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RESEARCH PAPER

The synthesis of silver nanoparticles using the water-in-oil biomicroemulsion method

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ABSTRACT

Objective(s): A combination of biological and microemulsion methods was used to synthesize silver nanoparticles for the first time. The applied method could be referred to as the biomicroemulsion method, which has the advantages of both biological and the microemulsion methods.

Materials and Methods: In the present study, silver nanoparticles were synthesized in a water-in-oil biomicroemulsion using silver nitrate, which was solubilized in the water core of one microemulsion as the source of silver ions. In addition, a bacterial culture supernatant solubilized in the water core of another microemulsion was employed as the biological reducing agent, dodecane was used as the oil phase, and sodium bis(2-ethylhexyl) sulfosuccinate was applied as the surfactant. Moreover, the antibacterial activity of the nanoparticles was investigated against gram-positive and gram-negative bacteria by disc-diffusion method.

Results: The UV-Vis absorption spectra, dynamic light scattering, and transmission electron microscopy were employed to characterize the presence, size distribution, and morphology of the nanoparticles, respectively. According to the results, the nanoparticles had the optimal conditions in terms of the size and distribution at the silver nitrate concentration of 0.001 M. In addition, the analysis of antibacterial activity indicated that the inhibition zone diameter of Staphylococcus aureus was higher compared to Escherichia coli.

Conclusion: Silver nanoparticles were synthesized successfully using biomicroemulsion method and showed significant anti-bacterial activities against S. aureus and E. coli.

Keywords: *Antibacterial, Biomicroemulsion, Synthesis, Silver Nanoparticles*

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INTRODUCTION

Top-down and bottom-up methods are the main approaches that are used in nanofabrication. The bottom-up approach is more advantageous compared to the top-down approach as the former has a better chance of producing nanostructures with fewer defects, better homogenous chemical composition, and superior short- and long-range ordering. In the bottom-up synthesis method, nanostructures are synthesized onto the substrate by stacking atoms onto each other, thereby resulting in the generation of crystal planes, which further stack onto each other and lead to the synthesis of the nanostructures. Therefore, the bottom-up approach could be viewed as a synthesis approach where the building blocks are added onto the substrate in order to form nanostructures [1-3].

Silver nanoparticles could be synthesized using several physical, chemical, and biological techniques. In chemical methods, a reducing agent is used to decrease silver ions [4-8]. The biological reduction method is an appropriate technique to produce silver nanoparticles within the shortest possible time [9-11]. Among various methods of silver nanoparticle synthesis, special attention must be paid to the microemulsion method. The microemulsion method is a recent and optimal technique for the preparation of nanoparticles [12, 13]. Oil and water are immiscible and separate into two phases when mixed, each saturated with the traces of the other component. An emulsion is formed when a small amount of an appropriate surfactant is mechanically agitated with the oil and water, thereby resulting in a two-phase dispersion

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in which one phase exists as droplets coated by a surfactant, which is dispersed throughout the other continuous phases [14, 15].

The preparation of nanoparticles in the microemulsion method involves the mixing of two sets of microemulsions, with one set containing the first reactant and the other containing the second reactant. The reaction occurs through the collision of two droplets during the following steps: 1) the movement of the aqueous phase droplets in the emulsion and their collision, 2) the opening of the protective active layer by surface collision and merging, 3) the penetration of the reactive molecules from one drop to the other, 4) the reaction between the reactants, nucleation, and growth, and finally 5) the separation of the droplets [12, 15].

In the present study, a combination of biological and microemulsion methods was used to synthesize silver nanoparticles for the first time. The method could be referred to as the biomicroemulsion method, which has the advantages of both biological and the microemulsion methods. The UV-Vis absorption spectra, dynamic light scattering (DLS), and transmission electron microscopy (TEM) were used to characterize the presence, size distribution, and morphology of the nanoparticles, respectively. Furthermore, the antibacterial activity of the nanoparticles was investigated against gram-positive and gram-negative bacteria.

Table 1. List of Chemicals with Details of Source, CAS Number, and Purity

Material	Source	CAS Number	Purity (%)
Silver Nitrate	Merk	7761-88-8	≥99.8
Polyvinylpyrrolidone (PVP)	Merk	9003-39-8	
Sodium Bis(2-ethylhexyl) Sulfosuccinate (AOT)	Sigma-Aldrich	577-11-7	≥97
N-dodecane	Merk	112-40-3	≥98.5
Tryptic Soy Broth (TSB)	Sigma-Aldrich		

MATERIALS AND METHODS

Experimental materials

Table 1 shows the chemical materials used in the study, as well as their CAS number, source, and the purities stated by the manufacturer. *Escherichia coli* (DH5α) and *Staphylococcus aureus* were obtained from the Microbiology Laboratory of Tehran University (Tehran, Iran).

