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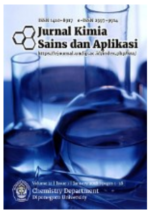

Synthesis and Characterization of Zirconium Dioxide Nanoparticles in Various Liquid Media by Nd:YAG Pulse Laser Ablation and Its Antibacterial Application

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Abstract

The issue of antibiotic resistance by bacteria has been studied to develop a new agent to inhibit bacterial activity. Recent studies have reported on nanoparticles promising antibacterial properties. Zirconium dioxide nanoparticles (ZrO_2 NPs) have emerged as potential antibacterial agents for gram-negative bacteria. Nevertheless, there remains a gap in research done on producing stable nanoparticles. Additionally, it studies the impact of the liquid environment in the synthesis to keep a small size. In this present work, ZrO_2 NPs have been successfully synthesized in various liquids by pulse laser ablation using the Nd:YAG laser. The laser was ablated on the surface of a zirconium metal plate in different liquid media, such as deionized water, ethylene diamine, and chitosan solution. Furthermore, the liquid media used has an effect on the characteristics of ZrO_2 NPs and their antibacterial properties. An investigation of scanning electron microscope images reveals that ZrO_2 NPs in deionized water, ethylene diamine, and chitosan solutions have a spherical morphology with diameters measuring around 24.33 nm, 19.76 nm, and 15.05 nm, respectively. The antibacterial effect of ZrO_2 NPs in chitosan solution against *E. coli* bacteria is assessed by measuring the diameter of the inhibition zone (DIZ), which has greater colloidal stability than the other liquid media. The findings indicate that the stability and small size of nanoparticles enhance the ability to inhibit the growth of bacteria.

1. Introduction

Antibiotic resistance represents one of the most pressing public health challenges. Initially, antibiotics were hailed as antibacterial drugs suitable for treating bacterial infections. However, the emergence and proliferation of antibiotic-resistant bacteria undermine the efficacy of these critical therapeutic agents [1]. Antibiotic resistance is the evolutionary development of bacterial mechanisms that enable them to survive the effects of previously successful medications in preventing their growth or causing their death. To solve this situation, efforts need to include the responsible handling of current antibiotics and the development of new pharmaceuticals [2, 3]. In recent years, metal and metal oxide nanoparticles have become candidates as antibacterial agents [4].

Nanoparticles are defined as small particles ranging from 1 to 100 nm. Nano-sized particles have a high surface-to-volume ratio for increased interaction with bacterial cells [5]. Several experiments have demonstrated the use of various metals and metal oxide nanoparticles in antibacterial applications, including copper oxide and tin oxide nanoparticles [6], silver nanoparticles [7], and zinc oxide nanoparticles [8]. Zirconium dioxide (ZrO_2) nanoparticles are a prominent class of inorganic nanoparticles with a wide range of applications due to their unique physicochemical properties. ZrO_2 nanoparticles have biocompatibility, mechanical strength, and corrosion resistance [9, 10]. Various methods have produced nanoparticles. Horti *et al.* [11] used the hydrothermal technique to produce ZrO_2 nanoparticles. Naik *et al.* [12] synthesized ZrO_2

nanoparticles using the sol-gel method. They demonstrated that ZrO_2 nanoparticles have antibacterial properties.

The most generally used bottom-up approach for synthesizing nanoparticles, such as hydrothermal and sol-gel, requires special preparation and complicated procedures [13]. Pulse laser ablation in liquid (PLAL) is offered as a top-down approach for synthesizing metal nanoparticles. PLAL utilizes the energy source from a laser to ablate a portion of the material and induce a luminous plasma. The plasma is condensed in the liquid medium, resulting in the dispersion of colloidal nanoparticles [14]. The advantages of synthesis using PLAL are simple preparation, high purity, and the ability to control the size of nanoparticles through liquid media and laser parameters, such as wavelength, laser energy, focal length, repetition rate, and ablation time [15, 16]. The choice of ablation media influences nanoparticle morphology, size, stability, and concentration [17, 18].

