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Proceeding Paper

Green Synthesis of ZnO-NPs by *Juglans regia* Green Husk Aqueous Extract †

Marzieh Dehghani and Parinaz Ghadam *

Department of Biotechnology, Faculty of Biological Sciences, Alzahra University, Tehran 1993893973, Iran; marzidehghani1990@gmail.com
* Correspondence: pghadam@alzahra.ac.ir

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Abstract: Nanobiotechnology is a broad science that provides nanomaterials to solve many medical, agricultural, engineering, biological, and chemical problems. Nanoparticles (NPs) are nanomaterials with zero dimensions. Due to its numerous applications in a variety of scientific fields, semiconductor NPs synthesis has received a lot of attention in recent times. There are three methods that can be used to make NPs including chemical, biological, and physical methods. ZnO-NPs has been synthesized by the biological method in this study. The green husk of *Juglans regia* aqueous extract was used to make NPs using the biological method. Transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and UV-Visible spectroscopy were used to examine the NPs' properties. The biosynthesized ZnO-NPs have a strong SPR band at 360 nm, as demonstrated by the UV-Vis analysis results. These NPs are crystalline. In addition, the results of the DLS analysis showed that the hydrodynamic diameter of 90% of the biosynthesized zinc oxide is less than 292.55 nm. Furthermore, the TEM analysis confirmed that these NPs have spherical and ellipsoidal shapes.

Keywords: ZnO-NPs; *Juglans regia*; green synthesis; agricultural waste material

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1. Introduction

Nanotechnology is a widespread science that manipulates atoms, electrons, protons, and neutrons to provide an accurate understanding of material development to solve many medical, agricultural, engineering, biological, and chemical problems [1,2]. NPs are a large group of nanomaterials [3]. Semiconducting NPs have properties between metals and non-metals [4]. As a result, photocatalysis, photo optics, and electronic devices all rely heavily upon them [5]. Zinc oxide is attractive for optoelectronics, electronics, and laser technology usage, according to materials science [6]. ZnO-NPs are photocatalytic, antioxidant, antibacterial, antifungal, and anti-inflammatory [7,8]. These NPs are utilized extensively in cancer treatment, drug delivery, and medical research [9]. ZnO-NPs are used to make sunscreen because they can effectively absorb ultraviolet rays from the sun [10]. Additionally, ZnO-NPs can be utilized for wastewater purification [11]. One green synthesis technique is NP biosynthesis. NPs are made from natural materials such as vitamins, sugars, plant extracts, microorganisms, and fungi in biosynthesis. Plant extracts are the most suitable reagents for the biosynthesis of NPs out of the aforementioned reagents [12]. Compared to other chemical and physical methods, the biological method has many advantages, such as its simplicity, cost-effectiveness, and absence of harmful effects on the environment, as well as the fact that NPs produced using this method will typically be more stable [13]. In order to make ZnO-NPs, numerous plant extracts have been used so far. Using the extract of the Trifolium pretense flower, Dobrucka and Dugaszewska produced ZnO-NPs. These NPs, with sizes ranging from 60 to 70 nm, had a strong antibacterial effect on the destruction of *E. coli*, *S. aureus*, and *P. aeruginosa* [14]. Thus, ZnO-NPs with these characteristics are referred to as nano-antibiotics [15]. As an agent for the production of NPs, an aqueous extract of the green husk of *J. regia* was utilized in this study. Then, these NPs' chemical and physical properties were examined. Transmission electron microscopy (TEM), X-ray diffraction spectroscopy (XRD), dynamic light scattering (DLS), and ultraviolet-visible spectrophotometry (UV-Vis) were utilized to investigate the NPs' properties.

2. Materials and Methods

2.1. Materials

Zinc sulfate, H_2SO_4, and NaOH were bought from Sigma–Aldrich. Furthermore, *J. regia* green husk was collected from Karaj Gardens (Karaj, Iran).

