FACILE SYNTHESIS OF HIGHLY UNIFORM SELENIUM NANOPARTICLES WITH ANTIBACTERIAL ACTIVITY ON BACILLUS SUBTILIS

[1]MAHSA VAHDATI, [2]TAHEREH TOHIDI MOGHADAM, [3]PARVANEH SAFFARIAN

[1,3]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Nanobiotechnology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Abstract - In the light of diverse applications of Selenium nanoparticles in biomedical applications, a facile procedure has been developed to synthesize highly stable, and monodisperse nanoparticles with Polysorbate 20 at room temperature. Transmission electron microscopy analysis revealed that the Selenium nanoparticles (Se NPs) were spherical in shape with uniform size of 43 nm. The antibacterial activity of Se NPs was investigated against bacillus subtilis as a model gram-positive bacteria which showed notable antibacterial activity. Results of this investigation suggest utilization of Se NPs in the next generation of nanohybrid systems with promising antibacterial efficiency.

Keywords - Antibacterial Properties; Monodispersity; Nanohybrid Systems; Selenium Nanoparticles

I. INTRODUCTION

Elemental Selenium plays a very important role in biological systems. It can easily be incorporated into proteins that can act as an antioxidant enzyme to inhibit cellular damages. It can also be used to control diseases like cancer or cardiac problems, arthritis, cystic fibrosis, muscular dystrophy, as well as application in form of a nutritional supplement [1][2]. When electrons are confined to a nanometer scale, the extent and type of electronic motion directs the physicochemical properties of the matter. Therefore, manipulating the size of materials within such dimensions leads to appearance of novel and unique properties compared to that of bulk material [3]. At nanoscale, Selenium is found in various shapes like spherical and belted morphologies [4], nanowires [5] and nanorods [6]. Formation of selenium particles within this scale provides unique physicochemical and functional properties. Such characteristic features of nanoparticles promise improvement in their biological activity when compared to their bulk counterpart [7]. Selenium nanoparticles (SeNPs) have recently drawn significant attention due to having variety of applications such as antioxidant [8], antidiabetic [9], antineoplastic [10], antimicrobial properties [11] and medical applications [12].

Although Se NPs have shown to be promising candidates in the next generation of nanohybrid systems with antibacterial activity, their function is extremely dependent upon their size and monodispersity. Herein, synthesis of highly monodisperse Se NPs is reported by a facile chemical reduction method, using polysorbat 20 as a stabilizing agent. Synthesized nanoparticles were characterized by Ultraviolet-visible spectroscopy (UV–vis), Transmission electron microscopy (TEM), and Dynamic light scattering spectroscopy (DLS) and inductively coupled plasma optical emission spectroscopy (ICP-OES) techniques. Antimicrobial effect of the nanoparticles was studied against Bacillus subtilis as a model for gram-positive bacteria.

II. MATERIALS AND METHODS

2.1 Chemicals and bacterial strain

Sodium selenite was purchased from Merck (Darmstadt, Germany). Ascorbic acid and Polysorbate 20 were procured from Sigma (St. Louis, MO, USA). Muller-Hinton broth/agar were purchased from HiMedia (Mumbai, India). Bacillus subtilis (ATCC 6633) were obtained from Microbiology Laboratory of Zakariya Razi Laboratory complex, Science and Research Branch, Islamic Azad University, Iran. Ultrapure deionized water (DI) was used during the whole experiments. Glassware was thoroughly cleaned with dilute sulfo chromic acid/detergent solution and rinsed with DI.

2.2 Synthesis and purification of Se NPs

Selenium nanoparticles (Se NPs) were prepared via reduction of sodium selenite by ascorbic acid and stabilized by polysorbate 20. Briefly, 51 mg of $Na_2SeO_3.5H_2O$ was added to 135 mL of Milli-Q water. 5µL of Polysorbate 20 was then added as a stabilizer agent. Ascorbic acid (15 mL, 116 mM) was added dropwise to sodium selenite solution with vigorous stirring. Selenium nanoparticles were formed after addition of ascorbic acid. All solutions were made in a sterile environment using a sterile cabinet and deionized water. Selenium nanoparticles were purified by centrifuging the solution at 12,000 rpm for 15 min. Pellets were sterilized by ultraviolet light exposure, and resuspended in MilliQ water before use in bacteria experiments.

