Research Article

Antibacterial Activity of Bismuth Oxide Nanoparticles Compared to Amikacin against Acinetobacter baumannii and Staphylococcus aureus


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In this work, bismuth oxide nanoparticles (Bi₂O₃ NP) were prepared by laser ablation of a solid target in distilled water. The optical and structural properties of Bi₂O₃ NPs were studied using UV–VIS spectroscopy, energy-dispersive X-ray spectroscopy (EDS), transmission electron microscope (TEM), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR). The results showed that the average particle size is less than 20 nm. The antibacterial activity of the Bi₂O₃ NPs was determined and compared with amikacin against Acinetobacter baumannii and Staphylococcus aureus. The activity of Bi₂O₃ NPs was higher than the amikacin. Furthermore, the antibacterial activity of Bi₂O₃ NPs against S. aureus bacteria was higher than Acinetobacter baumannii; this result was confirmed by studying the effect of Bi₂O₃ NPs in bacterial biofilm formation in eyes contact lenses using atomic force microscopy (AFM) technique. Taken together, Bi₂O₃ NPs could be used as a preservative for contact lenses as eye drops to prevent the formation of microbial biofilms in contact lenses.

1. Introduction

Antibiotics are the primary foundation for the treatment of bacterial diseases. The medical community was convinced that the discovery of these antibiotics and their usage as chemotherapeutic agents would lead to the eradication of infectious diseases in the future. Antibiotic misuse, on the other hand, has become a major role in the development and spread of multidrug-resistant strains of various bacteria [1]. Microorganism keratitis, which is commonly associated with contact lenses, is a significant risk factor for the progression of microbial keratitis [2].

Many patients have bad clinical results unless aggressive and adequate treatments are initiated quickly [3]. Even if effectively handled, microbial keratitis may lead to corneal scarring and neovascularization, leading to a loss of core corneal clarity, requiring a corneal transplant to restore sight. Successful leadership can lead to a continuous loss of visual function and possibly even eye loss [4]. This situation forced researchers to look for new antimicrobial and anti-
inflammatory substances from different sources, such as Bi$_2$O$_3$ NPs [5–7]. The growth of drug resistance, allergic reactions, and side effects has resulted in exploring alternative drugs for nosocomial infection treatment [8, 9]. Different researches have confirmed that the transitions in Bi$_2$O$_3$ are of the direct permissible type, and the width of the optical energy gap ranges between (2.80-2.95 eV). Bi$_2$O$_3$ is a crucial derivative in the fabrication of glass and ceramics, as also hydrocarbon oxidation catalysts. Sensors, microelectronics, and optical engineering are all frequent applications [7, 8]. Thus, it has a broad range of applications and may be used as a surface disinfectant in hospitals and the food, pharmaceutical, glass, and ceramic manufacturing industries [9].

The pulsed laser ablation technique is a simple, novel, nonpolluting, eco-friendly, low-cost, one-step, and quick method for producing pure metal nanoparticles with regular and minimum buildup [10–13]. The aim of this study was to synthesize Bi$_2$O$_3$ NPs by laser ablation technique and study their effect as a preservative of contact lenses against eye infections. The results showed the ability of Bi$_2$O$_3$ NPs as an antibacterial agent to prevent bacterial biofilm formation.

2. Materials and Methods

A pure plate of bismuth (Sigma Aldrich), contact lenses (Alcon-china), and DMSO was purchased from Sigma.

2.1. Preparation of Bi$_2$O$_3$ NPs. The Bi$_2$O$_3$-NPs were prepared via laser ablation of solids submerged in deionized distilled water [12, 14] as shown in the experiment illustrated in Figure 1. Then, the NPs were passed through a (0.22) m size filter for size uniformity by applying an Nd: YAG laser with a fundamental wavelength of (1064) nm., the target was irradiated for (15) min., using (150) pulses, a pulse length of (6) ns., a repetition rate of (2) Hz, spot size (10) mm, the focal length of the lens (8) cm, and the maximum output energy of (500) mJ.