2.2. Biosynthesis of ZnO-NPs

After being thoroughly washed with distilled water, some green husks of fresh *J. regia* were initially dried outdoors in the shade. A Meyer flask with parafilm and aluminum foil as the lid was filled with 0.4 g of dried husk powder of *J. regia* and 15 mL of boiling distilled water. After that, this was left for ten minutes in a water bath at 100 degrees Celsius. The Whatman paper no. 1 was used to filter the extract [16].

In order to achieve the highest possible concentration of zinc sulfate salt, concentrations of 0.25, 0.5, 0.75, 1, and 2 mM of the stock zinc sulfate salt were prepared. This test was carried out at room temperature with no alteration to the pH. The volume of *J. regia* extract was 30 L, and the solution had a final volume of 1 cc. For two hours at room temperature, all samples were stirred. The UV-Vis spectrophotometer's spectra were derived from the solutions, and then the best concentration of zinc sulfate salt was chosen.

Five solutions, of pH 5, 7, 8, 10, and 12, were made using 30 mL of *J. regia* extract and 1 mM zinc sulfate to adjust the pH. Room temperature was used for this test. The solution had a final volume of 1 cc. For two hours at room temperature, all samples were stirred. The UV-Vis spectrophotometer's spectra were taken from the solutions, and then the best pH was chosen.

Five solution series, containing 15, 30, 45, 60, and 75 L of *J. regia* extract, 1 mM zinc sulfate, and a pH of 12, were created in order to maximize extract volume. Room temperature was used for this test. The solution had a final volume of 1 cc. For two hours at room temperature, all samples were stirred. When making NPs, the extract was utilized as a producing and stabilizing agent. The UV-Vis spectrophotometer's spectra were obtained from the solutions, and then the optimal extract volume was chosen.

Achieving ZnO-NPs using the extract of this plant is possible even at room temperature. However, to obtain the desired NP, the reaction temperature was also evaluated and optimized. In order to optimize the reaction temperature, solutions with optimal conditions were stirred for two hours at room temperature, 40, 50, 60, and 70 °C. The spectra of the UV-Vis spectrophotometer were obtained from the solutions and, finally, optimum temperature was selected.

In order to optimize the reaction time, a solution of the mixture of extract and zinc sulfate salt with all the previous optimizations, at different times of 5, 15, 30, 60, 90, and 120 min, as well as 24 h and one week, was prepared. The spectra of the UV-Vis spectrophotometer were obtained from the solutions and, finally, optimum time was selected.

2.3. UV-Vis Spectroscopy

A SPEKOL 2000 spectrophotometer measured the obtained ZnO colloid's optical properties between 300 and 800 nm.

2.4. DLS Analysis

ZnO NPs' hydrodynamic diameter were measured by NanoPhox 90-246V instrument from Sympatec GmbH, Clausthal-Zellerfeld, Germany.

2.5. TEM Analysis

The Philips CM30 was used to measure the shape and size of dried ZnO NPs. Digimizer software version 4.1.1.0 was also used to calculate the size distribution of ZnO NPs.

2.6. XRD Analysis

ZnO NPs' crystal structure and presence were discovered through XRD analysis. Rigaka performed the analysis on the colloidal ZnO at 40 kV, 40 mA, and a step size of 0.02°.

2.7. FT-IR Analysis

A TENSOR 27 spectrophotometer was used to perform FT-IR analysis on a ZnO colloid.

3. Results and Discussion

Due to their diverse properties, the preparation of nanoscale materials has received significantly more attention in recent years [17]. A large group of nanoscale materials are called NPs. NPs have numerous applications, including chemical sensors, catalysts, medical diagnostic imaging, electronic components, medical treatment protocols, and pharmaceutical products [18]. There are a lot of physical, chemical, and biological ways to make NPs. It is possible to synthesize NPs using environmentally friendly methods. There are no hazardous, toxic, or expensive chemical agents used in green NP synthesis. This kind of synthesis can be referred to as green if green chemistry metrics are taken into account during the synthesis of NPs. Metal oxides is a class of minerals that has attracted much research attention because of its diverse structures and properties. One of the most well-known semiconductors in the family of metal oxides is ZnO. It has a large exciton binding energy and a wide band gap [19]. Over the past ten years, research has been integral to the creation of new zinc oxide applications. Catalysis, cosmetics, and optical devices are among zinc oxide's current uses [20].

