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Green Synthesis of Silver Nanoparticles in Biopolymer Stabilizer and Their Application as Antibacterial Efficacy

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Abstract. The synthesis of silver nanoparticles (Ag-NPs) has been conducted on the biopolymer media which is sodium alginate (Na-Alg) via the green method. Different times of ultrasound irradiation was used to reduce Ag⁺ to be Ag seed (Ag⁰) and produce Ag-NPs. The Ag-NPs was analyzed in their surface plasmon resonance (SPR), morphology, biomolecule responsible, and stability via UV-Vis, TEM, FTIR and Zeta potential, respectively. The application as antibacterial properties was studied against bacteria. Furthermore, this Ag-NPs could be synthesized using the green method and used as antibacterial efficacy for medical vehicles, would dressing, and so on.

INTRODUCTION

Nanomaterial with size in range 1-100 nm has been received high attention due to their different properties than bulk materials otherwise in the same element [1]. Silver nanoparticles (Ag-NPs) is one of the famous noble metal and also it was exhibited as an important nanomaterial due to physicochemical and inexpensive metal [2]. In addition, the application of Ag-NPs in our daily life has been used in many items like electronic, sensor, antibacterial, and medical devices [3], therefore the producing of this nanomaterial has been arising year-by-year because of high industry demand. However, today still many of the synthesized Ag-NPs have been used the chemical method and then affected some disadvantages for our environment [4].

Green synthesis method in the producing Ag-NPs can be an alternative which should be conducted in the three main parts. The capping agent, reductive agent, and reaction solvent are the three parts which can be called as a green synthesis approach [5]. Moreover, the necessary implementation of twelve principles of green chemistry approach in this nanoparticles synthesis is the fundamental reason on this particular chemistry studied [6]. Herein, a green synthesis of Ag-NPs has been applied using sodium alginate (Na-Alg), water, and ultrasound irradiation for the natural stabilizer, solvent, and reducing agent, respectively. Under different times of ultrasound irradiation, it can be shown that ultrasound played as the main reducing agent in this synthesis.

The aim of this research is a synthesis of Ag-NPs via green chemistry method and followed the application as antibacterial efficacy. The Ag-NPs was characterized using UV-Vis spectroscopy, TEM micrograph, FT-IR spectroscopy, zeta potential analyzer to study the surface plasmon resonance, morphology, responsible biomolecule binding, and their stability, respectively. In addition, the antibacterial efficacy of the Ag-NPs after ultrasound irradiation has been evaluated under Gram-positive bacteria (*Staphylococcus aureus* S276) and Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC15442).

EXPERIMENTAL METHOD

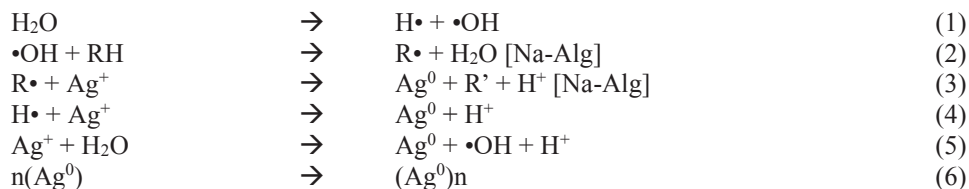
AgNO₃ with 10 mM (purchased from Bendosen C0721-2284551) was added to 400 mL of Na-Alg with 1.2 g (Acros, New Jersey, USA) in a beaker glass. The water was used as a solvent which prepared from ELGA Lab-Water/VWS (UK) purification system. Briefly, the Na-Alg solution was stirred for a few hours until complete dissolved. Then, the solution was exposed to a high-intensity ultrasound radiation ultrasonic processor probe (Hielscher UP-200S-RN 50/60 Hz, Germany/1 cm-Ti-horn) and used the amplitude of 70% and the cycle of 0.5. Different time's irradiation was monitored from 30, 60, 120, and 240 min. Finally, all solutions were kept in the freezer without any washing.

The characterization method was used UV-1800 Shimadzu spectrophotometer to analyze the surface plasmon resonance (SPR) of the Ag-NPs. The TEM microscopy was utilized Tecnai G2 F20 Series to study the morphology and size of the nanomaterial. Moreover, FT-IR spectroscopy and zeta potential analyzer have also been used by Nicolet 6700 using ATR method (scanned range of 600-4000 cm⁻¹) and Zeta NanoPlus Otsuka Electronics, Japan, respectively. The in vitro antibacterial efficacy of the Ag-NPs was studied using disc diffusion method on nutrient agar media (Merck) and determined the inhibition zone (in mm). This method was confirmed and recommended by the National Committee for Clinical Laboratory Standards.

