Determination of Antimicrobial Activity of Bi_2O_3 nanospheres Against Multi-Drug Resistant Pathogenic Bacteria

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Abstract:
The main objective of this study was to evaluate the bactericidal activity of Bi_2O_3nanospheres against the antimicrobial resistant isolates included *Staphylococcus aureus, Escherichia coli*and*Klebsiella sp.*Bismuth Oxide Nanospere particles was prepared by chemical reduction technique with (11-30)nm average diameter, the colloidal of Bi_2O_3nanospheres was characterized by using X-ray diffraction (XRD), TransmitionElectron Microscopy (TEM) and UV–Vis spectrophotometry. Three pathogenic strains of multi-drug resistant bacteria of each *Staphylococcus aureus, Escherichia coli*and*Klebsiella sp.*were isolated and identified from different clinical samples usingtraditional methods on bacteriological culture media. The antibacterial susceptibility of (Amoxicillin, Cefotaxime,Gentamicin, and Ciproflouxacin) were analyzed on Muller Hinton agar plate and the activity of Bi_2O_3nanospheres was assayed using well diffusion method against tested bacteria. The results of antibacterial susceptibility of bacterial isolates exhibited high resistance to the antibiotics that used in the current study. According to these findings thecolloidalof Bi_2O_3nanospheres revealed highest activity when using 100 µg/ml and the mean of inhibition zone was 18-22 mm for each *S. aureus, E. coli*and*Klebsiella sp*. However, the study showed slight effects (meanof inhibition zones ≤ 13 mm) when used50µg/ml of Bi_2O_3nanospheres. In same way, the *E. coli*and*Klebsiellasp* were more susceptible (mean of inhibition zones;15-12mm) for 150µg/mlofBi_2O_3nanospheres, than *S. aureus*. It is concluded that theBi_2O_3nanosphereshave *insitu* effectiveness an displayed a good antibacterial action against the tested bacterial isolates.

Keywords:Bi_2O_3nanospheres, Antimicrobial Activity,well diffusion method, *S. aureus, E.coli*and*Klebsiella sp*.

Introduction:

Escherichia coli, Staphylococcus aureus and *Klebsiella pneumoniae* are considered the most common multi-drug resistant bacteria associated with nosocomial infections [1,2]. After the great success of penicillin in the mid of 19th century, nowadays we are facing with problem of dissemination of pathogenic bacteria that resistant to many common antibiotics. Over the last twenty years, the field of nanotechnology have had a real development and advances [3]. The

field of nanoparticle-based medicine has special attention in medical treatment. In spite of several choices of nanoparticles exhibit medical features like less toxicity and smart therapeutics, this study showed the usefulness of bismuth nanoparticles as an alternative antimicrobial agent [4]. Bi_2O_3 has a broad spectrum activity and can be used as a surface disinfectant in hospitals, food and therapeutics industries also in the manufacture of glass and ceramic products [5,6]. Recently, the Bi_2O_3 nanospheres revealed a good performance in visible-light-driven photocatalysis for Cr (VI) and organic dye removal, for inactivation of Gram-negative and Gram positive bacteria [7]. For that reason this study aimed to determine the antibacterial activity of Bi_2O_3 on Multi-drug resistant bacteria local isolates.

Materials and methods:

Preparation Bi_2O_3nanospheres:

Bi_2O_3nanospheres was prepared by using 0.3g of Bismuth nitrate[Bi(NO_3)3.5H2O] with molecular weight (465.96 g/mol) was dissolved in 20mlof sterile distal water, the bismuth nitrate solution heated under magnetic stirring at 80^{o}C for 15min. 0.8ml of aqueous solution of KOH (0.025M) was added to the bismuth nitrate solution and the Bi_2O_3nanospheres was formed and isolated by using centrifugation with 4000rpm for 20min. The yellow powder of Bi_2O_3nanospheres was washed more than one time and dried at 120^{o}C for 20min. Finally the powder was re-suspended in distal water and sonicated for 2hrs to overcome the aggregation of the powder, the colloidal of Bi2O3 nanospheres ready to use in the measurement of antimicrobial activity. The characteristics of Bi_2O_3 was studied by XRD, AFM, TEM and UV-Vis spectrophotometer. As well as, the transmission electron (TEM) micrographs obtained using a JEOL 1200-EXII (JEOL Ltd, Tokyo, Japan) of 40–120 KV were used to determined the sphere-shaped morphology of Bi_2O_3 NPs. However, onto 200 mesh copper grids coated with a carbon/ collodion layer a single drop of the nanoparticle suspension were deposited. The Bi_2O_3 monoclinic phase identification was obtained from the X-ray diffraction pattern (PDF 050-1088 ICCD card),using (CuKα) radiation XRD-6000,Shimadzu X-ray diffractometer (Japan), 1.5406 Å (30 kV, 10 mA). The morphological properties was studied by using Angstrom AA 3000 atomic force microscopy andthe absorption spectra of synthesized Bi_2O_3nanospherewas studied by Cary 100 Conc plus UV–Vis spectrophotometer (Japan).