2.2. Characterizations of Bi$_2$O$_3$ NPs. The formation of Bi$_2$O$_3$ NPs was achieved by measuring the solution’s absorption spectrum with a UV-vis (UV/Vis, Seoul, Korea) in the 200-800 nm range [12, 15]. The microstructure and particle size of the nanocrystals were determined from (SEM) images (INSPECTS50-USA) and analyzed using Image J software [16]. FTIR was measured on the functional groups present in the nanoparticles and size distribution [18]. AFM was used to determine the beneficial groups and position of each atom on a three-dimensional model of the surface topography of a substance at the atomic level (CSPM-AA3000) [19]. TEM tests were done by (Inspect $50/FEI$ Company/Netherlands). These assays were achieved at the University of Tehran.

2.3. Evaluation of MIC of Amikacin and Bi2O3 NPs. The microdilution assay determined the minimal inhibitory concentration (MIC) of amikacin and bismuth oxide NPs [20, 21].

2.4. Antibacterial Activity. The well diffusion technique was utilized to identify the antibacterial effects of amikacin and bismuth oxide NPs against Staphylococcus aureus and Acinetobacter baumannii isolates at the concentration for sub-MIC (32) μg/ml according to [22, 23].

2.5. Fluorescence-Activated Cell Sorting Assay. Bacterial strains were colored by applying the LIVE/DEAD BacLight Bacterial Viability kit corresponding to the production command (Invitrogen AG, Basel, Switzerland). The dilution of SYTO9 was (1 : 60) allowing for prior titrations to get to the best dilution of pigments to be utilized for stain, propidium iodide (PI) (equipment instructions). The samples remained kept for (30) min. at (4°C) and was analyzed by applying a flow cytometer assay (FCA). One unit cell was confined on the up-scatter (FSC-A) versus up-scatter height (FSC-H) plots. The cell remains were rejected. Consequently, 20000 incidents were established in the entry, particularly for single incidents. Unblemished and particular-color controllers were utilized to set a door for PI (+ve cells). FCA was achieved on a BD Verse method, Biosciences, U.S.A., and statistics were examined by applying FACS DIVA computer software (BD), San Jose, CA, U.S.A.

2.6. Effect of Sub-MIC of Amikacin and Bi$_2$O$_3$ NPs in Biofilm Formation in Contact Lenses Visualized by AFM. This assay was performed according to previous work, with a few changes to produce biofilm on contact lenses. Briefly, lenses were put in (10) ml of nutrient broth containing bacterial cell growth in a concentration of $1.5 \times 10^{6}$ cfu/ml, which exhibited the highest reduction in biofilm formation.
capacity after treatment with NPs, and amikacin at sub-MIC was chosen for this experiment. For 24 hours, containers were incubated aerobically at (37°C). Decantation was used to remove the media and planktonic cells, followed by two washes with distilled water (DW). The adhering cells were allowed to dry for 30 minutes after being washed twice by DW. The lenses were then stained and prepared for examination under an atomic force microscope. Control positive was created by cultivating lenses with a bacterial culture free of NPs. At the same time, the control negative was prepared by cultivating lenses in nutrient broth without any treatment or bacteria.

2.7. Statistical Analysis. At a significance level of P 0.05, an unpaired t-test was used to evaluate the significance level in the observed values across the treatment groups [24].

3. Results and Discussion

3.1. Characteristics of Bi₂O₃ NPs. By decreasing bismuth ions in the solution, UV-visible spectroscopy verified the existence of nanoparticles (Figure 2). The Bi₂O₃ NPs were put in a quartz cuvette and monitored for wavelength scanning between (200 and 800) nm with distilled water as a reference. The absorption peak, which is typical of bismuth, was found at 245 nm.

SEM was used to examine the morphology of the produced Bi₂O₃ NPs, as shown in Figure 3. The size distribution of the Bi₂O₃ NPs sample was evaluated using Image J software, and most of the particles were in the 22-24-30 nm range.

