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Sonochemical Synthesis of Silver Nanoparticles and Its Nanomedicinal Activities Against *Staphylococcus aureus*

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ABSTRACT

The global rise of antibiotic-resistant bacteria due to the overuse of commercial antibiotics poses a significant public health threat. As a result, researchers have been exploring new antibacterial agents and approaches, including the field of nanomedicine. This study aims to produce silver nanoparticles (AgNPs) using lactose as a reducing agent through an ultrasound-assisted procedure and evaluate their anti-staphylococcal properties. AgNPs were synthesized using a one-pot ultrasound-assisted method with silver nitrate as the precursor and lactose as the reducing agent while the antibacterial activity was tested against *Staphylococcus aureus* using the agar well diffusion method. The synthesized AgNPs exhibited strong antibacterial activity, demonstrating potential as nanomedicine. These findings suggest that AgNPs could be valuable in combating antibiotic resistance and functional in the healthcare industry as an alternative antibacterial agent.

Keywords: Antimicrobial agents, Green synthesis, Lactose, Silver nanoparticles, sonochemical synthesis.

INTRODUCTION

The global outbreak of antibiotic-resistant bacteria and related infectious diseases as a result of indiscriminate intake of commercial antibiotics brought a threat to humans and was considered a serious public health issue[1]. Infections caused by pathogenic bacteria have been greatly reduced by the discovery and development of antibiotics. However, this widespread use of antibiotics led to abuse in the past 20 years and resulted in the decline of antibiotic efficacy and the emergence of superbacteria. For instance, the case of *Staphylococcus aureus*, the most common human pathogenic bacteria, caused the emergence of the highest number of multi-drug resistant strains[2]. *Staphylococcus aureus* is a *Gram-positive* bacteria that is responsible for hospital- and community-acquired infections affecting different parts of the body such as the lungs, skin, bone, mammary glands, blood, and heart[3,4]. According to the World Health Organization (WHO), bacterial infections resulted in increased healthcare costs and death[4]. Studies related to the development of new antibiotics are a difficult process as they require years of investigation for the safety and efficacy of the antibacterial candidates, costly

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raw materials, and time-consuming protocols, while the infections caused by antibiotic-resistant bacteria are increasing and resulting in deaths worldwide[5]. Encouraged by these facts, there is an urgent need for the development of novel and effective antibacterial agents[6].

The search for novel antibacterial agents and approaches gained the interest of different researchers worldwide such as the development of the field of nanomedicine[7]. Nanomedicine is an interdisciplinary area that utilizes nanoscience and technology in the field of medicine to improve disease management such as infections caused by human pathogenic bacteria[8]. The utilization of inorganic nanomaterials has become evident due to their unique and controllable properties, and without the development of antibiotic resistance, enables these nanomaterials to be useful as antimicrobial agents over organic compounds[9]. One of these nanomaterials is silver nanoparticles (AgNPs) which have demonstrated unique therapeutic properties and are therefore used in various applications in medical and health-related fields[10]. In this regard, fabrication of AgNPs through various reduction methods using chemical reducing agents[11], plant extracts[12], and biomolecules[13] have been reported. Recently, a simple protocol utilizing ultrasonic irradiation known as the sonochemical method, and in combination with lactose as the reducing agent produced AgNPs with potential applications[14]. A sonochemical approach for the synthesis of nanoparticles gained interest in the field of nanoscience gained interests of various researchers worldwide since the protocol is cost-effective, simple, rapid, and do not require high temperature and energy[14-15]. One advantage of using a sonochemical method for the preparation of nanoparticles is the production of well-dispersed nanoparticles with minimal agglomeration at ambient temperature conditions[14]. Further, a sonochemical method only requires mild reaction conditions such as room temperature and normal pressure conditions, and in some cases, only uses water as the solvent[15]. Therefore, the use of toxic and hazardous organic solvents is eliminated and thereby meet the criteria for green chemistry and possessed potential for environment-friendly industrial processes. Regardless of the numerous published papers about the anti-microbial activities of AgNPs, substantial research is still necessary to be explored to contribute to the scalable and sustainable fabrication of AgNPs with tailored characteristics for various applications. Therefore, the testing of AgNPs synthesized using lactose as the reducing agent via an ultrasound-assisted technique as an antimicrobial agent against *Staphylococcus aureus* is imperative.

The general objective of this work is to produce AgNPs using lactose as the reducing agent via an ultrasound-assisted procedure and test its anti-staphylococcal activities. The specific objectives include (1) to synthesize AgNPs using an ultrasound-assisted technique and lactose as the reducing agent; (2) to characterize the synthesized AgNPs using spectroscopic techniques, and; (3) to evaluate the antimicrobial activities of the synthesized AgNPs against *Staphylococcus aureus*.

