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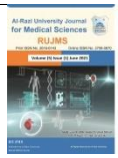

Influence of Zn^{2+} Ions Doping on the Antibacterial Activity of Barium-Nickel Ferrite Nanoparticles

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Abstract:

Background: The nanoparticle ferrites (NPFs) materials have recently attracted more attention. Due to their intriguing characteristics and wide variety of medical applications as an anticancer, antifungal, and antibacterial activity. **Aims:** In the current work, Zn doped barium-nickel ferrite with composition $BaNi_{2-x}Zn_xFe_{16}O_{27}$ (x=0.0, 0.4, 0.8, 1.2, 1.6, and 2) was synthesized for antibacterial activity using a ceramic technique. **Methods:** BNZF powder ferrite was characterized by X-ray diffraction. The Debye-Scherer formula has been used to calculate the average crystallite size from 43 to 56 nm. **Results:** The small crystallite size and the higher surface area of nanoparticles ferrite can enhance antimicrobial activity, causing an improvement in surface reactivity. Also, a higher ROS value generally depends on a larger surface area, and crystallite size. The sample prepared were investigated for their antibacterial activity against gram-negative bacterium Escherichia coli, and gram-positive bacteria Staphylococcus aurous using the agar well diffusion method. The antibacterial activity of the zinc doped barium nickel nanoferrites powder on both gram-negative bacteria and gram-positive bacteria was acceptable. The antibacterial results of the barium-nickel ferrites doped zinc nanoparticle on bacteria activity showed that the nanoparticles shows an inhibitory growth on gram-positive Staphylococcus aurous and gram - negative Escherichia coli with high antibacterial effect on Staphylococcus aurous of 21mm diameter of inhibition zone. Hence, the developed barium nickel ferrites doped by zinc nanoparticle shows high antibacterial effect on (Staphylococcus aurous) and Escherichia coli which makes it a potential material for biomedical application.

Keywords: Barium-Nickel ferrite; Antibacterial activities; Toxicity mechanisms; Nanoparticles.

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Introduction

Nanotechnology, unlike any other technology, can find applications in virtually all areas of human life owing to the unique and astonishing physical, electrical and mechanical properties of nano-sized materials. Ferrites nanoparticles (NPs) are a class of nanostructured materials that have recently received increased attention owing to their interesting properties and broad range of applications in the medical field as an anticancer, antifungal, biosensors, drug delivery, and antibacterial activity[1,2]. Development of ferrites nanoparticles has been extensively with various size and shapes as delivery Nano systems are considered effective new tools to tackle the current challenges in treating infections [3,4].

Diagnostic capability of iron oxide NPs due to their magnetic properties has brilliantly contributed to tumour detection methods[5]. In an MRI scan, the iron oxide NPs can yield enhanced images once they attach to tumour cells[6] . Attachment to tumour cells in the bloodstream is also regarded as a privilege of magnetic NPs where they can be used as a cancer diagnosis tool[7] . Hyperthermia approaches using iron oxide NPs have also been influential in the field of cancer treatment because of their higher specific absorption rate [8].

Researches have shown that the nanoparticles (NPs) ferrites have antibacterial activity against both gram-negative and gram-positive bacteria [9]. Some nanoparticles ferrites was show inhibitory effect on the bacterial growth activity when they are doped with other nano-powders[10].

Some studies even noted that Zn^{2+} , Ni^{2+}, Ba^{2+} cations are non-toxic on human cells and very essential for human health also, they present a good antibacterial truly effects[11,12].