Synthesis of silver nanoparticles by biomicroemulsion

Initially, *E. coli* was cultured in the TSB medium, followed by incubation at the temperature of 37°C for 36 hours. The solution was centrifuged at 5,000 rpm for 20 minutes in order to obtain the supernatant of the bacterial culture, which was used as the reducing agent. In addition, the silver nitrate solution was used as the silver precursor at three concentrations (0.01, 0.005, and 0.001 M). During a typical procedure, the microemulsions were prepared by mixing the same volume of the aqueous solution of silver nitrate and supernatant to the AOT/dodecane solution. The molar ratio of the supernatant and silver nitrate was kept constant in all the experiments (value: 3).

At the next stage, the microemulsion containing the supernatant was added to another microemulsion containing silver nitrate in a drop-wise manner.

After adding all the supernatant microemulsion, vigorous magnetic stirring was maintained for two hours until the resulting microemulsion mixtures changed color to stable light yellow after the reaction, indicating the formation of the silver nanoparticles. Finally, the nanoparticles were analyzed using the UV-Vis spectroscopy, DLS, and TEM.

Disc-diffusion method

In this study, the disc-diffusion method was used to assess the antibacterial activity against *E. coli* and *S. aureus* bacteria [16, 17]. The *Mueller-Hinton* agar (MHA) medium plates were prepared, sterilized, and solidified. The samples of the silver nanoparticles (0.01, 0.05, and 0.001 M) and two antibiotics were placed in the MHA plates and kept for incubation at the temperature of 37°C for 24 hours. Following that, the diameter of the inhibition zone of each sample was measured with a ruler. The experiment was performed in triplicate for each case study, and the obtained mean values were reported in millimeters.

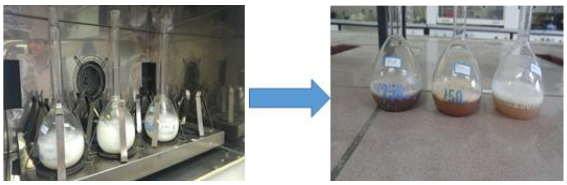

Fig 1. Color Change of Solution after Formation of Silver Nanoparticles

RESULTS AND DISCUSSION

Study of color change

The first sign of silver nanoparticle synthesis was the color change of the solution from milky to brown, which indicated the formation of the silver nanoparticles (Fig 1).

Table 2. Absorption Peak of Silver Nanoparticles

Silver Nitrate Concentration (M)	Absorption Peak (nm)	Absorbance (au)
0.01	436	0.906
0.005	415	0.876
0.001	412	0.819

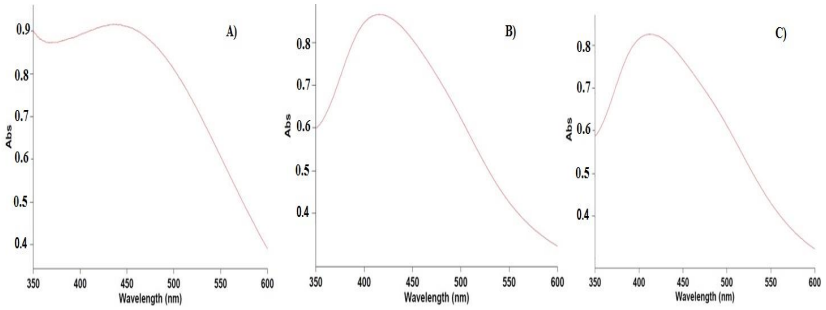

Fig 2. UV-Vis Absorption Spectra of Silver Nanoparticles at a) 0.01 M, b) 0.005 M, and c) 0.001 M

Table 3. Mean Size of Silver Nanoparticles (nm)

Concentration of Silver Ions (M)	Mean Size by Number Percentage	Mean Size by Volume Percentage
0.01	72.51-1256	94.78-1507-4739
0.005	93.24	131.7-50322
0.001	74.47	95.81-340