Methodology

Materials and reagents

Reagents such as silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), and lactose powder were prepared as aqueous solutions and used without further purification procedure. A PowerSonic 410 ultrasonic bath (Hwashin Technology, Korea) with a 40 kHz frequency was used for the synthesis. For the characterization and estimation of the size and concentration of the synthesized AgNPs, UV-Vis spectrophotometer (Lambda 365, PerkinElmer, USA) was used. The instrument parameters were as follows: (a) wavelength range = 300-650; (b) scanning rate of 120 nm/minute. A Fourier transform infrared (FTIR) spectrometer IRSpirit with Q-ATR accessory (Shimadzu, Japan) was used for the IR spectral characterization. The samples were scanned within the wavelength range 700-4000 cm^{-1}, with a resolution of 4 cm^{-1} for 20 scans. The apodization employed is Happ-Genzel. A background spectrum was recorded using the mentioned analysis parameters before every sample, and all analyses were conducted at room temperature.

Synthesis and characterization of silver nanoparticles (AgNPs)

The AgNPs were prepared via a one-pot, ultrasound-assisted synthesis procedure using silver nitrate precursor solution and alkaline lactose as the reducing agent following a reported protocol[14]. In brief, the mixture of silver nitrate and lactose solutions was irradiated through ultrasonication at room temperature for 10 minutes. Observable color change (colorless to yellow) is a signification of a

complete synthesis procedure. The synthesized AgNPs were then analyzed using spectroscopic techniques to determine the size, concentration, and functional groups. As such, using the absorbance values at the maximum absorption wavelength (λ_{max}) and the molar extinction coefficients, the AgNP size and concentration were estimated from a tabulated reference data set[16].

Antimicrobial activity screening

The screening of the nanomedicinal activities of the synthesized AgNPs against *Staphylococcus aureus* was performed using the agar well diffusion method[17] using *Staphylococcus aureus* UPCC 1143 as the test organism. The microbial suspension was prepared from the 18-24 h old culture and using 0.1% peptone water as the suspending medium. These microbial suspensions were inoculated by swabbing onto the surface of the pre-poured nutrient agar (NA) plates about 3 millimeters thick. This procedure is repeated twice to ensure the even distribution of the inoculum. Portions of 0.1-g samples were placed in three equidistant wells of 10 millimeters in diameter. The NA plates were incubated at 35°C for 24 h and the clearing zones were measured after the incubation. The positive control used in this assay is a commercially available antibiotic, chloramphenicol.

RESULTS AND DISCUSSION

Synthesis and characterization of AgNPs

The synthesis using the sonochemical procedure with lactose solution as the reducing and capping agents was successful as observed by the changes in colors of the reaction mixture after the reaction time at ambient laboratory conditions. The initial colorless solution turned into a yellow-colored solution (Fig. 1A). This color change was attributed to the reduction of Ag^+ to Ag^0 [13], indicating the formation of AgNPs[18]. Sugars such as lactose possess a large number of hydroxyl (-OH) groups, a hemiacetal-reducing end in its chemical structure that can reduce metal ions into metal nanoparticles with zero valency[19]. The capping of the AgNPs provided it protection against aggregation and was attributed to the abundance of -OH moieties and the oxygen molecules (Fig. 1B). Therefore, the pH adjustment of the lactose solution caused deprotonation events to the -OH groups that contributed to the stabilization and reduction of the AgNPs[12].

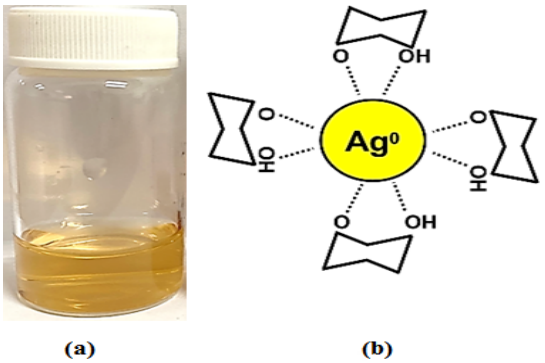

Fig. 1. (a) Photograph of the synthesized AgNP solution and (b) illustration of the AgNPs synthesized using lactose as the reducing and capping agent[14]

The UV-Vis spectroscopic analysis of the AgNPs exhibited a characteristic broad absorption peak at 403nm (Fig. 2) which is between 390 and 500nm and confirmed the synthesis of AgNPs[20]. Using the reference data set[16], the estimated concentration and size of the synthesized AgNPs were 0.2278 ± 0.0001 nM and 24.7 ± 0.6nm, respectively. The FTIR characterization of the pristine lactose and the AgNPs showed distinct peaks that can be attributed to the functional groups and moieties used in the synthetic procedure (Fig. 3). The stretching at 3346 cm^{-1} for the pristine lactose corresponds to the O-H groups[21] while stretching at 1032 cm^{-1} and 1069 cm^{-1} of the pristine lactose agree with the previous study as the characteristic peaks for lactose[22]. Moreover, the shift of the -OH band from 3273 cm^{-1} towards a higher wavenumber of 3346 cm^{-1} suggests the possible coordination of -OH groups onto the AgNP surface[19]. Overall, these characteristics proved the complete fabrication of the silver nanoparticles using the sonochemical procedure.