Gondal *et al.* [19] have synthesized ZrO_2 nanoparticles by neodymium yttrium aluminum garnet (Nd:YAG) pulse laser ablation in deionized water, ethanol, and acetone. The study resulted in different characteristics of ZrO_2 nanoparticles in each ablation medium. ZrO_2 nanoparticles in acetone showed a smaller distribution than in ethanol and deionized water [19]. However, acetone and ethanol have harmful effects on health. Contact with acetone can cause skin irritation, respiratory tract disorders, and eye damage [20]. Therefore, we need other media like polymers and organic compounds, which are more environmentally friendly and safe for health.

In this study, we address the existing gap in research regarding the synthesis of stable ZrO_2 nanoparticles for antibacterial applications. To achieve this, we employed chitosan and ethylenediamine as organic surfactants in the Nd:YAG pulse laser ablation process to produce ZrO_2 nanoparticles. Chitosan, a natural polymer, is known for its biocompatibility, non-toxicity, and biodegradability [21], while ethylenediamine, a colorless organic compound, is fully miscible with water, numerous organic liquids, and various polar solvents [22]. Both chitosan and ethylenediamine function as stabilizing, reducing, shape-directing, and sizing agents in the nanoparticle synthesis process [22, 23]. Additionally, this work investigates the antibacterial efficacy of the synthesized ZrO_2 nanoparticles against *E. coli* bacteria.

2. Experimental

2.1. Preparation of Zirconium Metal

The zirconium plate (99.99%, Nilaco Inc., Tokyo, Japan) was cut to 1.5 × 1.5 cm dimensions. The target was cleansed with 70% alcohol to eliminate impurities attached to the plate and then washed with distilled water to completely remove any residual alcohol. The glass beaker was also cleansed with 70% alcohol and washed with distilled water. After the washing procedure, the material was placed into a beaker containing 10 mL of deionized water (DIW), ethylenediamine (EDA), and chitosan solution (CS), respectively.

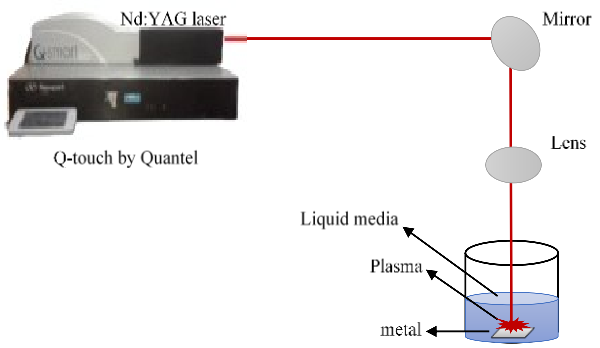

Figure 1. Schematic of the experimental process for synthesizing ZrO2 NPs using the PLAL method

2.2. Synthesis of ZrO_2 Nanoparticles

Zirconium dioxide nanoparticles (ZrO_2 NPs) were produced by Nd:YAG pulse laser ablation technique, in which a laser with a wavelength of 1064 nm was used. The Nd:YAG laser (Litron Series, Nano S 130-10) at 1064 nm is favored in nanoparticle synthesis due to its high efficiency, stability, and versatility with various target materials, making it popular in nanoparticle synthesis applications. The laser energy was set to 80 mJ, with a focal length of 10 cm, a pulse width of 7 ns, and a repetition rate of 10 Hz. The target surface was ablated by the laser for 3 hours in various liquid media. During the synthesis, the laser beam was redirected by the Nd:YAG mirror toward a convex lens, which focused it onto the target within the liquid media. This laser ablation on the metal surface generated plasma, resulting in the formation of nanoparticles that dispersed throughout the liquid media. Figure 1 shows the experimental setup utilized for the production of ZrO_2 NPs.

2.3. Characterizations

The optical characteristics of ZrO_2 NPs were evaluated using ultraviolet-visible (UV-Vis, Shimadzu UVmini-1240) spectroscopy and Fourier transform infrared (FTIR, Perkin Elmer/Spotlight 400 Frontier). The UV-Vis and FTIR were used to determine the absorbance spectrum and were also obtained to identify functional groups in ZrO_2 NPs. The morphology and size distribution of nanoparticles were obtained by scanning electron microscopy (SEM, JSM-6510LA). Furthermore, the successful outcome of producing ZrO_2 NPs can be verified by X-ray diffraction (XRD, Rigaku Miniplex 600). The characterization data obtained were analyzed using Origin and ImageJ software.