The antibacterial activity of these cations are highly significant as they stick on to the cell walls of bacteria and result destruction of DNA along with protein inhibition that further leads to cytolysis of bacterial cells[13]. Regarding the antibacterial activity, earlier studies have established that fairly ultrafine magnetic nanoparticles can easily penetrate intracellular into the bacterial cell, interact with cell membrane, produce oxidative stress and result in destruction of DNA[14]. Barium hexaferrite is one of the most important magnetic materials widespread uses, such as magnetic recording medium and in the creation of microwave components and devices such as circulators, microwaves absorbers, etc.[15,16] . The barium ferrite has attracted great interest in the magnetic material field and has been widely used as a permanent magnet because of its fairly large magnetocrystalline anisotropy and relatively large saturation magnetization, excellent chemical stability and corrosion resistivity[17]. A study on the antibacterial performance of BaFe12O19 composites on bacteria strains was evaluated. The results showed that the prepared nan composites possess antibacterial activity against Gram-positive Staphylococcus aureus (S. aureus)[18]. For the first time the antibacterial activity against Gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa of composite textile material, containing BaFe12O19 nanoparticles has been investigated and the results have shown good antimicrobial effect for both wound-related pathogens.[19]

The antibacterial activity of nanoparticles either directly interact with the bacteria cell or to produce secondary products that cause damage to the bacterial cell wall. There are several mechanisms behind the antibacterial activity of NPs. The responsible factors for antibacterial activity of the prepared are as follows[20] .

(i) Generation of reactive oxygen species (ROS).

(ii) The release of heavy metal ions. Oxidative process was followed through the attraction of hole in the valence band with electrons of water or hydroxyl anions to produce the most reactive radical (OH). Hydrogen peroxide (H2O2) was generated from the reaction between electron hole pairs and superoxide anions. The hydroxyl

radical (OH) and superoxide anion radical (O-2) caused high damage to DNA, nucleic acids, carbohydrates and lipids. Among the ROS, (H2O2) and (OH) are the most powerful oxidizing agents, which can directly penetrate the bacterial cell membrane to cause injuries and prevent the growth of cells, thereby leading to the bacteria death.

A higher ROS value generally depends on a larger surface area, appropriate crystallite size, increase of oxygen vacancies and the facilitation of diffusion and mass transportation of reactant molecules. Other mechanisms are also involved in the antibacterial activity. The bacteria cell membrane is damaged by nanocomposite, which binds to the mesosome. The mesosome processes of cell respiration, DNA replication, and cell division are disturbed, which increases the surface area of the bacterial cell membrane. The oxidative stress caused by ROS formation as a result of cell death is introduced by these intracellular functional alterations.

When the heavy metal (Ag^{2+}, Ni^{2+}, Co^{2+},Zn^{2+} and Cu^{2+}) released by nanocomposite surface come into contact with the cell membranes of the microbe, the cell membranes with negative charge and (Ag^{2+}, Ni^{2+}, Co^{2+},Zn^{2+} and Cu^{2+}) with positive charge mutually attract, and the (Ag^{2+}, Ni^{2+}, Co^{2+},Zn^{2+} and Cu^{2+}) penetrates into the cell membrane and react with the thiol groups (–SH) of the proteins present on the bactericidal cell surface. The nanomaterial inactivate the proteins and decrease the membrane permeability leading to the death of the microbe[21,22,23]. A schematic picture is shown in Figure 1.

Based on these findings, as previously mentioned, the use of Barium ferrites may be a promising alternative to minimizing antibacterial activity. In addition, zinc ferrites also, it is possible to showed antibacterial activity with various sizes. Based on the relevant information presented in this section, the recent research will be showing the influence of antimicrobial activity obtained with the introduction of dopants in Ba NPs. The study about these parameters is very important because of Ba NPs structural changes result in new properties that can enhance the antibacterial activity of Ba with ZnO NPs.

Therefore, the aims of these study investigation effect and efficiency of Zn with Ba-Ni against antibacterial activity.

Materials and Methods

Study design and period

The samples used in this work with the starting materials barium carbonate (BaCO3), zinc oxide (ZnO), ferric oxide (Fe2O3), nickel oxide (NiO), and ferric oxide (Fe2O3), were mixed according to their molecular weight ratio to obtain different compositions system $BaNi_{2-x}Zn_xFe_{16}O_{27}$. Synthesis procedure for the preparation of zinc doped W-type hexagonal barium -nickel ferrite nanoparticles has been prepared by the well-known ceramic method. The preparation processes of the sample are described detailed elsewhere [24,25].

