stage, Ag atoms are continuously deposited along the {111} plane and inhibit the growth of the lateral {111} plane due to the preferential absorption of PVP and Cl^- on the {100} plane [31]. Tang *et al.* (2009) classified mechanisms for the formation of Ag nanostructures and the cases: [NaCl]/[$AgNO_3$] = 0.0065, the major products are five-twinned 1D particles because the concentration of Ag^+ ions is sufficient to provide a high probability for twinning [32].

In our study, the fabrication of silver nanomaterials using either 1.5 min or 3 min of microwave provided them with a better anti-bacterial activity than with 5 min. Silver nanomaterials were found to be effective against *Bacillus cereus* and *Bacillus megaterium* bacteria. Together with the results of TEM and X-ray analysis, it was demonstrated that when the microwave time is 1.5 min, there are still many $AgNO_3$ crystals in the solution. Therefore, the measured antibacterial activity must be due to both $AgNO_3$ and AgNPs. However, when the microwave time was increased to 3 min, the $AgNO_3$ crystals disappeared and formed AgNPs with various shapes, whose bactericidal properties are enhanced by the presence of the (111) and (200) facets. The silver nanowires (AgNWs) created after 5 min of microwave time did not show high antibacterial activity. It might be because their shape hinders their ability to bind to the membrane, and thus their ability to penetrate it. Many possible mechanisms have been proposed to explain the antibacterial activity of silver. Silver nanoparticles could accumulate in pits in the cell wall and cause cell membrane denaturation leading to cell death [33]. They are also capable of penetrating bacterial cell walls and subsequently altering the structure of cell membranes [34]. AgNPs with the same surface area but different conformations show different bactericidal activity, possibly due to differences in effective surface area and active aspects of AgNPs. However, there is currently little information on how the shape of the nanoparticles affects the biological activity of AgNPs.

5. Conclusions

In this study, we reported a simple, time-saving, environment-friendly method for the synthesis of silver nanoparticles by using microwaves. Different microwave durations created different nanosilver shapes. The formation of nanosilver was confirmed through UV-Vis, XRD, and TEM. The catalytic ability of the synthesized nanosilver was investigated by the addition of NaCl and PVP. The inhibition zones obtained in the antibacterial assay demonstrated the gram-positive antimicrobial properties of the nanosilver produced by this method. Microwave nanoparticles synthesis has several promising assets and could be used for large-scale production.

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