Original Article

Green Synthesis of Silver Nanoparticles Using Nisin and its Antibacterial Activity against *Pseudomonas aeruginosa*

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Abstract

Background: Green synthesized silver nanoparticles (AgNPs) have been used in a wide range of biological applications, including their use as antimicrobial agents. The aim of this study was to evaluate the antibacterial activity of green synthesis AgNPs using nisin against *Pseudomonas aeruginosa* (*P. aeruginosa*). **Materials and Methods:** In order to synthesize Ag-nisin, a 1 mg/ml nisin solution was mixed with a 1-mM silver nitrate solution and incubated. The Fourier transform infrared spectroscopy (FTIR) analysis was employed to determine the presence of various biomolecules around AgNPs. The AgNPs were morphologically observed and characterized using field emission scanning electron microscopy assessment, dynamic light scattering (DLS), and zeta potential analysis. The microdilution broth method based on CLSI principles was used for the assessment of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nisin on *P. aeruginosa* isolates. **Results:** Field emission scanning electron microscope showed spherical shaped nanoparticles. DLS revealed that the average size of nanoparticles was 37.2 nm. The zeta potential of AgNPs was − 13.3 mV. FTIR findings revealed that nitrogen atoms of nisin's amine and amide groups are responsible for the capping and stability of the nanoparticles. The MIC and MBC showed that Ag/nisin nanoparticles had higher antimicrobial activity than nisin or AgNPs alone. **Conclusion:** The findings of this study show that the antibacterial activity of nisin can be increased by assembling it into the AgNP interface using a green chemical synthesis method. As a result, the technique may be used to develop an antibacterial formulation to enhance the effectiveness of nisin.

Keywords: *Green synthesis, nisin, Pseudomonas aeruginosa, silver nanoparticles*

Introduction

In recent years, the application of nanoparticles (NPs) in the medical and pharmaceutical sectors has emerged as an alternative drug delivery system with great prospects.[1]

Silver nanoparticles (AgNPs) have played a significant role in the development of nanoscience. The AgNPs are among the most studied nanoparticles due to their characteristic features and their wide variety of utilization. Among silver NP applications, its antibacterial activities toward various bacterial strains have long been considered as a potential solution to antibiotic resistance.[2]

Pseudomonas aeruginosa (P. aeruginosa) is among the most prevalent opportunistic pathogens in immunocompromised patients. Due to high susceptibility to genetic mutations and biofilm formation, *P. aeruginosa* is considered one of the most important multidrug-resistant agents of inhospital infections.[3]

Nisin is produced as a 34 amino acid precursor peptide (NisA) by Gram-positive *Lactococcus lactis* strains and is widely used as a natural antimicrobial polycyclic peptide. Nisin's antimicrobial activity is mediated by the formation of pores and the inhibition of cell wall synthesis by binding to lipid II (bacteria cell wall precursor).[4]

Physical, chemical, and biological approaches can all be used to synthesize nanoparticles. Each of these approaches can have specific advantages and disadvantages.[5]

While chemical and physical synthesis may generate nanoparticles with defined size, dimension, composition, and structure, these methods are accompanied by higher expense, energy, pressure, temperature, and

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most significantly, the production of toxic chemicals that can be absorbed or attach to nanoparticle surfaces, finding them unsuitable for medical applications.[6]

Natural chemicals and plants, due to their natural origin, are ideal candidates to replace synthetic compounds, which are considered to have toxicological consequences.[7] Green synthesis is a low-cost, low-energy approach with the ability to control the size, shape, and stabilization of NPs, which is both environmentally friendly and nontoxic.[8] The biological products used in the biosynthesis of nanoparticles are derived from natural sources such as plants, bacteria, fungi, and yeast.[9]

Green synthesized AgNPs have been extensively used in a variety of biological applications. The biosynthesized AgNPs have shown excellent antimicrobial properties.[10]

In this report, we investigate the *in vitro* antimicrobial activity of AgNPs synthesized by a green method using nisin against *P. aeruginosa*. We also have described the biological synthesis and characterizations of nisin/AgNPs.