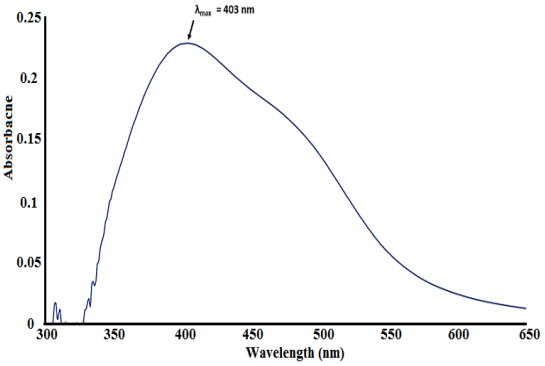

Fig. 2. UV-Vis spectra of AgNPs with a maximum absorption wavelength (λ_{max}) of 403 nm

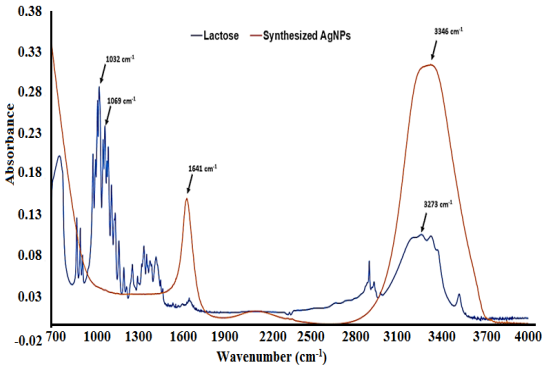

Fig. 3. Overlaid FTIR spectra of lactose and AgNPs

Nanomedicinal activities of AgNPs against *Staphylococcus aureus*

The synthesized AgNPs were tested for its nanomedicine activity against a *Gram-positive* bacteria, *Staphylococcus aureus*. As can be seen in the three wells in the agar plates, clearing areas were observed where the test organism did not grow (Fig. 4). The measured zone of bacterial inhibition of the synthesized AgNPs is 12±1mm for *Staphylococcus aureus* (Table 1). These results confirmed that the synthesized AgNPs had a potential as antimicrobial nanomedicines against *Staphylococcus aureus*. The results of the study correlate with the previous reports on the antibacterial activity of AgNPs against *Staphylococcus aureus*[21,23-27].

Fig. 4. Agar plate showing the zones of inhibition against *Staphylococcus aureus*

Table 1: Results of the agar well diffusion of the synthesized AgNPs as antimicrobial nanomedicines against *Staphylococcus aureus*

	Antimicrobial activities of the synthesized AgNPs (zone of inhibition diameter, mm)
Synthesized AgNPs	12 ± 1*
Chloramphenicol, 30 µg	31 ± 1

*The statistical significance is indicated as $p < 0.05$ according to paired samples t-test.

The mechanism of action of AgNPs against pathogenic bacteria is not completely understood yet but several hypotheses have been reported to explain the antimicrobial activity such as the generation of reactive oxygen species, the release of silver ions from the AgNPs that can denature proteins by bonding with sulfhydryl groups, and the attachment of AgNPs on the bacterial cell wall that can cause subsequent damage to the bacteria[23]. These results suggest the potential of the synthesized AgNPs as antimicrobial nanomedicines with high application value to combat pathogenic strains.

CONCLUSION

Herein, a facile and one-pot method for the fabrication of AgNPs using lactose as the reducing agent was presented and its antimicrobial activities against *Staphylococcus aureus* were screened. Without the use of extreme reaction conditions such as strong chemical reducing agents and boiling temperatures, lactose and ultrasound irradiation were used for the fabrication of the AgNPs. Thus, the sonochemical procedure offered another route in the fabrication of AgNPs. The antimicrobial activity screening showed the nanomedicinal potential of the synthesized AgNPs against the test organism, *Staphylococcus aureus*. It is recommended that the synthesized AgNPs be further analyzed using other test organisms such as other *Gram-positive* bacteria, *Gram-negative* bacteria, and fungi. Thus, the initial results of this study paved the way for the application of AgNPs as nanomedicines in the healthcare industry to help in the fight against antibiotic resistance.

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Conflict of Interest

The author declares that there is no conflict of interest related to this article.

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