Isolation and Identification of Bacterial Isolates

From different clinical samples; *S. aureus*, *E. coli* and *Klebsiella sp.* were isolated and identified using MacConkey agar and manitol salt agar (Difco, USA). Cultures were incubated at 37^{o}C for 18-24 hrs. The methods of bacterial identification were used according to the Bergeys Manual [8] .

Determination of Antibiotic Susceptibility

Muller-Hinton agar medium (Oxoid, UK) was used to assay the susceptibility of *S. aureus* and *E. coli* and *Klebsiella sp.* using disk diffusion method. The disks of gentamicin (10µg), cefotaxime (30µg) ,Ciproflouxacin (5µg) and Amoxicillin(25µg) (Himedia, India) were applied as standard antibiotic. MacFarland tube number 0.5 was used as standardforinoculums preparationwhich approximately contain 1×10^8 CFU/ml. After overnight incubation at 37°C according to Kirby-Baur [9]. Zones of inhibition were measured and interpreted as the National Committee for Clinical Laboratories Standard guidelines. In this study, *Escherichia coli* (ATCC 25922) used as the reference strain for antimicrobial susceptibility testing [10].

Antimicrobial Activity of Bi_2O_3 nanospheres

The activity of Bi_2O_3 nanospheres against isolates of *S. aureus*, *E. coli* and *Klebsiella sp*. were assayed using an agar-well diffusion method [11]. Bacterial suspension were prepared in sterile water and diluted to 1×10^8CFU/mL . The inoculum was spread by sterile cotton swab onto the surface of Mueller Hinton agar, after 15 min, holes with (8mm) were punched into the cultured agar plates. To avoid leakage of nanomaterials outside, the bottom of holes were sealed with one drop of molten agar [12]. Using a micropipette, an equal volume 100µl of (150,100,50 µg/mlof Bi_2O_3nanospheres) poured separately on to wells. Plates were left overnight incubation at 37°C, inhibition zones were measured for evaluating the antibacterial activity. Tetracycline used as a positive control.

Results and Discussion:

Physicalproperties of Bi_2O_3nanospheres

The structural and morphological properties of prepared Bi_2O_3nanospheres was characterized by using XRD and TEM technique as illustrated in figure (1), which exhibits that all diffraction peaks are well indexed to Bi_2O_3 according to (PDF 050-1088 ICCD card), indicative to high purity of Bi_2O_3, the TEM image further reveals that particles have spherical shape and their diameter is in the range of 11-30 nm. By the 2-D and 3-D AFM image of Bi_2O_3 Figure 2, shows a film with distinct surface morphology. The average surface roughness and root mean square roughness values were observed at Bi_2O_3 film 1.84 nm and 2.14 nm, respectively. The absorption spectra of synthesized Bi_2O_3nanospheres are shown in Figure 3. It can be seen that a strong absorption for prepared sample at the wavelength range from 320 to 400 nm. as well as Figure 3 shown that the Bi_2O_3 sample has obvious absorption in the visible region (>400 nm).

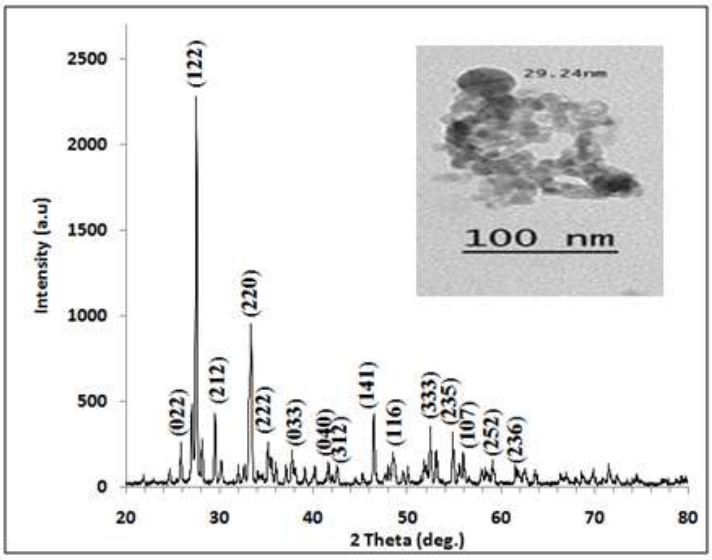

Figure 1: The XRD spectrum of investigated Bi2O3 nanospheres and TEM image showing their morphology.