Materials and Methods

Synthesis of Ag-nisin

For the synthesis of Ag-niacin nanoparticles, first, 50 mg nisin purchased from Sigma-Aldrich (Germany) was combined with 50 ml of one millimolar silver nitrate solution in a dark bottle and then the solution was stirred at 150 rpm at room temperature for 24 h. The incubated solution was then centrifuged twice at 3500 rpm at room temperature for 20 min, and the pellet was washed two times with phosphate-buffered salt (PBS) and then lyophilized for 24 h in a freezer (Mars, Germany). For controls, we used a 1 mg/ml nisin solution and a 1-mM $AgNO_3$ solution.[11]

Characterization of Ag-nisin

Fourier transform infrared spectroscopy (FTIR) (FT/IR-6300, JASCO Corporation, Tokyo, Japan) was used to investigate the composition and structure of Ag-nisin. Field emission scanning electron microscopy assessment (FESEM) (MIRA3, Tescan, Czech Republic) was used to examine the particle sizes and morphology of the AgNP samples that had been prepared.

The nanoPartica SZ-100 HORIBA made in Japan was employed to investigate the size distribution and stability of AgNPs (dynamic light scattering [DLS]), as well as zeta potential measurements.

Investigation of the antimicrobial properties of nisin

The microdilution broth method based on CLSI principles was used for the assessment of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nisin on *P. aeruginosa* isolates. In this method, first, double successive dilutions (range from 2.32 μg/ml to 2400 μg/ml) were prepared from the plant extract in a liquid culture medium (nutrient broth). Then, in a 96-house microplate, 100 μl of diluted dilutions was added to the wells in each row. Before 24 h of culturing the bacteria, a suspension with a standard concentration of half McFarland was prepared, and 100 μl was added to the wells. The plates were placed in the incubator at 37°C for 18–24 h, and then, 15 μl from triphenyltetrazolium chloride with a concentration of 5 mg/ml was added to the wells. The discoloration caused by this substance was examined after 3 h of storage at 37°C. The reddish-pink discoloration indicates the growth of the bacteria in the plate. The first well in which no discoloration was observed was considered as the MIC. To evaluate the MBC of the study groups, 2 μl of wells in which growth was not observed was added to the solid culture (Müeller-Hinton agar) and was determined after 24 h at 37°C based on observation of microbial colony formation or nonformation. To confirm the results of each study, each evaluation was repeated three times. In this study, bacteria and culture medium (without nisin and nano-nisin) were employed as positive controls, whereas culture medium (without bacteria and nisin, etc.) was utilized as a negative control.[12]

Results

Field emission scanning electron microscopy assessment

According to FESEM images, the Ag nanoparticles are well dispersed and attached to the surface of nisin. The formation of relatively spherical nanoparticles with an average particle size of 34.83 nm is illustrated using FESEM images[Figure 1].

Dynamic light scattering and zeta potential

According to the analysis, the mean size of AgNPs at optimal condition was 37.2 nm. In the frequency diagram, a single peak is observed, which indicates the purity of the substance. The last residual diagram is showing the stability of the material [Figure 2].

The biosynthesized AgNPs' zeta potential was observed to be a sharp peak at −13.3 mV [Figure 3].

It is thought that the nanoparticles' surfaces are negatively charged and scattered in the medium.

The negative value indicates the particles' repulsion and demonstrates their stability.

Fourier transform infrared spectroscopy

The appearance of several peaks, as shown in Figures 4 and 5, suggests the presence of several functional groups in various contexts. Figures 4 and 5 demonstrate the FTIR spectra of control nisin and synthesized Ag-NPs, respectively. For control nisin, strong bands were found at 3527 cm^{-1}, 3381 cm^{-1}, 2934 cm^{-1}, 2899 cm^{-1}, 2663 cm^{-1}, 2538 cm^{-1}, 2067 cm^{-1}, 1653 cm^{-1}, 1434 cm^{-1}, 1387 cm^{-1}, 1261 cm^{-1}, 1093 cm^{-1}, 899 cm^{-1}, 875 cm^{-1}, 775 cm^{-1}, 605 cm^{-1},

549 cm^{-1}, and 466 cm^{-1}, and for synthesized AgNPs, strong bands were found at 3388 cm^{-1}, 3196 cm^{-1}, 2923 cm^{-1}, 2454 cm^{-1}, 1650 cm^{-1}, 1457 cm^{-1}, 1266 cm^{-1}, 1122 cm^{-1}, 1055 cm^{-1}, 996 cm^{-1}, 892 cm^{-1}, 645 cm^{-1}, and 541 cm^{-1}.