Antibacterial activity Test

1.Preparation of Muller Hinton agar plates

A required amount (38 g) of the Muller Hinton agar (MHA) is weighed accurately and dissolved in 1000 ml of distilled water in a 2000 ml conical flask. It gently stirred using a glass rod to dissolve the contents uniformly. Then the conical flask sealed with a tight cotton plug after ascertaining that the pH of the medium is adjusted to the required pH (7.3 ± 1) for effective bacterial growth. Thus, dissolved Muller Hinton agar is sterilized by autoclaving principle at 121 °C temperature and 15 lbs. pressure for 15 min. It is permitted for the sterilized medium to cool to 45 °C. The medium is then poured in sterile petri plates in an aseptic manner using around 15 ml (under Laminar Air flow). Wells were made with 4 mm diameter and 4 mm depth. Then the poured Muller Hinton agar plates are allowed for solidification. The bacterial inoculums were swabbed on the solidified plates. Antibacterial activity of the synthesized NPs was tested

against the human pathogenic bacteria. Gram negative (Escherichia coli) and gram positive (Staphylococcus aurous).

2.Preparation of samples for antibacterial test:

Different concentrations were prepared from nanoparticles ferrites to study their effects against various kind of negative and positive bacteria. Various concentrations of the test solution are (0.75 mL) (1.5) mL and (3mL) were loaded in the respective wells along with Gentamycin as standard control. The plates were incubated at 37 °C for 18 to 24 h. The zone of inhibition was evaluated using Vernier's calipers. After incubated, the antimicrobial potential of the synthesized ferrites with respect to standard antibacterial agents was determined by measuring the diameter of inhibition zones the diameter (mm) of the inhibition zone created around the preformed wells[25].Tests were performed in triplicate, and the results were interpreted and discussed elaborately in the discussion part.

Results

The structural composition of the samples nanoparticles was determined by x-ray diffraction (XRD) analysis. The average crystallite size of Zn substituted barium-nickel ferrite has been calculated for the different compositions from the X-ray diffraction (XRD), by using the Scherrer formula[26] equation.1, and was summarized in Table I.

$$D = 0.9\lambda/\beta\cos\theta \quad (1)$$

Where “D” is the average crystallite size (nm), “β” is the broadening of diffraction peaks measured at half maximum intensity (radians), “λ” is the X-ray wavelength(1.54Å). From Table I, it is seen that the crystallite size was influenced by the Zn ions substitution as Zn ions concentration increases in the $BaNi_{2-x}Fe_{16}O_{27}$ the crystallite size decreases up to the 0.4 substitution of Zn. The obtained crystallite size was fluctuated between 35-36.7 nm. The minimum crystallite size of 35.18 nm for the 0.4 Zn substitution was obtained. thereafter it increases slowly up to 36.66nm. This is due to the transfer of Ni^{2+} ions from octahedral sites to tetrahedral sites with increasing concentration of the Zn^{2+} ions. Also, Surface area has been calculated using the equation.2[26], and summarized in Table I.

Table.1. Structural parameters of barium-nickel ferrite doped zinc.

Zn	Crystallite Size(nm)	Surface area	Porosity%
0	36.50	31.02	20.92
0.4	35.18	32.23	19.10
0.8	35.53	32.16	15.87
1.2	35.81	31.91	13.99
1.6	36.37	31.45	13.098
2	36.66	31.37	11.32

$$S = 6000/D\,\rho x, \quad (2)$$

where “D” is the crystallite size and “ρ_x” is the x-ray density.

The structural parameters such as surface area (S) and porosity (P %) shows an inversely proportional relation with crystallite size, as reported in Table 1. The crystallite size is the dominant factor for variation in surface area. As the crystallite size increases, the smaller number of atoms appear at the surface, while the porosity decreases with the increases of Zn^{2+} ions concentration due to the creation of oxygen vacancies with the reduction of more cation vacancies. The production of reactive oxygen species (ROS) by metal oxide NPs is one of the mechanisms responsible for antimicrobial activity most commonly reported. ROS include superoxide anions (O-2 hydroxyl radicals (HO-2) and hydrogen peroxide (H2O2) which can cause

the destruction of cellular components such as DNA, proteins and lipids[27].

