Nanomed. J. 6(4): 258-262, Summer 2019

RESEARCH PAPER

Green synthesis and antibacterial activity of zinc selenide (ZnSe) nanoparticles

Majid Darroudi [1*], Niloofar Khandan Nasab [2], Himen Salimizand [3], Alireza Dehnad [4]

[1]Nuclear Medicine Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; Department of Biotechnology, Higher Education Institute of Rabe-Rashid, Tabriz, Iran
[3]Liver and Digestive Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran; Department of Microbiology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
[4]Department of Biotechnology, Higher Education Institute of Rabe-Rashid, Tabriz, Iran; Department of Biotechnology, East Azerbaijan Agricultural Education Center, Tabriz, Iran

ABSTRACT

Objective(s): In this study, zinc selenide nanoparticles (ZnSe NPs) were prepared via green synthesis as a simple, fast, and eco-friendly method at an ambient temperature and various reaction pH (11, 12, and 13). Also ZnSe NPs antibacterial activity was investigated.

Materials and methods: The ZnSe NPs were characterized using instruments such as UV-Vis spectrophotometry within the range of 360-610 nanometers and transmission electron microscopy (TEM). The antimicrobial activity of various concentrations of ZnSe NPs (1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1,024 µg/ml) was examined against Gram-positive bacteria (Staphylococcus epidermidis, Staphylococcus lugdunensis, Enterococcus faecalis, and Staphylococcus aureus), Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, and Enterobacter aerogenes), and Staphylococcus aureus biofilms using the broth microdilution MIC method.

Results: The results of UV-Vis spectrum and TEM confirmed the successful synthesis of ZnSe NPs with the mean diameter of approximately 50 nanometers. According to the results of broth microdilution MIC method, there were differences in the resistance of the bacterial strains. In addition, Staphylococcus aureus biofilms were observed to be completely resistant to various concentrations of ZnSe NPs.

Conclusion: It seems that synthesized ZnSe NPs can be capable of inhibiting growth of bacterial strains especially Gram-positive strains.

Keywords: *Antimicrobial activity, Green chemistry, Nanoparticles, Zinc selenide*

How to cite this article
Darroudi M, Khandan Nasab N, Salimizand H, Dehnad AR.Green synthesis and antibacterial activity of zinc selenide (ZnSe) nanoparticles. Nanomed J. 2019; 6(4): 258-262. DOI: 10.22038/nmj.2019.06.000003

INTRODUCTION

Nanoparticles are groups of materials with unique properties due to the larger surface area compared to macro-sized particles [1, 2]. These properties have been reported to increase the reactivity of nanoparticles. Nanoparticles have significantly different properties with bulk materials depending on their size [3, 4]. Nowadays, nano-sized materials have extensive applications in various fields, including biotechnology, chemistry, physics, and electronics [5-10]. One of the main applications of nanoparticles (NPs) is their antibacterial activity. Bacterial infections are among the most important health concerns in humans. The spread of the infectious diseases

* *Corresponding Author Email: majiddarroudi@gmail.com*
Note. This manuscript was submitted on June 3, 2019; approved on May 15, 2019

that are caused by pathogenic strains, outbreak of bacterial antibiotic resistance, and development of new bacterial mutations have attracted the attention of researchers to discover new methods and materials to confront these organisms. Biofilm formation is another issue associated with bacteria, which could cause severe medical and industrial problems [11].

According to the literature, biofilm-producing bacteria are significantly more resistant to antibacterial agents compared to planktonic cells [12]. Zinc selenide (ZnSe) is a semiconductor material with a bulk band gap of 2.7 eV and luminescence properties [13]. Various methods are available for NP synthesis, such as solvothermal synthesis [14], hydrothermal synthesis [15, 16], wet chemical reaction [17], and microwave [18]. Among these methods, green synthesis has been reported to have numerous benefits as it is simple, economical, and eco-friendly. ZnSe NPs have several applications in various fields, such as biological molecules and cell labeling [19], photocatalysis [20], and wastewater treatment [21]. In our previous study, we synthetized ZnSe NPs and evaluated their chemical characteristics and cellular toxicity [22]. The present study aimed to investigate the antibacterial activity of synthesized ZnSe NPs.

MATERIALS AND METHODS

Synthesis of ZnSe NPs

In this study, a ZnSe NP solution was prepared *via* green synthesis as previously described [22]. The solutions of the reacting materials were prepared in distilled water. For the preparation of the zinc solution, zinc nitrate hexahydrate powder (16×10^{-3} M; Merck, Germany) was dissolved in 100 milliliters of distilled water, and the solution was stirred to dissolve the zinc nitrate powder. Afterwards, an aqueous solution of ascorbic acid (16×10^{-2} M; Merck, Germany) was placed in a flask (50 ml), constantly stirred, and added to the zinc nitrate solution.

The pH of the solution was regulated from 2.50±2 to 11, 12, and 13 by the addition of 0.1 M NaOH solution. At the next stage, the process continued by adding a colorless sodium selenide solution (16×10^{-3} M; Sigma-Aldrich, USA), which was prepared in 50 milliliters of distilled water. The color of the solution gradually changed from colorless to light yellow (pH=11) and dark yellow (pH=13).