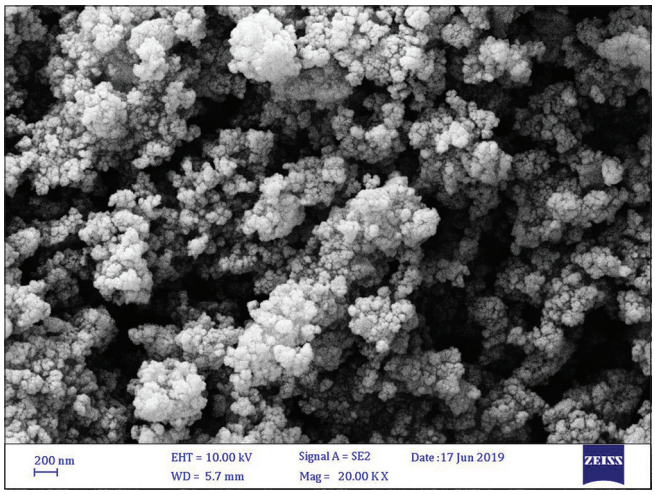

Figure 1: Field emission scanning electron microscopy assessment images of green synthesis silver nanoparticles

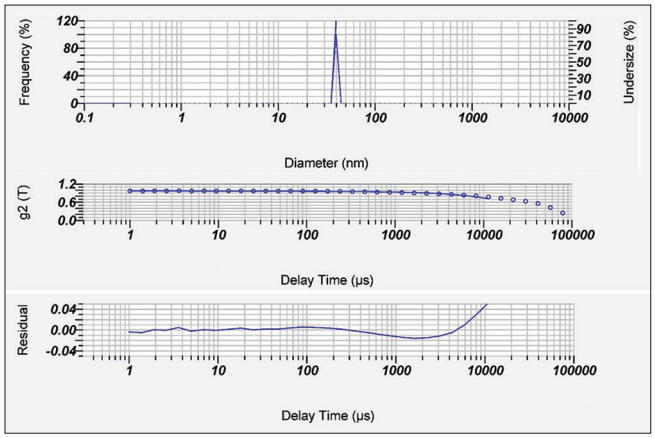

Figure 2: Dynamic light-scattering analysis of silver nanoparticles

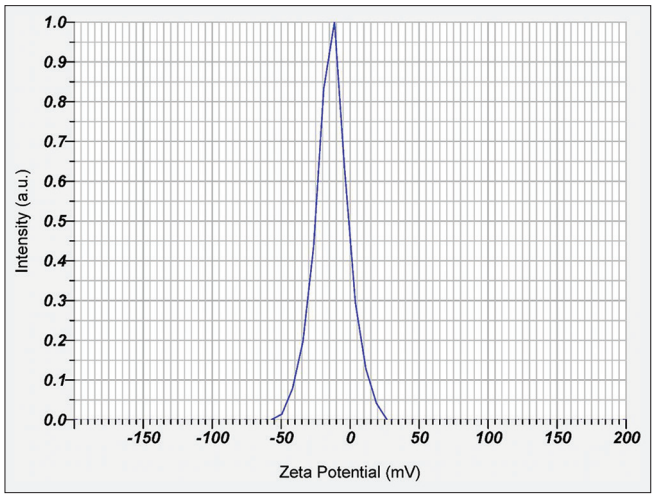

Figure 3: Zeta potential of nisin/silver nanoparticles

The nisin FTIR spectrum has a fingerprint region in the range of 400–1500 cm^{-1}, which is shown in Table 1. The characteristic peaks of this spectrum are related to the areas above 3000 cm^{-1} and between 1700–1600 cm^{-1}, which are specified in Table 1.