Here, we provide some basic advice for formatting your mathematics, but we do not attempt to define detailed styles or specifications for mathematical typesetting. You should use the standard styles, symbols, and conventions for the field/discipline you are writing about.

RESULTS AND DISCUSSION

Ultrasound irradiation has been utilized to reduce AgNO₃ became Ag⁰ during processing treatment. Evidently, the changing color of the solution appeared after irradiation which colorless before irradiation then finally brown color after 240 min as shown in Fig. 1(B). Due to the high intensity of the ultrasound irradiation, the producing Ag⁰ could be formed in this synthesis method. The detail mechanism of producing Ag seed has been suggested by Nagata *et al.* [7] which shown in Eq. (1-6). In addition, surface plasmon resonance (SPR) from UV-Vis spectra of the solution has been showed that the Ag characteristic in around 450 nm according to Kumar *et al.* [8] as shown in Fig. 1(A). Indeed, this phenomenon could be indicated the presence of the Ag-NPs on the Na-Alg as the biopolymer stabilizer.



The TEM image has been given the size and shape information of the Ag-NPs on this synthesis. From the image, the size of the Ag-NPs was calculated in the range of 5 until 52 nm with an average of 24.41 nm and the shape was in spherical after 240 min irradiation (Fig. 2). This average size and shape of the Ag-NPs also have similarity with Sathyavathi *et al.* [9] However they did on *Coriandrum Sativum* leaf extract as the biopolymer stabilizer. This image could be seen that the Ag-NPs was in surrounded by Na-Alg as the biopolymer stabilizer. The binding interaction of the Ag-NPs with Na-Alg has also been detected using FT-IR for their biomolecule responsible.

The functional group of the Ag-NPs was measured on the different FT-IR absorption peaks after 240 min ultrasound irradiation (Fig. 3). The spectrum shows the peak at 3256 cm⁻¹ is attributed for O–H stretching, the peak at 1589 and 1405 cm⁻¹ are represented to the –COO– (asymmetric) and –COO– (symmetric) [10]. Furthermore, the band at 1081 and 1025 cm⁻¹ is assigned for the stretching of C–O–C group, however, C–C–H stretching, C–O–C glycosidic linkage (ring breathing), and –C–C (C–C–O ring) could be associated with the peak of 949, 887, and 814 cm⁻¹ [11], respectively. The FT-IR spectrum has been concluded that the responsible bonding was carried out by van der Waals forces based on many researchers discussed [12–14]. The oxygen atom with their negative charge from the biopolymer stabilizer was interacted with the positive charge of the Ag-NPs [3].

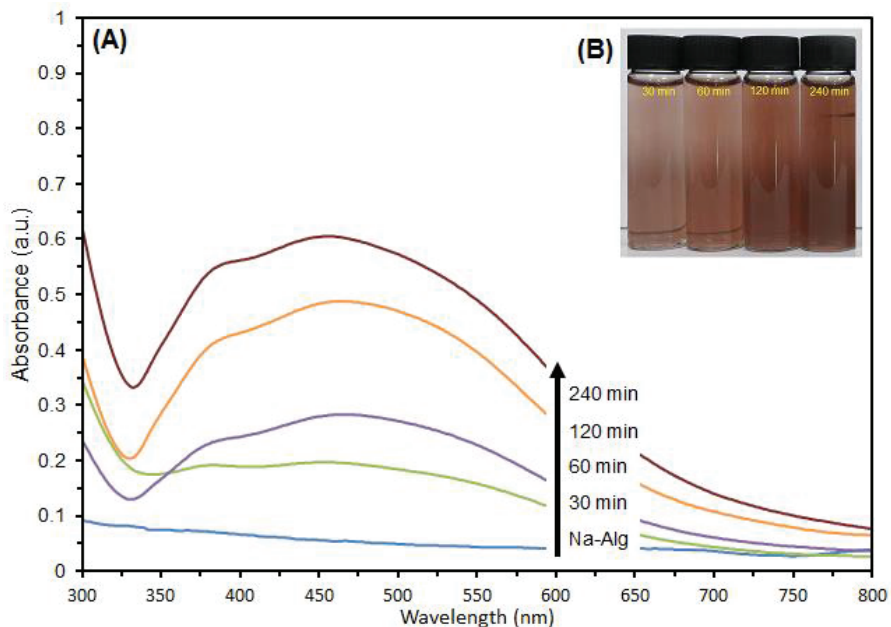


FIGURE 1. UV-Vis absorption spectra (A) and the photograph of Ag-NPs after different time irradiation (B)