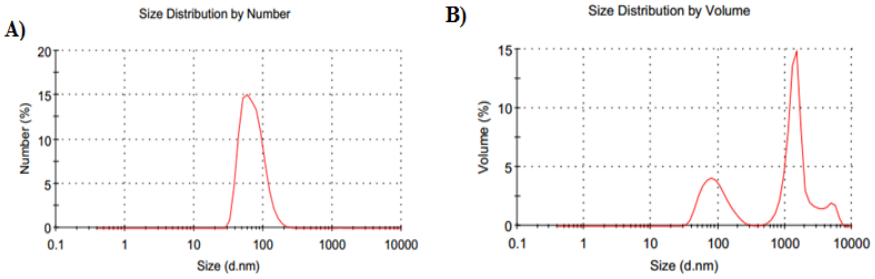

Fig 3. Curve of Size Distribution by a) Number and b) Volume at 0.01 M

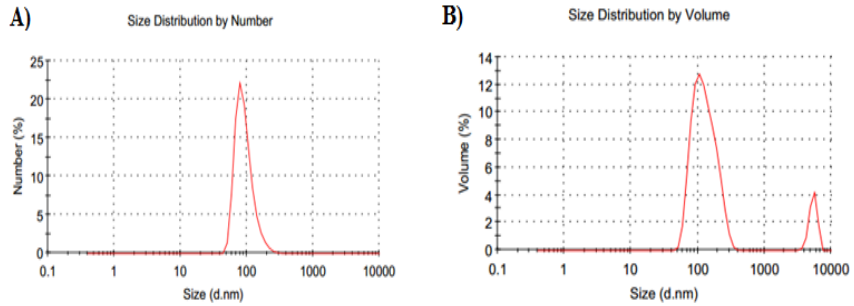

Fig 4. Curve of Size Distribution by a) Number and b) Volume at 0.005 M

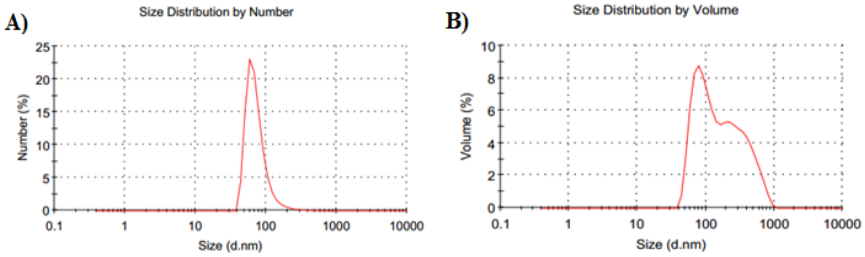

Fig 5. Curve of Size Distribution by a) Number and b) Volume at 0.001 M

Assessment of the silver nanoparticles by uv-vis spectroscopy

UV-Vis spectroscopy is considered to be the simplest technique for confirming the presence of silver nanoparticles. In the current research, the UV-Vis absorption spectra of the silver nanoparticles indicated that the absorption peak was within the range of 400-450 nanometers. The observed absorption peak confirmed the presence of the silver nanoparticles in the solution [18, 19]. Table 2 shows the absorption peak of the silver nanoparticles at various concentrations.

As is shown in Fig 2, the absorbance peak wavelength increased at the higher concentration of the silver ions, indicating the increased size of the silver nanoparticles. Moreover, the size distribution of the nanoparticles increased at the higher concentrations and decreased at the lower concentrations of the silver ions. The smallest size and size distribution of nanoparticles was obtained in the solution containing 0.001 M of silver nitrate, which was selected as the optimal concentration.

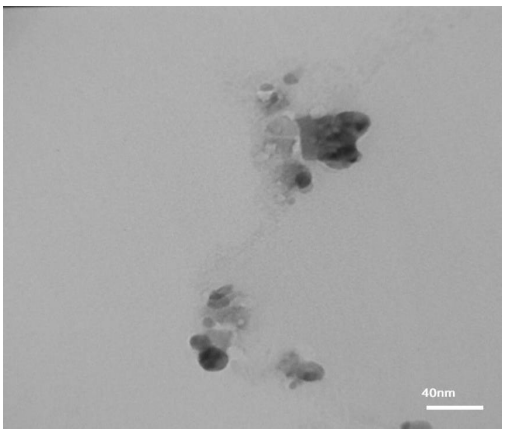

Fig 6. TEM Image of Silver Nanoparticles

Assessment of the silver nanoparticles by DLS

At this stage, DLS analysis was used to measure the size of the silver nanoparticles. Figs 3-5 depict the size distribution of the obtained silver nanoparticles. Table 3 shows the mean size of the silver nanoparticles at various concentrations.

According to the results of the DLS analysis, the most appropriate concentration was 0.001 M. As is shown in the tables and Figs, no peaks were observed in the microparticles area. Moreover, the highest number of the nanoparticles was observed at the mentioned concentration. These findings are consistent with the results obtained by the UV-Vis spectroscopy.

Assessment of the silver nanoparticles by TEM

Fig 6 shows the TEM image of the sample of 0.001 M. As is observed, the particle morphology was quasi-spherical; such morphology has also been reported in the other studies in this regard. In addition, the accumulation of the silver nanoparticles was observed in some areas.