When the spectrum of this material is scanned in the presence of AgNPs, changes in the spectrum are observed due to the presence of nanoparticles. The presence of these particles has caused the index peaks to change. For example, the width of the spectrum has decreased in the areas above 3000 and 1600 cm^{-1}, which indicates that AgNPs have interacted with nitrogen atoms of amine and amide groups, which has reduced the amount of adsorption by nisin. The peaks also shifted to shorter wavelengths, which again confirms the effect and interaction of AgNPs with nisin.

The antimicrobial effect of Ag/nisin was investigated on *P. aeruginosa* using the microdilution broth method, and by determining the MIC and MBC in µg/mL, the results were compared to those obtained by AgNPs and nisin [Table 2].

Discussion

In recent years, AgNP synthesis has emerged as a new method to improve several biomedical applications.[13] Green synthesis is a modern method that employs natural compounds or plant components to produce metal nanoparticles.[10] In our previous study, we investigated the effect of green synthesized Ag-nisin particles on macrophage cells. Our findings showed that the synthesized nanoparticle could induce inflammatory responses in macrophage cells by raising interleukin-12 levels by leaving tumor necrosis factor levels unchanged.[11]

To the best of our knowledge, for the first time in this study, the antimicrobial potential of green synthesized Ag/nisin was investigated against *P. aeruginosa*. One of the benefits of AgNPs is that they are reported to have antibacterial properties.[14]

The FESEM images revealed that spherical AgNPs with an average size of 34.83 were formed on the surface of nisin. The DLS results showed that nanoparticles were stable and that the average size of nanoparticles was 37.2 nm. The difference between FESEM and DLS particle size is linked to the fact that microscopic techniques can only measure a limited number of particles, whereas DLS analyzes all particles in the sample.[15]

The zeta potential value indicated the high stability of the synthesized nanoparticles, and the negative potential value might be due to the capping action of nisin with AgNPs.[16]

The FTIR spectrum showed that nitrogen atoms of nisin's amine and amide groups are capable of acting as reducing or capping agents and interact with AgNPs, which is consistent with previous observations.[17]

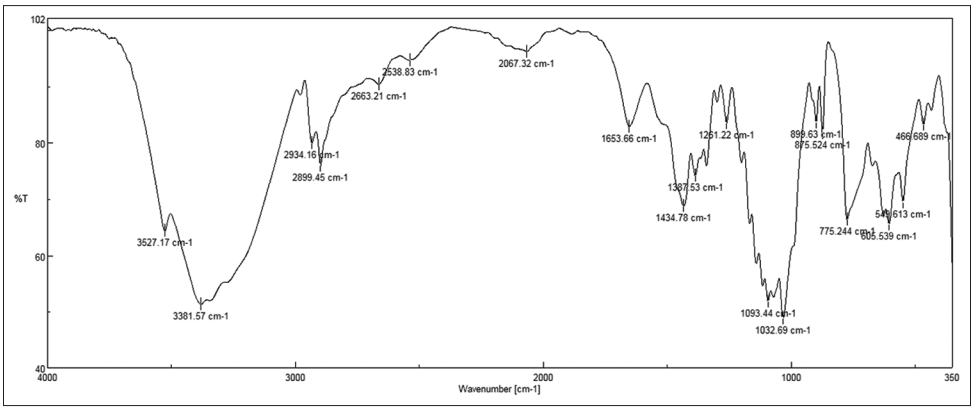

Figure 4: Fourier transform infrared spectroscopy spectra of nisin before reaction with $AgNO_3$