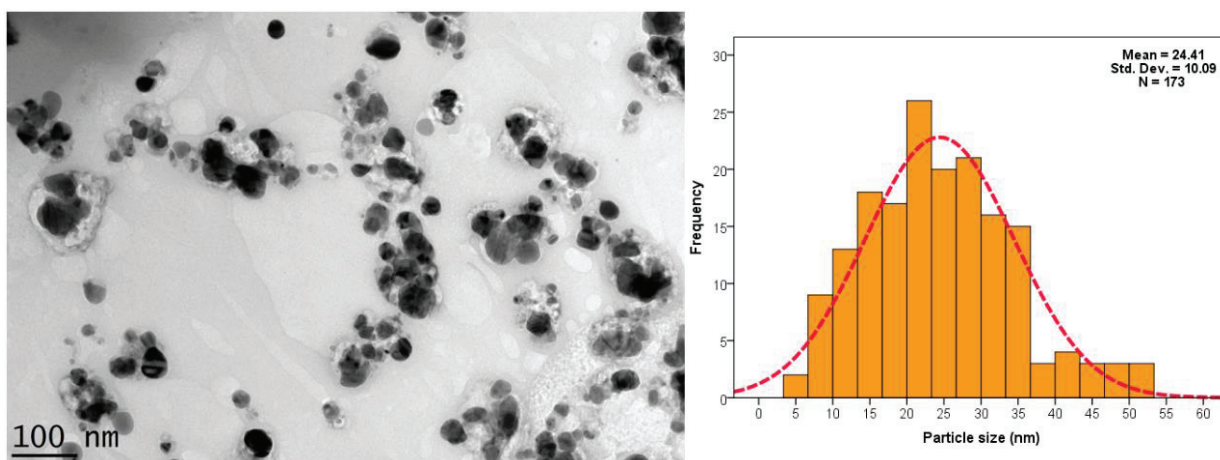


FIGURE 2. TEM image and the size distribution of Ag-NPs after 240 min ultrasound irradiation

The zeta potential of the Ag-NPs after 240 min ultrasound irradiation has been measured to study the stability of the solution. This equipment is an important parameter for characterizing the electrical properties of the interfacial layer in solution [15]. With the value at between 30-40 mV (either positive and negative) shows moderate stability, however, if around 40-60 mV could be indicated as a high stability [16]. Therefore, the Ag-NPs after 240 irradiation was found of -49.43 mV (Fig. 4) which is indicated that the nanomaterial has a high stability. This value was also supported by the previous study of the Ag-NPs on bio-stabilizer from plant extract by Shameli *et al.* [17].

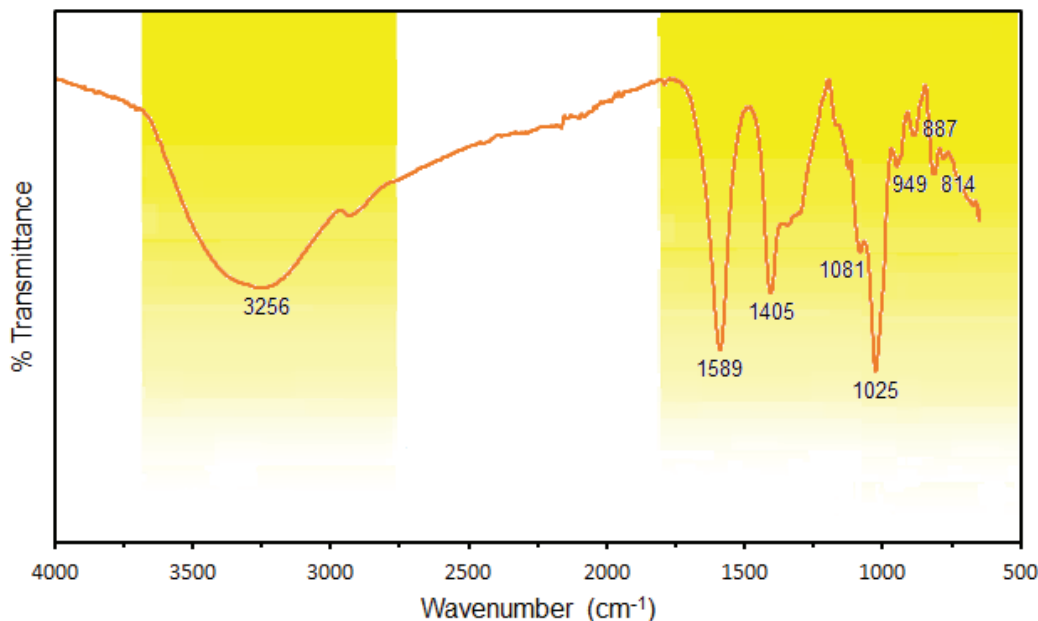


FIGURE 3. FT-IR spectrum of the Ag-NPs on Na-Alg stabilizer after 240 min ultrasound irradiation