2.3 Characterization of Se NPs

UV-Vis absorption spectroscopy, scanning electron microscopy and Dynamic light scattering were used to

characterize formation of the nanoparticles. The spectra were recorded within 200–700 nm wavelength range using Perkin Elmer, Lambda 25). To determine and study the size, morphology and uniformity of Se NPs, TEM images were analysed. Size distribution and surface charge of the nanoparticles was determined using Malvern Zetasizer Nano-ZS instrument (Worcestershire, UK). Selenium contents of nanoparticles were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES, model Arcos EOP from Spectro, Kleve, Germany).

2.4 Antimicrobial activity of Se NPs

Se NPs were tested for minimum inhibitory concentration leading to the inhibition of bacterial growth by standard microdilution method (provided by the CLSI, 2018). A 96-well microtiter plate dilution was applied to determine the MIC. MIC was performed by inoculating the indicator bacteria at a concentration of 10^6 CFU/mL into a series of wells with two-fold dilutions of the synthesized Se NPs with 100 µL of Mueller–Hinton broth. After proper mixing, the 96-well microtitration plate was incubated at 37 °C for 20 h. Bacterial growth inhibition was determined by monitoring the optical density at 630 nm by ELX 808 microplate reader (Biotek Instruments, Winooski VT, USA).
Absorbance at 630 nm showed the bacterial growth. The MIC value was defined as the lowest concentration of se NPs that inhibits the growth of the microorganism (no turbidity in the broth).

2.5 Statistical analysis

All experiments and analysis were made in triplicate with data reported as mean ± standard deviation. Experimental statistics were performed using SPSS 16 software. Analysis of variance (ANOVA) followed by Tukey's multiple-comparison post hoc tests were exploited for the comparison of three or more groups. The significance level (P) was considered at 0.05.

III. RESULTS AND DISCUSSION

3.1. Synthesis and characterization of selenium nanoparticles

In this study, a facile wet chemical method was utilized for synthesis of monodisperse Selenium nanoparticles (Se NPs) by ascorbic acid with biocompatibility and good reducing properties. Selenium oxoanion (SeO_3^{2-}) was formed in aqueous medium. When this solution reacted with ascorbic acid, selenium was reduced to elemental selenium (Se^0), and dehydroascorbic acid was formed as an oxidized form of ascorbic acid [13]. Change in the appearance of the solution (from colorless to orange) indicated the reduction reaction. Polysorbate 20 was used as a stabilizing reagent to suppress the abnormal growth and to prepare monodisperse Se NPs in water. This reagent is found to have played a critical role in controlling the size of SeNPs and their stability in the solution. We observed that in the absence of polysorbate 20, the nanoparticles are less stable and tend to aggregate over time. Chemical reduction of selenium ions to Se NPs was evaluated by UV–vis spectroscopy, which is the most simple and indirect method. Figure 1a depicts the strong absorption peaks about 265 nm, which is related to formation of SeNPs during the reduction process [1]. Figure 1b shows the color of the colloidal suspension after synthesis. Characterization of the purified samples by Transmission electron microscopy depicted the spherical morphology of the nanoparticles. Analysis of TEM by Image J software showed that the size of the nanoparticles was 43 nm (Figure 1c).

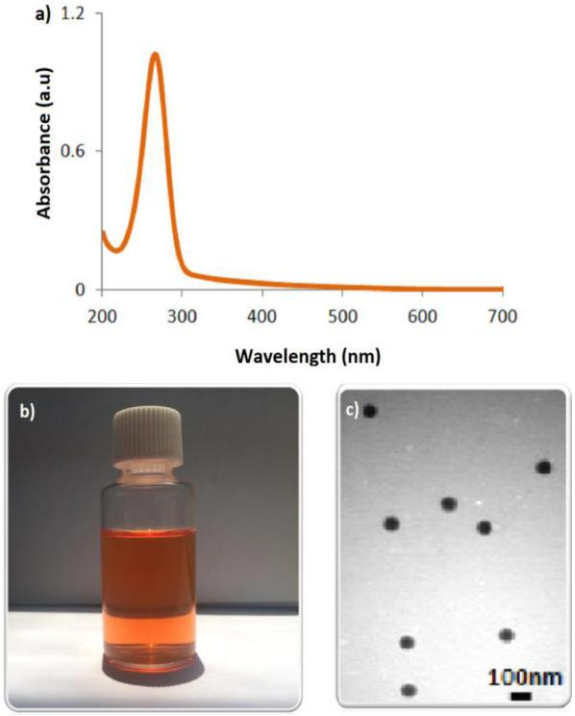


Figure 1. Characterization of Selenium nanoparticles (Se NPs) by UV–Vis spectroscopy (a) appearance of the colloidal suspension (b) and Transmission electron microscopy image (c)

Dynamic light scattering is among the most common light scattering techniques for size distribution studies [14]. Figure 2a presented a peak centered at 59.41 nm with a very low polydisperisty index (PDI=0.031). Such uniformity in size facilitates conducting a conclusive series of experiments to precisely correlate the size and dose effects to the antibacterial activity of the nanoparticles. The electrostatic stabilization of Se NPs was estimated by measuring their zeta potential values. The zeta potential value was found to be -22.2 mV (see Figure 2b). It should be noted that zeta potential is an important indicator of the stability of colloidal suspensions of the nanoparticles [15]. Surface charge of the nanoparticles also plays a key role in determining the type of interaction with bacteria[16].