2.4. Testing of ZrO_2 Nanoparticles as Antibacterial

The disc diffusion method is a technique used to analyze antibacterial activity by utilizing disc paper as a medium to absorb antibacterial compounds. Initially, it is crucial to ascertain the quantity of bacteria present in the media. The bacterial suspension was diluted until it reached a viscosity level or optical density of 0.5 McFarland. The 0.5 McFarland standard represents a bacterial concentration of 1.5×10^8 bacteria per milliliter of solution. The paper discs were positioned on agar media previously coated with a microbial culture. After that, it was incubated at a temperature of 37°C for 24 hours [24, 25]. The microorganisms examined in this investigation were *E. coli*, a strain of gram-negative bacteria. The presence or lack of bacterial growth was identified by viewing the transparent area surrounding the bacteria media.

3. Results and Discussion

3.1. UV-Vis Analysis

The optical properties of produced ZrO_2 NPs are determined by UV-Vis spectroscopy, as shown in Figure 2. The absorbance spectra are obtained from ZrO_2 NPs in deionized water (ZrO_2NPs-DIW), ethylenediamine (ZrO_2NPs-EDA), and chitosan solution (ZrO_2NPs-CS). with the same ablation time. The absorption spectra of ZrO_2 NPs in DIW are significantly lower than those in EDA and CS. Based on Lambert's Law, the concentration of the sample solution is directly proportional to its absorbance. A sharp absorbance peak appeared at 212 nm for ZrO_2NPs-DIW, 210 nm for ZrO_2NPs-EDA, and 207 nm for ZrO_2NPs-CS. Mehta *et al.* [26] confirmed this result, indicating that Mie's theory predicts a shift in peak position at a lower wavelength, which corresponds to a decrease in the size of nanoparticles.

3.2. FTIR Analysis

FTIR spectroscopy was used to classify the functional compounds of ZrO_2 NPs. Figure 3 presents the transmittance spectra of ZrO_2 NPs obtained in various liquid media. The primary absorption peak with the broadest width appears at 3435 cm^{-1}, indicating the presence of O–H groups [27]. The C=C bond is observed at around 1634 cm^{-1} across all media. In EDA and CS media, additional peaks appear at 1073.06 cm^{-1} and 1387.86 cm^{-1}, corresponding to the C–N vibrations of phenolic and alcohol groups. Furthermore, ZrO_2 NPs in EDA exhibit distinct peaks at 2091.15 cm^{-1} and 1484.74 cm^{-1}, associated with the stretching vibrations of N=C=N and N–H, respectively [28, 29]. Absorption peaks at 640.97 cm^{-1}, 655.33 cm^{-1}, and 659.35 cm^{-1} are observed for each ZrO_2 NP in different media, representing Zr–O bond vibrations. Andiyappan and Ramalingan confirmed the characteristics of Zr–O in the range of 472–779 cm^{-1} [30].