Characterization of ZnSe NPs

A UV-Vis spectrophotometer (PerkinElmer, USA) was used to investigate the formation of ZnSe NPs at the wavelengths of 360-610 nanometers. The size of the ZnSe NPs was measured via transmission electron microscopy (TEM; Philips).

Antibacterial activity of the ZnSe NPs

The *in-vitro* antibacterial activity of the ZnSe NPs was determined using the broth serial microdilution method in a 96-well microtiter plate [1] to determine the planktonic and biofilm formation at various concentrations of the NPs (1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1,024 µg/ml) in Gram-positive bacteria (*Staphylococcus epidermidis, Staphylococcus lugdunensis, Enterococcus faecalis,* and *Staphylococcus aureus)*, Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa,* and *Enterobacter aerogenes*). To this end, the powder of NPs was provided by freeze-drying, dissolved in distilled water, and diluted on each well of the microtiter plates from the highest concentration (1,024 µg/ml) to the lowest concentration (1 µg/ml). At the next stage, the bacterial suspension was prepared overnight using the saline suspension of the isolated colonies obtained from the Muller-Hinton agar plate. The suspension was adjusted to the 0.5 McFarland turbidity standard and diluted one-tenth. Afterwards, five microliters of the diluted bacterial suspension encompassing the colony-forming unit (CFU) of the bacteria was transferred to each well. Finally, the microtiter plates were incubated at the temperature of 37˚C for 24 hours. After incubation, the bacterial growth at each concentration of the NPs was visually evaluated.

Biofilm formation assay

S. aureus was cultured on the Muller-Hinton broth at the temperature of 37˚C for 18 hours in an incubator. Following that, five microliters of the cultured bacterium was transferred to the 96-well microtiter plate containing 200 microliters of the Muller-Hinton broth and 0.45% glucose and incubated for 24 hours in order to perform the biofilm formation assay. The main steps in this assay included the removal of the medium from each chamber, washing the wells with normal saline three times, adding 100 microliters of 99% methanol to fix the bacteria, elimination of methanol after 15 minutes, adding 100 microliters of 0.5% crystal violet stain, removal of the stain

after 20 minutes, washing the wells with normal saline three times, adding 150 microliters of acetic acid/ethanol solution in order to release the crystal violet, and reading the absorption at the wavelength of 590 nanometers using the ELISA plate reader kit (PerkinElmer, USA) [23]. In order to investigate the effects of the ZnSe NPs on biofilm elimination, the bacterial biofilm was subjected to minimum inhibitory concentration (MIC).

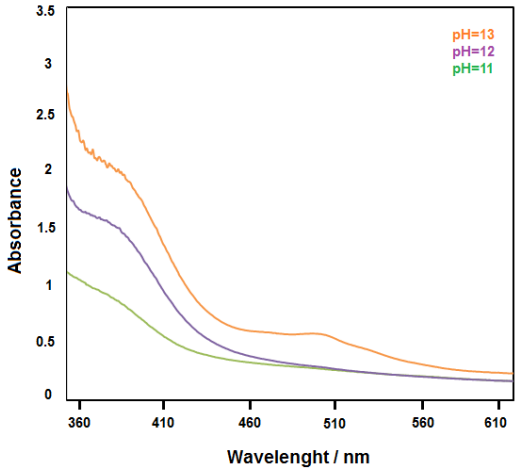

Fig 1. UV-Vis spectrum of ZnSe NPs at pH of 11, 12, and 13

RESULTS AND DISCUSSION

In the present study, the ZnSe NPs were synthesized *via* green synthesis at the pH of 11, 12, and 13. After one hour, the color of the resulting ZnSe NPs changed from colorless to yellow, implying the synthesis of the ZnSe NPs.

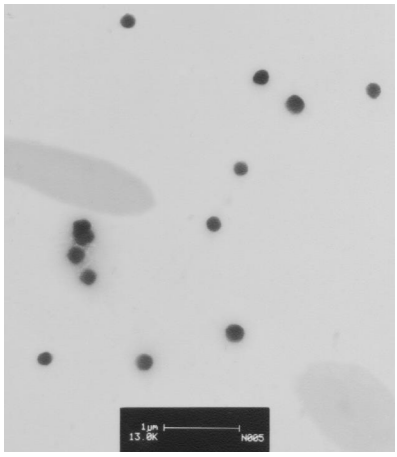

Fig 2. TEM Image of ZnSe NPs

The resulting UV-Vis spectrum was within the range of 360-440 nanometers (Fig 1), which confirmed the synthesis of the ZnSe NPs at different pH. However, the spectrum indicated that the optimum synthesis occurred at the pH of 12. Due to the UV-Vis results, increased pH caused changes in the size of the NPs and their aggregation.

Therefore, the sample with the pH of 12 was selected for TEM imaging and antibacterial tests. In addition, the synthesized ZnSe NPs were scanned using TEM as spherical with the mean diameter of approximately 50 nanometers (Fig 2).