As well as the results showed that the sample $BaNi_{1.6}Zn_{0.4}Fe_{16}O_{27}$ had a smaller crystallite size than other, because it has a very small nanoparticles, they had a high specific surface area, as shown in table 1. Which enables more contact with the microorganism surface had bigger surface area antibacterial activity. These findings might potentially be used to antibacterial qualities. Because it has a small nanoparticle and a high specific surface area, which enables more contact with the microorganism surface. Another reason is related to the hexagonal structure. Due to its unsaturated oxygen coordination and positive charge, the (001,008) face is able to adsorb oxygen molecules and OH- ions. The small particle size and the high surface area of ZnO NPs can enhance antimicrobial activity, causing an improvement in surface reactivity. This results in a higher rate of H2O2 and OH• radical production, and hence increased antimicrobial activity.

The antibacterial activity of the prepared samples against two types of bacteria depicts in Figure,2. Inhibition zone of antibacterial activity against gram negative bacterial growth. As shown in Figure. 2. According to the results of figure 2, there is a significant effects for the sample $BaNi_{1.6}Zn_{0.4}Fe_{16}O_{27}$, For gram-negative E. coli, at concentration (3ml), with inhibition zone 16 mm, due to the accumulation of zn ions pitted the cell wall structure causing the continual release of membrane proteins and lipopolysaccharides which leads to the death of the bacteria[28], this is due to small crystallite size and higher surface area, these results is useful to find a relationship between nanoparticles size and antibacterial activity. From figure.3. $BaZn_2Fe_{16}O_{27}$ at concentration (3ml), explain significantly the highest antibacterial activity against gram- negative bacteria Staphylococcus aurous with zone of inhibition 21 mm, due to the presence of zinc ions in huge ratio retarded the antibacterial life time[29]. According to the results, change of microstructural NPs can influence the release of Zn^{2+} and lead to the production of ROS and thereby influence antibacterial activity. Bacteria died with or without surface modifications. The increase in Zn^{2+} was also, related to increased activity, but some time lower Zn^{2+} concentrations were associated with better antibacterial properties[30]. whereas BaNi2Fe16O27 gave the lowest inhibition zone (19mm) which lead to the low interaction between the sample and the cell membrane. Disk diffusion assays showed that the inhibition zone was increased with smaller sized nanoparticles, which may be related to the ability of these particles to rupture the bacterial membrane.

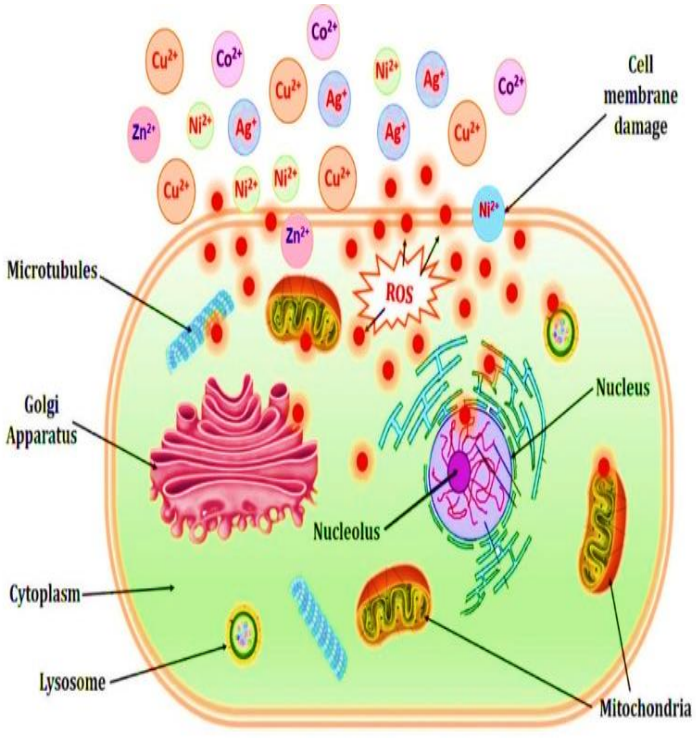

Figure 1: Characteristic diagram of antibacterial activity mechanism.