Finally, the application of the Ag-NPs was used as antibacterial efficacy against Gram-positive and Gram-negative bacteria. These bacteria are *Staphylococcus aureus* S276 (Gram-positive) and *Pseudomonas aeruginosa* ATCC15442 (Gram-negative) which are usually as an infectious disease in our daily-life [18]. The inhibition zone around the paper disc could be concluded that the Ag-NPs has an antibacterial efficacy (Fig. 5). Moreover, the average inhibition zone of the antibacterial efficacy was also calculated (Table 1). The inhibition zone could be indicated that the bacteria was killed after the paper disc immersed on the Ag-NPs. This was caused the accumulated of Ag ions on the cell-wall of the bacteria, therefore, it disrupted the bacteria grown [19]. Consequently, this Ag-NPs could be used as an antibacterial agent for many application. In addition, the green method of this synthesis has an advantage due to environmental friendly and also following green chemistry approach.

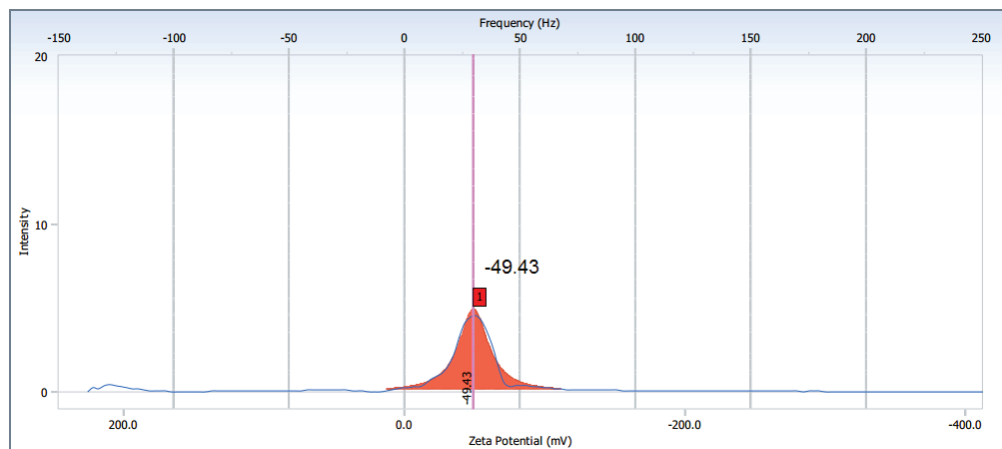


FIGURE 4. Zeta potential value of the Ag-NPs after 240 min ultrasound irradiation

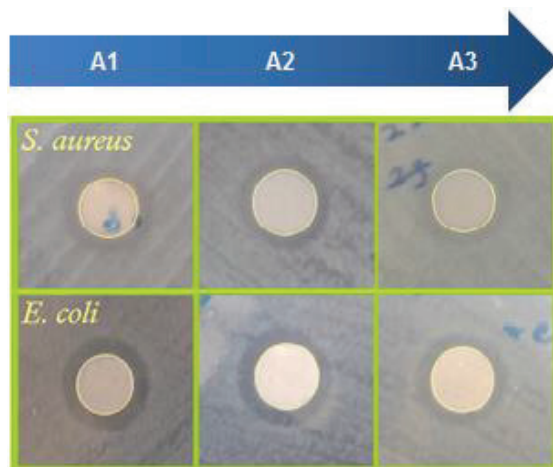


FIGURE 5. Comparison inhibition zone in the different time ultrasound irradiation of the Na-Alg/AgNO₃, 120, and 240 min for A1, A2, and A3, respectively

TABLE 1. Average inhibition zone of the Na-Alg/AgNO₃, 120, and 240 min ultrasound irradiation (in mm)

Bacteria	A1	A2	A3	Streptomycin (control)
<i>S. aureus</i> (S276)	8.3	8.4	8.6	23.6
<i>E. coli</i> (E266)	8.9	9.0	9.1	21.8

CONCLUSION

The Ag-NPs have been produced via green synthesis method under biopolymer media (Na-Alg) for different ultrasound irradiation times from 30, 60, 120, and 240 min. The solution concluded that the SPR is the presence of the Ag-NPs oscillation, size at an average of 24.41 nm, and spherical shape. FT-IR concluded that the van der Waals interaction has been responsible between Ag-NPs and the Na-Alg. The zeta potential showed a high stability nanomaterial with a value of -49.43 mV. Finally, the application as the antibacterial efficacy of the Ag-NPs has been showed an antibacterial agent both for Gram-positive and Gram-negative bacteria. Therefore, the Ag-NPs could be used as a new antibacterial nanomaterial for medical devices, wound dressing, and others.

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