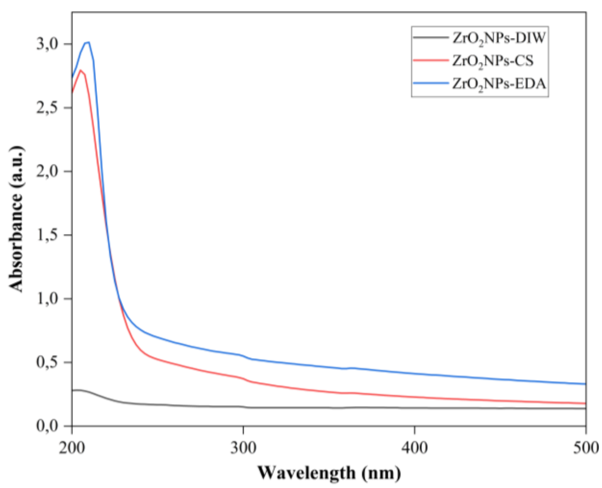

Figure 2. The absorption spectrum of ZrO_2 NPs in various liquid media

3.3. SEM Analysis

The morphology and size distribution of ZrO_2 NPs were characterized using SEM, with the resulting images shown in Figure 4 for colloidal ZrO_2 NPs in various liquid media. As illustrated in Figures 4(a), (b), and (c), ZrO_2 NPs in DIW, EDA, and CS exhibit a consistent spherical shape. However, the size distribution varies by medium. Based on SEM images, the size distributions of 100 ZrO_2 NPs were analyzed using ImageJ software, with the results presented as histograms in Figure 4 (right). The average diameters measured were 25.5 nm for DIW, 19.9 nm for EDA, and 12.9 nm for CS. Notably, ZrO_2 NPs in CS medium are smaller and more uniform than those in EDA and DIW. In Figures 4(a) and (b), SEM images of ZrO_2 NPs in DIW and EDA appear agglomerated and are clearly visible, whereas the ZrO_2 NPs in CS are smaller, more uniform, and less distinct.

The polymer functions as a surfactant, reducing surface strain in the nanoparticles, which enhances nanoparticle dispersion and minimizes the likelihood of particle interactions [23]. In contrast, ZrO_2 NPs produced in DIW and EDA tend to form clusters. This clustering is due to Van der Waals forces and Brownian motion, causing particles to collide and adhere to one another, eventually leading to agglomeration [31].

3.4. XRD Analysis

The crystal structure characteristics of ZrO_2 NPs were analyzed using XRD. Figure 5 displays the diffraction patterns of ZrO_2 NPs synthesized in DIW, EDA, and CS. Prominent peaks were observed at angles of 27.84°, 29.81°, and 31.18°, corresponding to the (111), (101), and (-111) crystal planes, respectively. These peaks align closely with reference data provided by JCPDS under data files No. 01-072-0597 and No. 00-024-1164, confirming the coexistence of monoclinic and tetragonal phases in the ZrO_2 NPs. This result supports the findings of Prasad *et al.* [32], who reported peaks at 29° and 31° for the monoclinic phase, while the peak at 30° signifies the tetragonal phase.

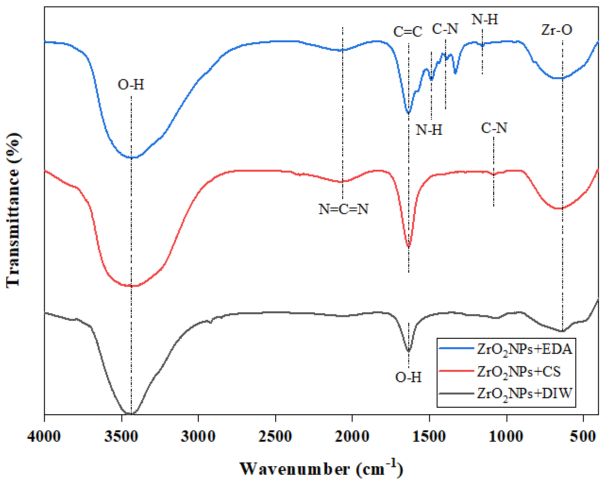

Figure 3. FTIR spectra of ZrO_2 NPs in various liquid media

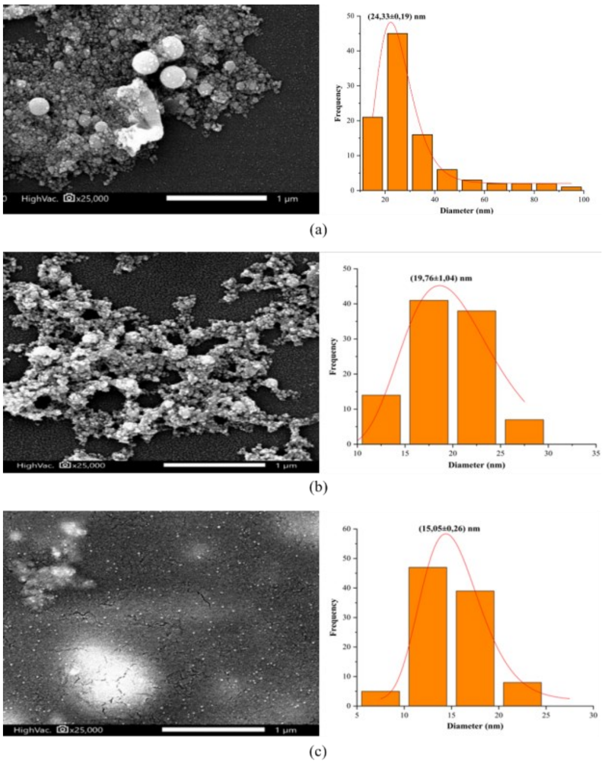

Figure 4. Morphology and size distribution of ZrO_2 NPs in (a) DIW, (b) EDA, and (c) CS