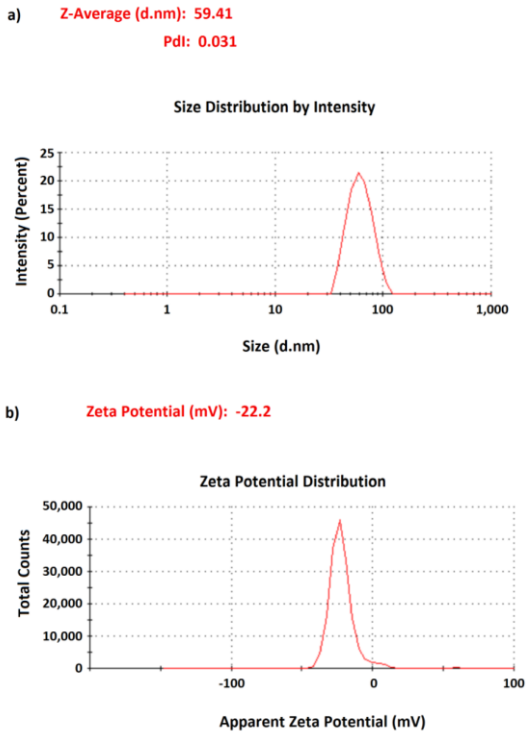


Figure 2. Polydispersity index (PDI) and Z-Average value of SeNPs (a), with zeta potential analysis of the colloidal suspension by Dynamic light scattering (b)

3.2 Effect of Se NPs on bacterial growth

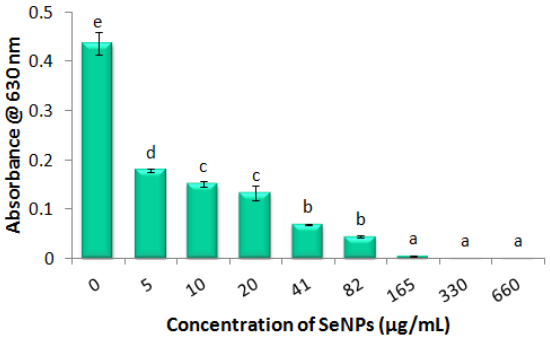


Figure 3. The antibacterial activity of Se NPs against B. subtilis after 20 hours in terms of OD @ 630nm. Each bar indicates mean ± standard deviations of three replications. Bars not labeled by the same letter represent statistical significance at P ≤ 0.05 using ANOVA followed by Tukey's HSD test.

The antibacterial activity of the synthesized SeNPs was investigated against the gram-positive bacterium, B. subtilis. The nanoparticles were incubated with B. subtilis and the bacterial growth was monitored via optical density measurements. The concentration-related effect of Se NPs against the growth of B. subtilis is depicted in Figure 3. The bacteriostatic effects of Se NPs were determined in terms of their MIC values. Absorbance of the samples was recorded at 630 nm, and zero values represented complete inhibition of the bacterial growth. After 20 h of incubation, all concentrations were found to effectively reduce the bacterial growth. The minimum inhibitory concentration was calculated to be 165 µg/mL. The obtained results were in agreement with the previous work done on the antibacterial activity of Se NPs [16][17][18]. Subburaman et al. observed the morphological changes occurred in the bacterial cells by SEM. Compared to untreated B.subtilis, the cells were shrunk and fragmented, and sizes of the cells were comparably irregular[4].

IV. CONCLUSION

Selenium nanoparticles have attracted significant attention due to showing good biocompatibility with promising antimicrobial, anticancerous, antidiabetic, and anti-inflammatory effects. Herein, facile synthesis of uniform SeNPs has been reported with proper antibacterial efficiency. Results of this study emphasizes synthesis of monodisperse nanoparticles to control the physicochemical properties which, in turn, result in conclusive correlation of size to their antibacterial function as well as biocompatibility. This paves the way to design the next generation of stable, uniform nanohybrid systems with promising biomedical and biomedicine applications.

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