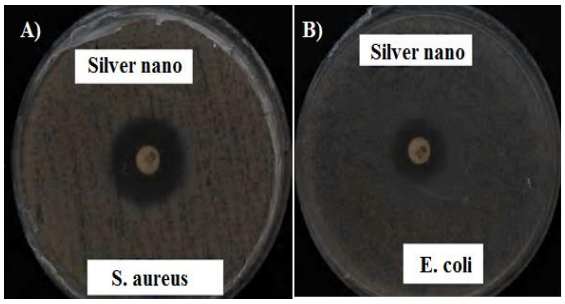

Fig 7. Inhibition Zone of Silver Nanoparticles against a) S. aureus and b) E. coli

Antibacterial activity of the silver nanoparticles

Fig 7 depicts the obtained results regarding the antibacterial activity of the silver nanoparticles on *S. aureus* and *E. coli*.

The inhibition zone of the samples indicated that the prepared silver nanoparticles had antibacterial properties. It has previously been reported that silver nanoparticles are able to anchor to and infiltrate the bacterial cell wall, causing physical changes in the bacterial membrane (e.g., membrane damage) and leading to the leakage of cellular contents and bacterial death [17, 20].

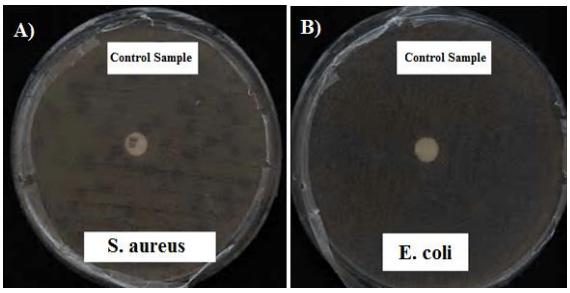

Fig 8. Inhibition Zone of Control Sample against a) S. aureus and b) E. coli

According to the current research, the diameter of the inhibition zone for *S. aureus* (gram-positive) was higher compared to *E. coli* (gram-negative) (Fig 9a-b). Similar findings have also demonstrated the greater resistance of gram-negative bacteria to gram-positive bacteria against silver nanoparticles. The antibacterial activity of silver nanoparticles has been shown to be more

significant against *S. aureus* due to the differences in the cell wall structure between gram-positive and gram-negative bacteria (lipids, protein, and liposaccharide).

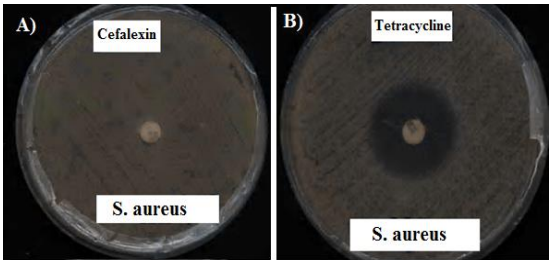

Fig 9. Inhibition Zone of a) Cefalexin and b) Tetracycline against S. aureus

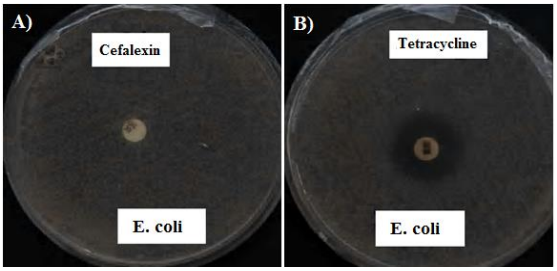

Fig 10. Inhibition Zone of a) Cefalexin and b) Tetracycline against E. coli

Figs 8-10 depict the obtained results for two antibiotics and a control sample.

Table 4 shows the total results regarding antibacterial activity.

Table 4. Diameter of the Inhibition Zone at Various Concentrations of Nanoparticles

Sample	Diameter of Inhibition Zone (mm)	
	S. aureus	*E. coli*
Control Sample	0	0
Silver Nanoparticles (0.001 M)	8.7	5.6
Silver Nanoparticles (0.05 M)	9.1	5.9
Silver Nanoparticles (0.01 M)	9.3	6.0
Tetracycline	10.7	8.8
Cefalexin	0	0

CONCLUSION

Silver nanoparticles are of great interest owing to their applications as catalysts, antimicrobial agents, and biosensors. The use of silver nanoparticles is largely dependent on the size distribution and structure of the particles. In this study, a combination of biological and the microemulsion methods was used for the first time to synthesize silver nanoparticles. The method could be referred to as the biomicroemulsion method, which has the advantages of both microemulsion and biological methods (e.g., green synthesis). For this purpose, silver nanoparticles with high purity were prepared at three concentrations (0.01, 0.005, and 0.001 M) and characterized using DLS, TEM, and UV-Vis spectroscopy. In addition, the antibacterial activity of the samples was determined using the disc-diffusion method. According to the findings, the diameter of the inhibition zone against *S. aureus* (gram-positive) was higher compared to *E. coli* (gram-negative).

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