3.5. Antibacterial Activity

The morphology of bacterial cell membranes changes upon exposure to nanoparticles. Generally, nanoparticles can penetrate bacteria due to their unique morphology, including their spherical shape and small size. The ability of nanoparticles to diffuse through bacterial membranes is directly related to their size; smaller nanoparticles have a greater capacity to penetrate and disrupt bacterial membranes [16]. Additionally, antibacterial properties are influenced by the type of bacteria, classified as gram-negative or gram-positive. In gram-negative bacteria, such as *E. coli*, a lipopolysaccharide and peptidoglycan layer covers the cells, affecting the interaction between the bacterial cell wall and nanoparticles or their released ions [33]. Tabassum *et al.* [34] reported that gram-negative bacteria exhibit high susceptibility to antibacterial activity due to the electrostatic attraction between their negatively charged cell walls and the positively charged Zr^+ ions, ultimately leading to cell wall rupture and bacterial death.

This study aimed to evaluate the effectiveness of ZrO_2 NPs in inhibiting antibacterial activity. Table 1 presents the diameter of the inhibition zone (DIZ) for ZrO_2 NPs in DIW, EDA, and CS, along with positive and negative controls for *E. coli* activity. The results indicate that ZrO_2 NPs inhibited *E. coli* growth, as evidenced by DIZ values. The positive control effectively inhibited *E. coli* growth with a DIZ of 27.10 mm. The DIZ values for ZrO_2 NPs suspended in DIW, EDA, and CS were 6.40 mm, 7.15 mm, and 8.10 mm, respectively. The CS medium yielded the largest DIZ, likely due to the smaller nanoparticle size and the presence of positively charged molecules, which interact with gram-negative bacterial cell membranes and tend to damage them [35, 36].

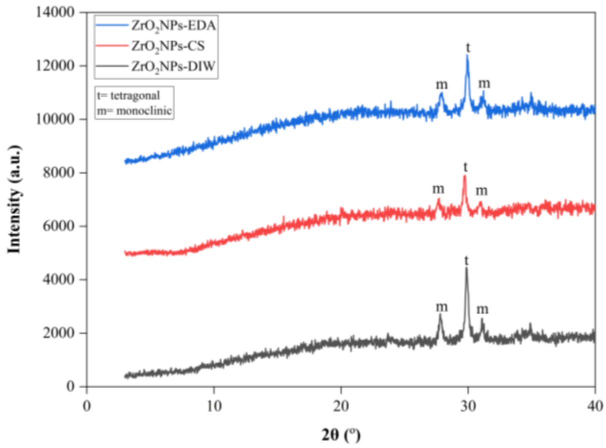

Figure 5. XRD patterns of ZrO_2 NPs in various liquid media

Table 1. Diameter of inhibition zone for the activity of ZrO_2 NPs against *E. coli* bacteria

Sample	DIZ (mm)
ZrO_2NPs-DIW	6.40
ZrO_2NPs-EDA	7.15
ZrO_2NPs-CS	8.10
Positive control	27.10
Negative control	0

4. Conclusion

The pulsed laser ablation method has successfully facilitated the synthesis of nanoparticles in various liquid media, influencing both the physical and antibacterial properties of ZrO_2 nanoparticles. Among these, the ZrO_2 nanoparticles synthesized in chitosan solution exhibited the greatest stability, maintaining their condition for over two weeks, compared to those synthesized in ethylenediamine and deionized water. Additionally, SEM images revealed that the ZrO_2 nanoparticles in chitosan maintained a spherical shape with minimal agglomeration, resulting in the smallest average size of 15.05 nm. These results suggest that the enhanced stability and smaller size of ZrO_2 nanoparticles contribute to their ability to inhibit *E. coli* bacteria growth.

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