ZnO-NPs were made using a biological method in this study. ZnO-NPs were produced using the biological method using green husk extract of *J. regia*. Juglandaceae is a large family that includes Persian walnuts and is predominantly found in temperate climates. Walnut trees can be found growing today in a wide range of locations, including Asia, Europe, and America [21]. As a waste product, it has a green husk that is loaded with antioxidants. As a result, the aqueous extract of dried green husk was chosen for ZnO-NP synthesis in this study. Numerous materials in the extract are capable of transforming Zn^{2+} into ZnO-NP [22].

ZnO-NPs are in the form of colloidal systems. The colloids of these NPs are white [22], white-milky [23], and yellow [24]. In this study, the color of the ZnO-NPs was white-milky (Figure 1).

Figure 1. ZnO-NPs synthesized by biological method.

UV-Vis spectroscopy is used to verify their synthesis and investigate the NP shape in colloidal systems. Surface plasmon resonance, or SPR, is the process by which the free electrons of metallic NPs in colloidal systems fluctuate, resulting in their intense coloration. As a result, the coordinated and cumulative oscillations of metallic electrons triggered by radiation constitute the exacerbation of surface plasmon [25]. The first person to use Maxwell's equations to explain the colors of colloids was Gustav Mie [26]. In 1908, Mie presented his theory. He explained the height of the plasmon oscillations and the position and width of the SPR band using his theory. According to Mie's theory, the SPR band's characteristics are influenced by the environment's dielectric constant and the size and shape of the particles [27]. ZnO-NPs have a surface plasmon resonance band between 300 and 400 nm, according to previous research. Aloe vera leaf extract was used by Sangeetha and colleagues to make ZnO-NPs in 2011, for instance. The strong peak of the synthesized NPs was located between 375 and 358 nm [28]. Using the root extract of Polygala tenuifolia, Nagajyathi and his colleagues produced ZnO-NPs in 2015. The broad peak of these NPs was 314 nm [9]. Elumalai and Velmurugan were able to use Azadirachta indica plant extract to create ZnO-NPs in 2015, which had a strong peak around 370 nm [24]. Additionally, Carissa edulis was used to synthesize ZnO-NPs in 2016 by Fowsiya and colleagues. The strong peak of these NPs was located between 330 and 400 [29].

The SPR peak in biosynthesized ZnO-NPs measured by UV-Vis spectrometry was located at 360 nm in this study. Additionally, the biosynthesis of ZnO-NPs was influenced directly by extract volume, time, temperature, and pH. The concentration of 1 mM zinc sulfate salt was chosen as a result. Additionally, five pH 5, 7, 8, 10, and 12 solutions, containing 30 mL of *J. regia* extract and 1 mM zinc sulfate, were prepared to optimize the pH. The extract was unable to produce NPs at an acidic pH. Due to the increased synthesis of ZnO-NPs, the sample color changed with pH, and the sample SPR peak became higher. pH 12 was the ideal for the synthesis of NPs. Additionally, five solution series, containing 15, 30, 45, 60, and 75 L of *J. regia* extract, 1 mM zinc sulfate, and with a pH 12, were created in order to maximize extract volumes. The solution's SPR peak was 30 L higher than that of the other solutions. As a result, this extract volume was chosen as the best volume. The effect of temperature revealed that, in the synthesized NPs, the optimal temperature also has the highest SPR peak, which occurs at room temperature. Additionally, a solution containing the mixture of extract and zinc sulfate salt was prepared with all of the previous optimizations at various times of 5, 15, 30, 60, 90, and 120 min, in addition to 24 h and one week. ZnO-NP synthesis was continuously observed. The following are the ideal conditions for the synthesis of ZnO-NPs: 30 mL of extract, room temperature, pH 12, 1 mM sodium salt, and a five-minute incubation period (Figure 2).