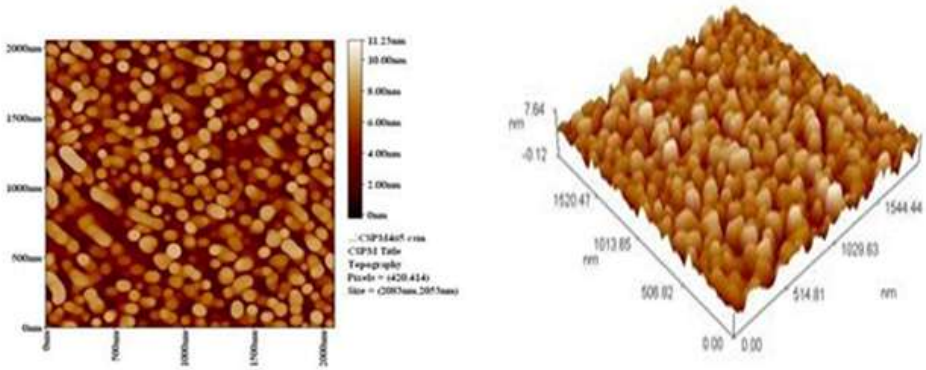

Figure 2: Two-D and three -D AFM image of Bi_2O_3nanospheres

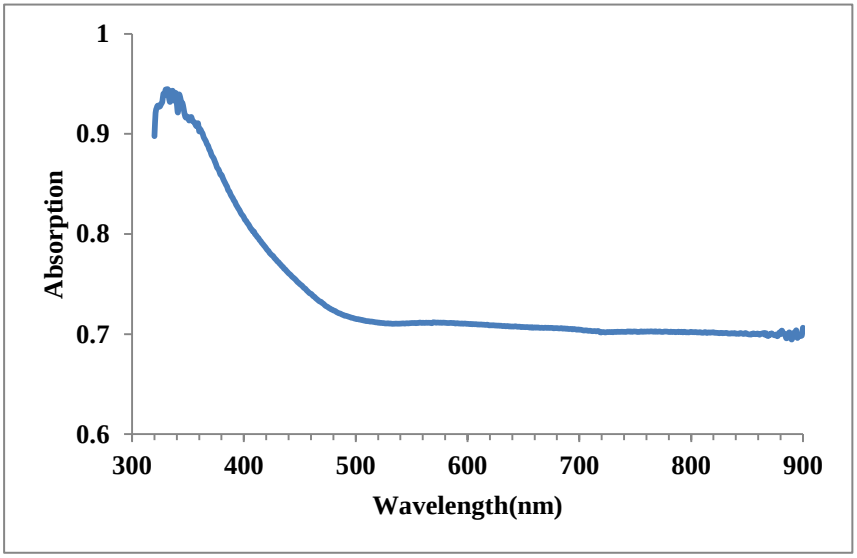

Figure 3: UV–Vis spectrum of suspension Bi_2O_3

Antimicrobial assay of Bi_2O_3nanospheres:

The Bi_2O_3nanospheres revealed a good antibacterial action against all tested isolates (Table 1). This may because that bismuth characterized as diamagnetic crystalline, brittle metal. Moreover, the colloidal chemistry provides s synthetic routes of bismuth nanoparticles with well-controlled size distributions and high crystallinity, that make bismuth nanoparticles had been employed in antimicrobial agents of broad-spectrum [13,14].The greatest antibacterial activity appearedagainst *E.coli*with zone of inhibition reaching to 22 mm. However,thezone of inhibition was 18 mm against *S. aureus*followed by 20 mm for *Klebsiella sp.* when used 100 µg/mlof Bi_2O_3nanospheres, while the Bi_2O_3nanospheres revealed less effect against isolates at 50 and 150 µg/ml, respectively.This result agree with (Abdulkadir*et al* ,2015)[15], in compare with the other antibiotics activity. Also Luo*et al*, 2013[16] mentioned that Bi_2O_3nanospheres have potential in treating of drug-resistant bacteria. Recently, it was published that bismuth nanoparticles cause alteration in nucleotide and amino acid metabolism may inhibited bacterial growth [17]. As well as, the highly reactive oxygen species (ROS), can be formed in the presence of metallic nanoclusters is a potential mechanisms for the antibacterial action may lead to DNA damage in bacteria and cell membranes dysfunction [18,19].