The infrared spectrum of the Bi₂O₃ NPs is shown in Figure 4. Four peaks appeared, with wavenumbers of 624.87 and 3455.67 cm⁻¹, which are attributed to variation group vibrations of -C=C-, C=O, S-H, and N-H groups. A broad peak at 624.87 cm⁻¹ is associated with an alkyne group (−C=C−), the peak at 1637.06 cm⁻¹ is associated with a carboxylic acid group, the peak at 2084.05 cm⁻¹ is related with the thiol group, and the peaks at 3455.67 cm⁻¹ are associated with amine groups.

TEM was used to evaluate the size distribution and morphology of nanoparticles. Figure 5(a) explains the TEM images of Bi₂O₃ nanoparticles are spherical-like shape, while Figure 5(b) shows histogram size distribution, most of the particles are in the 5–40 nm, and this was comparable with the SEM result. Figure 5(c) shows energy dispersive spectrum (EDS) explains the chemical composition of the as-synthesized Bi₂O₃ NPs samples. The NPs are mainly composed of Bi and O atoms. The ratio of weight (%) of Bi and O in the as-prepared final NPs sample is 89.8 and 10.2, respectively.

The AFM images for the nanostructures deposited utilizing 150 pulses are shown in Figure 6. The topography of the surface of Bi₂O₃ NPs particles was studied, and measurements of surface roughness, particle size nanoparticles rate, and particle size distribution were taken. The average surface roughness is 1.454 nm, with diameters ranging from 5 to 40 nm and a quasispherical shape. These findings are in good agreement with the TEM measurements.

3.2. Evaluation of MIC of Amikacin and Bi₂O₃ NPs. The MIC is used to identify which antibiotic class is the most
Figure 4: Infrared spectrum of Bi$_2$O$_3$ nanoparticles.

Figure 5: TEM image of (a) Bi$_2$O$_3$NPs, (b) size distribution, and (c) EDS spectrum of nanoparticles.
effective. The MICs of amikacin and Bi$_2$O$_3$ NPs for *S. aureus* and *A. baumannii* isolate examined varied from 64 μg/ml in *S. aureus* isolate, in *A. baumannii* isolate, and amikacin MICs 16 μg/ml. For *S. aureus*, the MIC of Bi$_2$O$_3$ NPs is 128 μg/ml (Table 1). Bi$_2$O$_3$ NPs had MICs 64 μg/ml in *A. baumannii* isolate.

### 3.3. Antibiofilm Activity of Amikacin and Bi$_2$O$_3$ NPs

The well diffusion technique occurred used to investigate the synergistic impact of Bi$_2$O$_3$ NPs with antibiotics (Amikacin) at a concentration of 32 μg/ml. The synergistic impact of Bi$_2$O$_3$ NPs against *S. aureus* is shown in Figure 7. The inhibitory zones were (16) mm, followed by (13) mm for antibiotics alone and (23) mm for Bi$_2$O$_3$ NPs. In addition, Bi$_2$O$_3$ NPs with amikacin have a synergistic impact against *A. baumannii*. As seen in Figure 7, the zone of inhibition was (12) mm, followed by antibiotics alone (15) mm and Bi$_2$O$_3$ NPs (20) mm. The interaction between antibiotics and nanoparticles is most likely to blame for this synergistic impact [25]. For example, silver nanoparticles and amoxicillin were
shown to have the most significant synergistic impact in suppressing *E. coli* growth [26]. This showed that Bi$_2$O$_3$NPs might significantly enhance amikacin invasion and absorption into the cell membrane.

Furthermore, since NPs need a wide surface area for interactions, they have a higher bactericidal effect than bigger particles, increasing bacterial cytotoxicity. For the identical treatments, the inhibiting area in G$^+$ve bacteria is larger as compared to G$^-ve$ bacteria. G$^+$ve bacteria have a different cell wall structure than G$^-ve$ bacteria, which could explain why G$^+$ve bacteria are more antibiotic-resistant. Only the exterior peptidoglycan layer of G$^+$ve bacteria is present. G$^-ve$ bacteria, on the other hand, have an exterior polysaccharide barrier that keeps all polysaccharide structures intact [27]. Many active compounds, such as hydroxy and amido groups, are found in antibiotic compounds. Chelation is a simple reaction that these groups have with nanoparticles. Through van der Waals contact and other weak interactions, antibiotic molecules can connect. Finally, antimicrobial groups are formed, made up of a nanoparticle core and antibiotic molecules around it. Antimicrobial groups induce more destruction when they act on one location on the surface of bacterial cells [28].