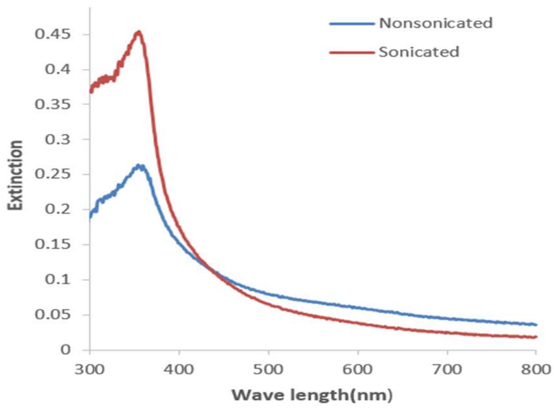

Figure 2. SPR band of non-sonicated and sonicated sample.

In the next step, 1 mL of the optimized sample was centrifuged for 20 min at 10,000 rpm, and the white-milky precipitate was washed twice in order to remove the extra salt and extracts. The precipitate was dried at 50 °C for 4 h and disperse in distilled water and, finally, its SPR peak was read against distilled water (Figure 3). The sample had a considerable SPR peak at 360 nm.

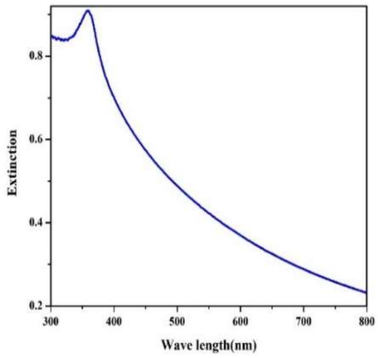

Figure 3. SPR band of dried precipitate dispersed in distilled water.

In a colloidal solution, the Brownian motion of the dispersed NPs is constant. The NP hydrodynamic diameter in the solution was measured using DLS measurements of light scattering over time [30]. Figure 4 depicts the DLS results for biologically synthesized ZnO-NPs. According to the DLS analysis, the hydrodynamic diameter of 90% of biologically synthesized ZnO was less than 292.55 nm.

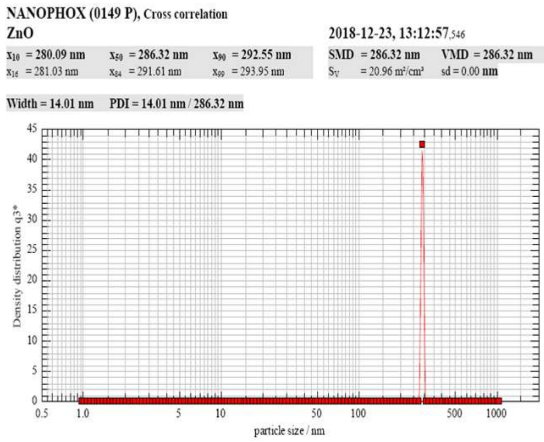

Figure 4. The DLS diagram of ZnO-NPs synthesized by biological method.

The DLS diagram revealed the hydrodynamic diameter, but the TEM images should be used to determine the precise size distribution, size, and shape of ZnO-NPs. It showed that, in using the biological method, ZnO-NPs are spherical and oval in shape (Figure 5). Oval NPs range from 50 to 60 nm, and spherical NPs are between 60 and 70 nm (Figure 6).

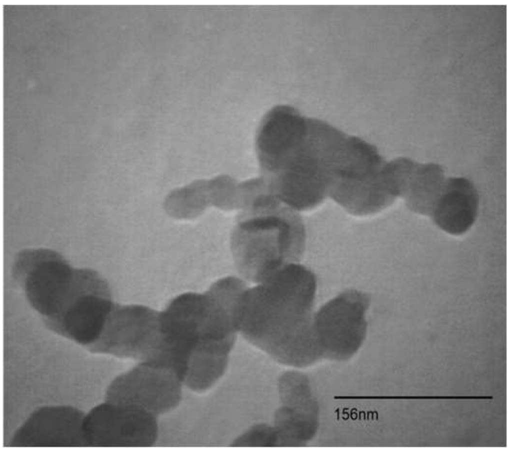

Figure 5. TEM images of biosynthesized ZnO-NPs at optimum conditions.