Bacterial Type	Mean of zone diameters (mm) 50µg/ml	Mean of zone diameters (mm) 100µg/ml	Mean of zone diameters (mm) 150µg/ml
Klebsiella sp.	**10**	**20**	**12**
Staphylococcus aureus	**11**	**18**	**13**
E.coli	**13**	**22**	**15**

Table 1: Antibacterial effects of the Bi_2O_3nanospheresusing well diffusion method

Figure 4, shows thediameters of inhibition zone for antibiotics susceptibility against *S. aureus* and *E. coli*and*Klebsiellasp.*Isolatesdetermined by a disk diffusion method. According to the standard of CLSI, all isolates showed high resistant rate (inhibition zone ≤ 5 mm) to most antibiotics that used in this study includedGentamicin, Ceftriaxon and Amoxicillin. However, *E. coli* and *Klebsiellasp.*were susceptible to Ciprofloxacinonly(inhibition zone 29 mm and 30 mm respectively). One of the goals of the current study was to evaluate the antibiotic resistance of clinical isolates. In general bacteria have different ways to be resistant against antimicrobials. They include; resistance to β lactam antibiotics

(Amoxicillin and Ceftriaxone) was mainly caused by production of β-lactamases, which detected among all isolates of current study. Although the β lactamases enzyme play a major role in the resistance to β-lactam antibiotics, also *S. aureus* is conferring resistance to β-lactam drug by reducing the penetration across outer membrane, because low affinity to penicillin binding protein [20,21,22]. Alternatively, ciprofloxacin, some aminoglycosides may showed in vitro activity against the *E. coli* and *Klebsiella sp.* isolates, leaving far behind the β-lactam antibiotics. In both Enterobacteriaceae and other Gram-positive bacteria, gentamicin resistance, may due to the production of a variety of effective enzymes including aminoglycoside modifying enzymes (AME) which can impair the power of antibiotics [23], several fluoroquinolones antibiotics. There was a high activity of fluoroquinolones including ciprofloxacin. Quinolones target is the DNA gyrase in order to inhibit bacterial growth. More recently, researches identified that plasmid-borne resistance genes (qnr) have been improved [24].

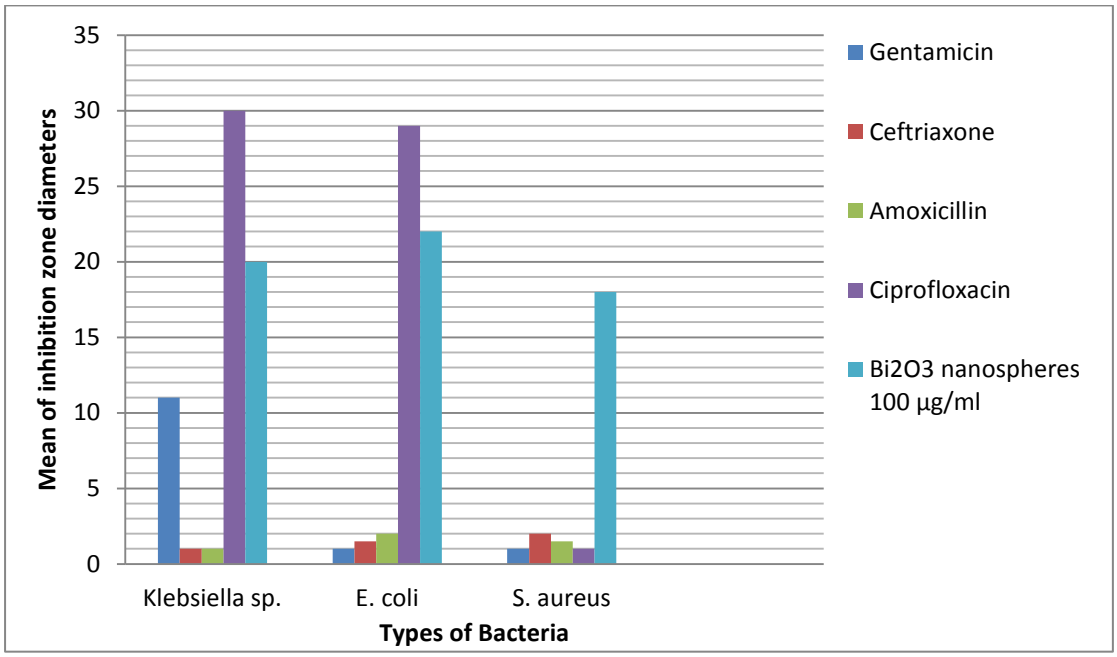

Figure 4: Mean of inhibition zone diameters of 100 µg/ml of Bi_2O_3nanospheresin compared with the antibiotics