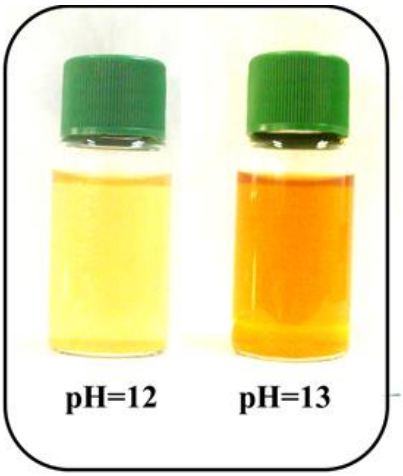

Fig 3. ZnSe samples at pH of 12 and 13

According to the findings regarding the antibacterial activity of the ZnSe NPs (Table 1), the Gram-positive bacteria (*S. aureus* and *S. epidermidis)* had average susceptibility to the NPs. However, *S. lugdunensis* was totally sensitive to the ZnSe NPs and had no growth at any of the concentrations.

Another Gram-positive bacterium (*E. faecalis*) and the Gram-negative bacteria (*E. coli, S. aeruginosa,* and *E. aerogenes)* showed high resistance to the ZnSe NPs. On the other hand, the Gram-negative bacteria and *E. faecalis* had no growth only at the highest concentration of the ZnSe NPs (1,024 µg/ml).

The biofilm assay indicated that the bacteria that were in the biofilm form were completely resistant to the ZnSe NPs. This finding was predictable since the formation of bacterial biofilms largely influences the biological activities of bacteria, so that it could not be easily predicted based on the current knowledge.

Table 1. Results of bacterial growth at various concentrations of ZnSe NPs

ZnSe-NPs (µg/ml)	*Enterobacter aerogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Enterococcus fecalis*	*Staphylococcus lugdonensis*	*Staphylococcus epidermidis*	*Staphylococcus aureus*
1024	-	-	-	-	-	-	-
512	+	+	+	+	-	-	-
262	+	+	+	+	-	-	-
128	+	+	+	+	-	-	-
64	+	+	+	+	-	-	-
32	+	+	+	+	-	-	-
16	+	+	+	+	-	+	+
8	+	+	+	+	-	+	+
4	+	+	+	+	-	+	+
2	+	+	+	+	-	+	+
1	+	+	+	+	-	+	+

+ Symbol indicates growth and – symbol indicates lack of growth.

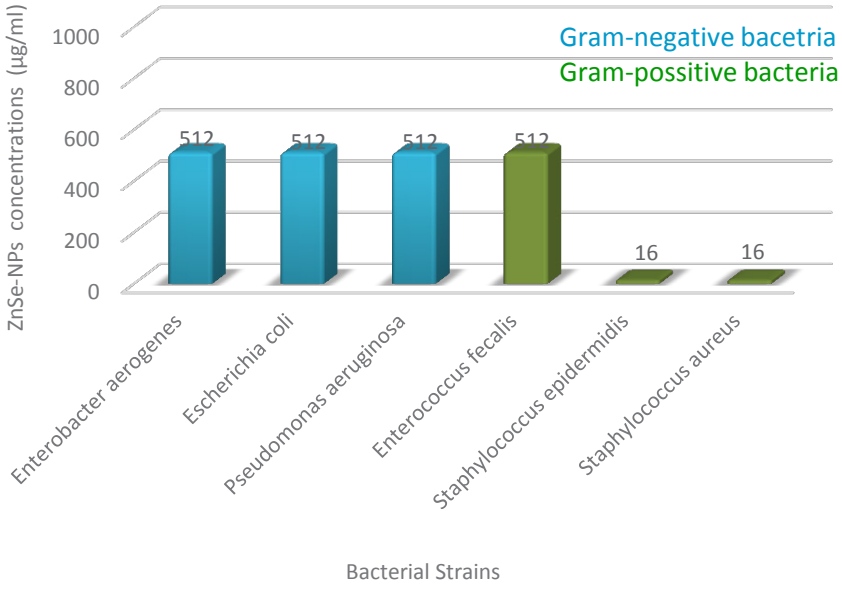

Fig 4. Comparison of growth rate of various bacterial strains at different concentrations of NPs

CONCLUSION

We could successfully synthesize ZnSe nanostructures using the simple, rapid green synthesis method by changing the pH of the reaction. The present study aimed to investigate the antibacterial effects of ZnSe NPs on Gram-positive bacteria (*S. epidermidis, S. lugdunensis, E. faecalis,* and *S. aureus*), Gram-negative bacteria (*E. coli, P. aeruginosa,* and *E. aerogenes*), and biofilm-forming *S. aureus.* Evaluation of the antibacterial activity of the ZnSe NPs in the Gram-positive and Gram-negative bacteria and MIC determination indicated the variable effects of these NPs, which could be attributed to the differences in the cell wall structure of Gram-positive and Gram-negative bacteria or the specific properties of each bacterial species. Furthermore, the results of the biofilm assay indicated that the *S. aureus* biofilm was completely resistant to ZnSe NPs.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mashhad University of Medical Sciences, for providing technical and instrumental support.

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