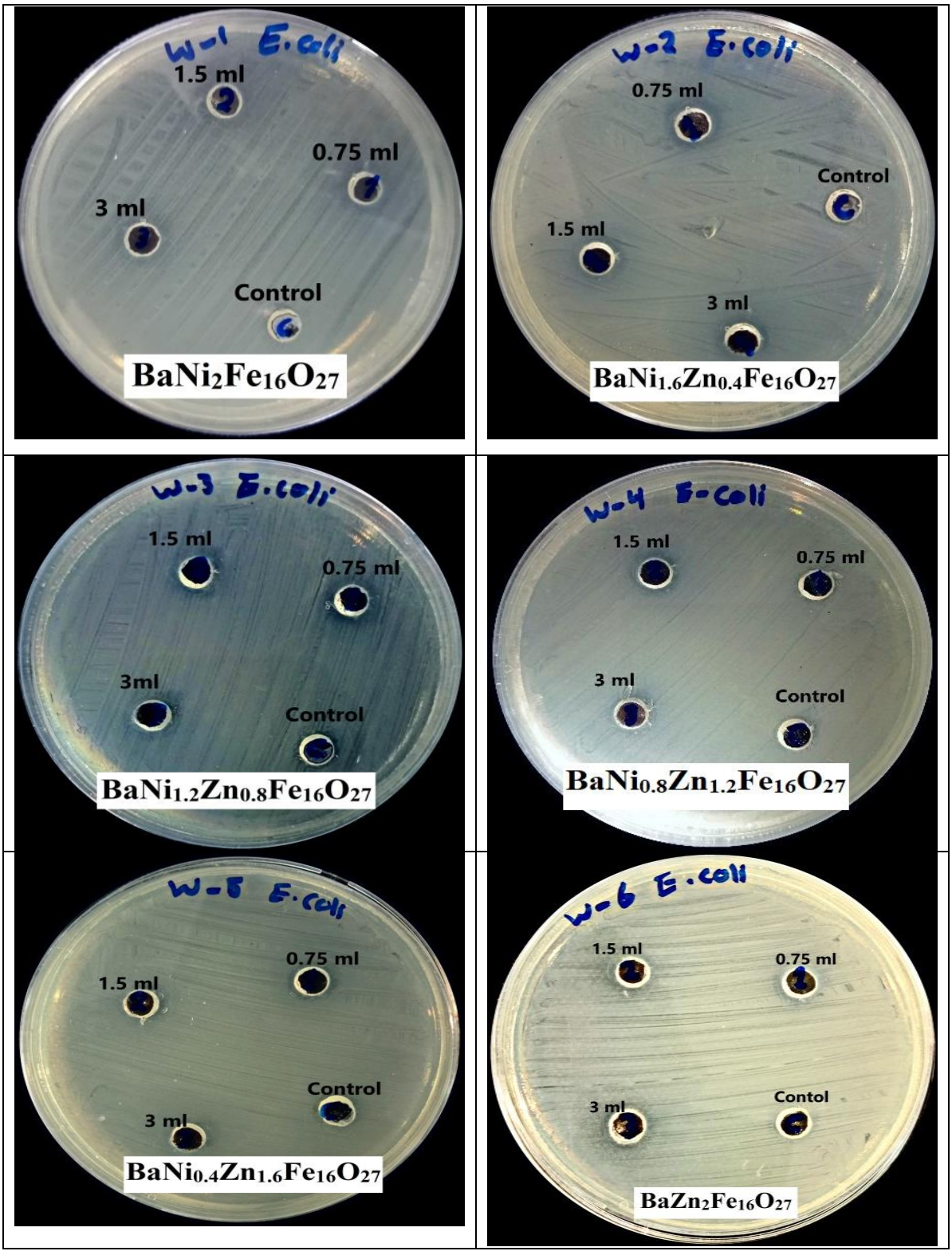

Figure 2. Antibacterial activity of $BaNi_{2-x}Zn_xFe_{16}O_{27}$ (x=0.0, 0.4, 0.8, 1.2, 1.6, and 2) against E. coli bacteria