### 3.4. Viability Assay for Bacterial Strains

Flow cytometry of microbial cells was also presented to confirm the bactericidal activity of the prepared nanoparticles. The stains which are applied in this research were SYTO9 and (PI) as DNA-binding pigments, depending on their cell-penetrative property. SYTO9 enters both live and dead cells as various to PI, which can only enter cells whose membrane integrity is conceded, as death/killing cells. The observed FCA is extra receptive compared with conventional plate analysis in the identification of death/killing bacterial cells as the failure in pathogenic cell penetrability is immediately found by PI integration. Unblemished cells as in Figure 8 were run to quantitate autofluorescence and set standard energy. At zero time, the experiments have been run to calculate the early proportion of PI (+ve cells). After 6 hr. of treatment with nanoparticles, the possibilities of these bacterial species were examined. Bi$_2$O$_3$NPs determined the maximum cell death ratio as studied by quantification of PI-positive cells as well as the nanoparticles as shown in Figure 8.

Flow cytometry of bacterial cells was also used to confirm the bactericidal activity of the nanostructured surfaces. SYTO9 and propidium iodide (PI), the DNA-binding dyes used in this investigation, have different cell-penetrating properties. SYTO9 can infiltrate both live and dead cells, whereas PI can only infiltrate cells whose membrane integrity has been disrupted, i.e., dead cells. It should be noted that the flow cytometric technique is more sensitive than standard plate assay in the detection of dead bacterial cells as the loss in bacterial cell permeability is readily detected by PI incorporation. Unstained cells (Figure 8) were run to quantitate autofluorescence and set baseline voltage. Samples were taken during the zeroth hour to determine the
initial percentage of PI-positive cells. The viability of these cells was determined after 3 hours of exposure to nanostructured surfaces. Quantification of PI-positive cells among nanostructured surfaces revealed that Bi$_2$O$_3$NPs had the highest cell death percentage (Figure 8).

3.5. Effect of Sub-MIC Amikacin and Bi$_2$O$_3$ NPs on Biofilm Formation Visualized by AFM. AFM also looked at the contact lenses to see how efficient amikacin and Bi$_2$O$_3$ NPs prevented biofilm development. As demonstrated in Figures 9(a)–9(g), Bi$_2$O$_3$ NPs outperformed amikacin in preventing biofilm development on the lenses. The results of an atomic force microscope (AFM) study of biofilm development in lenses with S. aureus bacteria was (29.6 nm) of high lens surface Figure 9(b). The outcome which did not have any bacteria was (2.14 nm) of lens surface in Figure 9(a). Figure 9(c) shows just A. baumannii bacteria or treatment on the high lens surface (21.4 nm). In Figure 9(d), the most significant surface was (9.9 nm) with S. aureus and treated with amikacin antibiotic and with S. aureus and treated with Bi$_2$O$_3$ NPs (3.06 nm) in Figure 9(e). In Figure 9(f), the most significant surface was (12.4 nm) with A. baumannii and treated with Amikacin antibiotic. In comparison, the lowest surface was (8.06 nm) with A. baumannii and treated with Bi$_2$O$_3$ NPs in Figure 9(g). These findings revealed that Bi$_2$O$_3$ NPs were more effective as an antibiofilm agent than amikacin. AFM revealed no S. aureus colonization on any Bi$_2$O$_3$ NP-treated lenses (Figure 9(g)). On the other hand, the untreated contact lenses exhibited colonization of lenses as a multilayer on this lens contact Figure 9(a). AFM was used to measure the adhesion and biofilm stiffness [29]. Surface roughness [30] and topography have all been effective in microbial biofilm research [31]. A few studies have shown that Bi$_2$O$_3$ NPs have strong antibiofilm actions against various pathogens in vitro and have indicated various antibacterial mechanisms for Bi$_2$O$_3$ NPs, including impairing cell division, disturbing the cell wall cytoplasmic membrane, and decomposing DNA [32–34]. By employing AFM image analysis [29], the biofilm formation and surface roughness of P. aeruginosa were investigated by AFM.