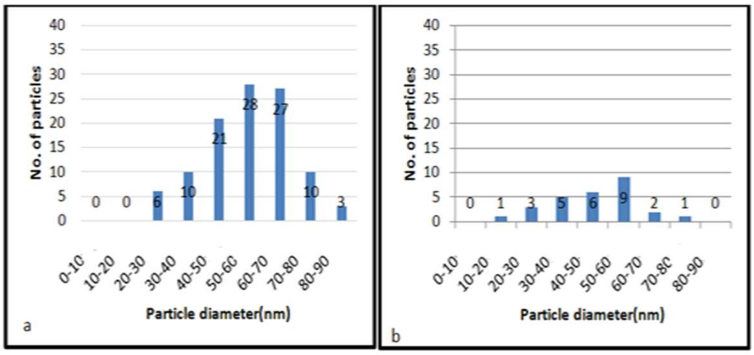

Figure 6. Histogram of size of (**a**) oval and (**b**) spherical ZnO NPs synthesized by the biological method.

In general, the stability of the synthesized NPs will affect their features and function. Therefore, NPs are stabilized using polymers or surfactants [31]. FTIR analysis was performed to determine the surface properties and determination of functional groups on the surface of ZnO-NPs.

All previous studies have shown that ZnO-NPs have a crystalline nature. For example, in 2016, Colak and colleagues synthesized ZnO-NPs using lemon peel extract. The XRD analysis results showed that these NPs are crystalline and have a crystalline size of 45 nm [32]. Furthermore, in 2014, Ramesh and colleagues were able to synthesize ZnO-NPs using the *Solanum nigrum* extract. These NPs had a crystalline nature, and the crystalline size of these NPs was 29.99 nm [33]. In this study, NPs synthesized by biological method had a crystalline nature.

The XRD has nine peaks of diffraction or refraction at angle 2θ. Indeed, diffraction peaks of 31.74, 34.42, 36.24, 47.54, 56.64, 62.9, 66.52, 67/88, and 69/06 degrees can be attributed to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) Miller's indexes, respectively, which reflected the hexagonal structure of metallic ZnO [34] (Figure 7).

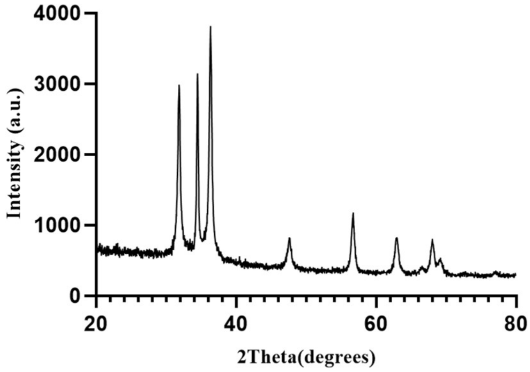

Figure 7. The XRD spectra of ZnO-NPs synthesized by biological method.

4. Conclusions

There are three general ways to make NPs: chemical, physical, and biological. One example of green synthesis is the production of NPs through biological means. There are no hazardous, toxic, or expensive chemical agents used in green NP synthesis. The synthesis of ZnO-NPs by plants is currently receiving a lot of attention. The biological method was used to synthesize ZnO-NPs in this study. As a salt source, zinc sulfate salt was used, and extracts of the green husk of *J. regia* were used to produce and stabilize the NPs. The crystalline structure of the synthesized NPs was also revealed by the XRD analysis. The biosynthesized ZnO-NPs that were analyzed using UV-Vis spectroscopy had a significant SPR peak at 360 nm. The synthesized NPs were spherical and oval, as shown by the TEM analysis results.

Author Contributions: M.D. performed the study, analyzed the data and wrote the original draft. P.G. conceived, proposed and designed the study and also as corresponding author, sponsored the study, and made all the arrangements. All authors reviewed and edited the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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