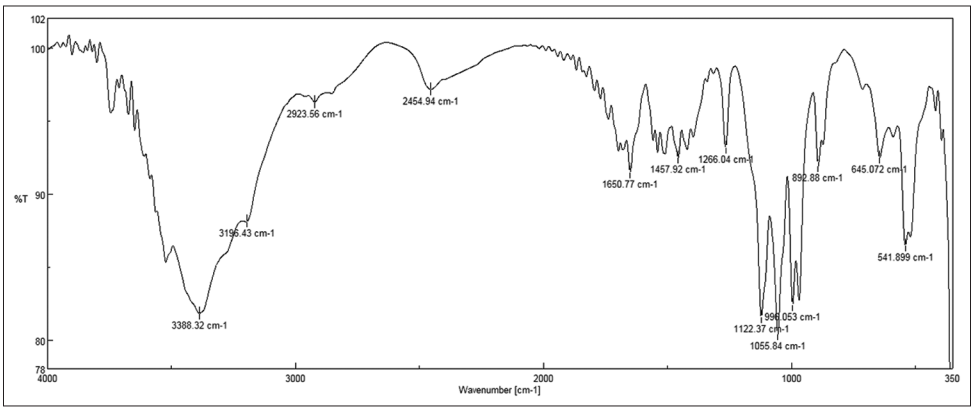

Figure 5: Fourier transform infrared spectroscopy spectra of after reaction with $AgNO_3$

Table 1: The characteristic peaks of Fourier transform infrared spectrum

Chemical structure	Absorbance (Cm^{-1})
Fingerprint area	1500-400 cm^{-1}
Bending C-H-C	1900-1450 cm^{-1}
C-O-C tensile	1032.69
Tensile methylene group	1090 cm^{-1}
Tensile CH and flexural CH2	1434 cm^{-1}
Tensile C-S	659 cm^{-1}
Tensile NH hydroxyl group and OH group carboxylic acid	3381.57 and 3527 cm^{-1}
Asymmetric tensile strength of the carbonyl group	1653.66 cm^{-1}
Asymmetric tensile OH	3288 cm^{-1}
Asymmetric tensile CH3	2934 cm^{-1}
Tensile C-N amide-type II coupled with NH flexion	1550 cm^{-1}
Tensile CH in CH2 and tensile C=N	2067 cm^{-1}
Symmetric tensile CH3	2899 cm^{-1}
CH2 vibrational oscillation	775.2 cm^{-1}
Everton peak 1032.69	1032.69×2≈2067.32

The results of our study show that the MIC of AgNPs and nisin was 18.75 and 37.5, respectively. In contrast, Ag/nisin showed the highest antibacterial activity against *P. aeruginosa* by the MIC of 4.58. The MBC also demonstrated that the chemical synthesized Ag/nisin has the highest bactericidal activity with 18.75 compared to AgNPs and nisin (37.5 and 75, respectively).

These results demonstrated that Ag/nisin has a high potential of antimicrobial activity, and chemically synthesized nisin could be a potential.

In a 2017 study, Pandit *et al*. investigated the antibacterial activity of nisin bioconjugated with AgNPs against *Listeria monocytogenes*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Aspergillus niger*, and *Fusarium moniliforme*.

Table 2: Antibacterial activity of the silver nanoparticles against *Pseudomonas aeruginosa*

Samples	MIC	MBC
Ag	18.75	37.5
Ag-nisin NP	4.58	18.75
Nisin	37.5	75

The antimicrobial effect of Ag/nisin was investigated on *Pseudomonas aeruginosa* using the microdilution broth method, and by determining the MIC and MBC in µg/mL, the results were compared to those obtained by AgNPs and nisin.NP: Nanoparticles, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, AgNPs: Green synthesized silver nanoparticles

The results of the Pandit *et al*.'s study demonstrated that silver bioconjugate with nisin had higher antibacterial activity than nanoparticles and nisin alone, which is in the same pattern as our results.[18]

Arakha *et al*. investigated the antibacterial efficacy of AgNP-nisin conjugates using growth kinetics against *Bacillus subtilis*, *S. aureus*, *Proteus vulgaris*, and *Escherichia coli*; the findings of this study also demonstrated significant growth inhibitions against the mentioned bacteria, whereas AgNP and nisin alone had no significant effect.[19]

Conclusion

According to the findings of this report, by assembling nisin into the AgNP interface using the green chemical synthesis method, the antibacterial efficacy of nisin can be enhanced. As a result, the method could pose as a possible formulation to improve nisin's antibacterial activity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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