The production of harmful ions, shading impact on photosynthetic bacteria, cellular absorption of NPs, physical restraint and cell wall destruction, and oxidative stress are the key processes behind the toxicity of metallic-based nanoparticles, as shown by several biological models. Furthermore, the biological models are used to assess the potential hazards of nanomaterials (cell wall damage, inhibition of cell proliferation, gene expression changes, genotoxicity, and cell death, alteration of function and morphology of organelles, and the presence of oxidative stress bioindicators) [35]. Environmental nanotechnology is defined as the use of biotechnological applications as a low-cost, environmentally friendly, and simple method of producing, manipulating, and utilizing materials with dimensions ranging from 1 to
Contact Lenses (Control Negative)
Roughness average = 2.14 nm

S. aureus in Contact Lenses (Control Positive)
Roughness average = 29.6 nm

A. baumannii in Contact Lenses (Control Positive)
Roughness average = 21.4 nm

S. aureus with Amikacin
Roughness average = 9.9 nm

A. baumannii with Amikacin
Roughness average = 12.4 nm

Figure 9: Continued.
100 nm [36, 37]. Antimicrobial drug resistance in bacterial infection has driven the development of new treatment options. Among these efforts, nanomaterials have emerged as notable and novel antibacterial agents [38]. In this work, the effectiveness of bismuth nanoparticles in inhibiting the growth of A. baumannii and S. aureus was investigated using the broth microdilution method, which is a standard method that is more accurate, reliable, and easy to interpret than other methods such as the well diffusion method. Because of its poor water solubility, the inhibitory action of elemental bismuth can only be noticed at quite high doses. Nonetheless, our findings revealed that Bi$_2$O$_3$ NPs had more efficient antibacterial action against A. baumannii and S. aureus than amikacin, with a MIC of bismuth nanoparticles that was higher than amikacin. Overall, the experimental data suggest that bismuth nanoparticles could be an interesting alternative to combat A. baumannii and S. aureus, which, given the benefits mentioned for bismuth nanoparticles such as inhibiting A. baumannii and S. aureus biofilm formation and higher antibacterial activity compared to amikacin, could be suggested for use in various fields of keratitis, which is commonly associated with contact. Furthermore, additional study is needed into the compatibility of nanoparticles with nature as well as the way of their recovery.

### 4. Conclusions

In this study, Bi$_2$O$_3$ NP was prepared by laser ablation technique. The antibacterial activity of the Bi$_2$O$_3$ NPs was determined and compared with amikacin against A. baumannii and S. aureus. S. aureus and A. baumannii, the predominantly bacterium of eye infection, displayed the different capabilities to create biofilms, Bi$_2$O$_3$ NP concentration of 32 µg/ml had antibacterial activity against S. aureus and A. baumannii. This result was confirmed by studying the effect of Bi$_2$O$_3$ NPs in bacterial biofilm formation in eyes contact lenses using the atomic force microscopy (AFM) technique. Taken together, Bi$_2$O$_3$ NPs could be used as a preservative for contact lenses as eye drops prevent the formation of microbial biofilms in contact lenses. Furthermore, Bi$_2$O$_3$ NPs were more effective as antibiofilm agents than amikacin in vitro by AFM, and the use of Bi$_2$O$_3$ NPs as contact lens preserving instead of chemicals preservatives. Taken together, Bi$_2$O$_3$ NPs could be used as promising therapeutic or preservative material for contact eye lenses to prevent the formation of microbial biofilms.

### Data Availability

All data are presented within the article.

### Conflicts of Interest

The authors report no conflicts of interest.

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