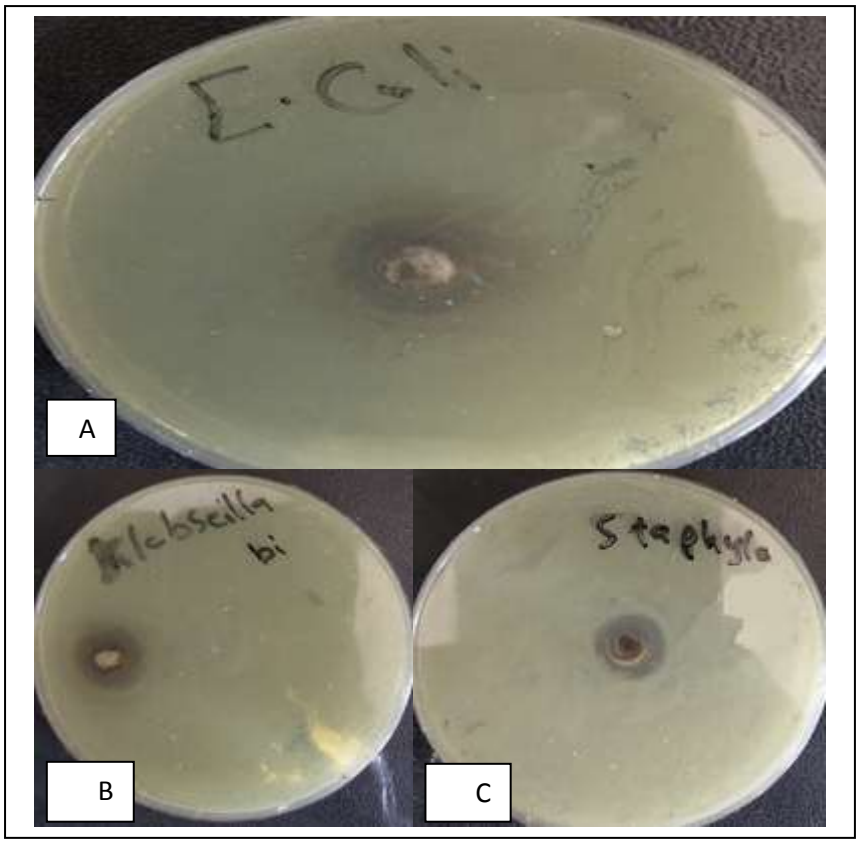

Fig.5 : Inhibition zone of bacterial isolates against (100 mg/mlconcentration) from Bi_2O_3nanospheres, A:*E.coli*,B:*K. pneumonia* and C:*S. aureus*.

It is clear from Figure (4), Figure (5) and Table (1) that the inhibition zone of *Klebsiella sp*. and *E.coli* larger than *S. aureus* and this attributed to a fact of *Klebsiella sp*. and *E.coli* have the outer cell membrane after a thin layer of murine wall, Which is firmly constructed from packed lipopolysaccharide molecules, which provides an effective permeability barrier [25]. Although, the isolates recorded high resistant rate to almost antibiotics (Figure 4), bacteria showed inhibition zone around wells after using Bi_2O_3 nanosphere and this may caused by the change that took place by using some nanoparticles, in its membrane structure that produced an increase in its permeability leading randomly transport through the plasma membrane, with no regulating, resulting into cell death [26]. The opposite charges of on bacterial surface and nanoparticles are attributed to their adhesion and bioactivity due to electrostatic forces. The enhancement of bactericidal effect getting by the large surface area are available during nanoparticles interactions, hence Bi_2O_3 increases the cytotoxicity to the bacteria [27]. As well as, according to the quantum of Bi_2O_3 nanosphere confirmed that high surface-to-volume ratios, so the nanoscale size allow more active to interact with biological systems [28].

Conclusion:

This study conclude that Bi_2O_3 with concentration 100µg/ml have an excellent activity and potential effect in reducing pathogenic bacterial growth in practical applications.

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