As shown in figure. 4.b, the antibacterial activity of $BaZn_2Fe_{16}O_{27}$ NPs gave high effect against the resistant gram- positive bacteria Staphylococcus with zone of inhibition 21 mm which was the highest between the whole samples, the higher activity of $BaZn_2Fe_{16}O_{27}$ NPs may be due to the deposition of Zn^{2+} and Ba^{2+} nanoparticles on the surface of membrane would enter into the cell membrane or even the cells such as DNA, RNA, enzyme, protein and lipids in microbial cells. This leads to inhibition of bacterial cell growth as well as bacterial proliferation finally cause lysis of bacterial cells. As shown in Figure.4.a, the most effective against gram-negative bacteria E. coli with inhibition zone 16 mm was for the sample $BaNi_{1.6}Zn_{0.4}Fe_{16}O_{27}$ NPs. For the gram-negative E. coli, the accumulation of Ba^{2+}/ Ni^{2+} and Zn^{2+} ions pitted the cell wall structure causing the continual release of membrane proteins and lipopolysaccharides which leads to the death of the bacteria. The test sample exhibits significant antibacterial activity against all the bacterial strains, especially at higher concentrations (3 mL) as shown in figure 3. The rate of bacterial growth appears to be an additional factor that affects how sensitive bacteria are to nanoparticles and medicines. It has been noted that bacteria with slower growth rates are more resistant to nanoparticles than those with faster growth rates. The test sample were able to produce clear zones on all the bacterial strains used indicating bacterial activity, withe variations in diameter size inhabitation zone. The resistance of nanoparticles the effect antibacterial activity for Gram-positive and Gram-negative activity depends on the susceptibility of the microorganism, the difference in sensitivity of $BaNi_{2-x}Zn_xFe_{16}O_{27}$ nanoparticles ferrites. The differences in the cell walls of the two bacteria may provide an explanation. Thus, nanoparticles ferrites can interact with carboxylic acid and amino groups and thereby inhibit cellular processes. However, the peptidoglycan layer of Gram-negative bacteria is thinner than the membrane of Gram-positive bacteria, so rupture of the cell membrane is easier[31]. The difference between the components of Gram-positive and Gram-negative cell membranes dependent on the basis's microstructure sensitivity. The Gram-negative bacteria are less susceptible to attack by nanoparticles than Gram-positive bacteria. The minimum inhibitory concentration (MIC) of nanoparticles observed in S. aureus, a Gram-positive bacterium, and E. coli, a Gram-negative bacterium at 3ml concentration, was 16mm and 21mm, respectively, which shows that the inhibition of Gram-negative bacteria less susceptible to attack by nanoparticles ferrite. This is likely because the peptidoglycan layer that surrounds Gram-positive bacteria can promote nanoparticles ferrites attack inside the cell, while the cell wall components of Gram-negative bacteria, such as lipopolysaccharides can counter this attack. The results showed that Gram-negative bacteria was less sensitive to peroxide hydrogen than Gram-positive bacteria. Higher concentrations (3mL) of the W-type BHFs are proven antibacterial agents. So, these materials can become a useful member of recommended clinical settings by physicians for bacterial infections. All Samples were able to produce clear zones on all test strains used indicating bacterial activity, withe variations in diameter size inhabitation zone, which 21mm with Staphylococcus aurous and 16mm with Escherichia coli, both at higher concentration, while at lower concentration the inhibitory zone fluctuate between 14 -16mm of the Staphylococcus aurous, while the lower inhibition zone of the Escherichia coli, varied between 9 - 13 mm at lower concentration.

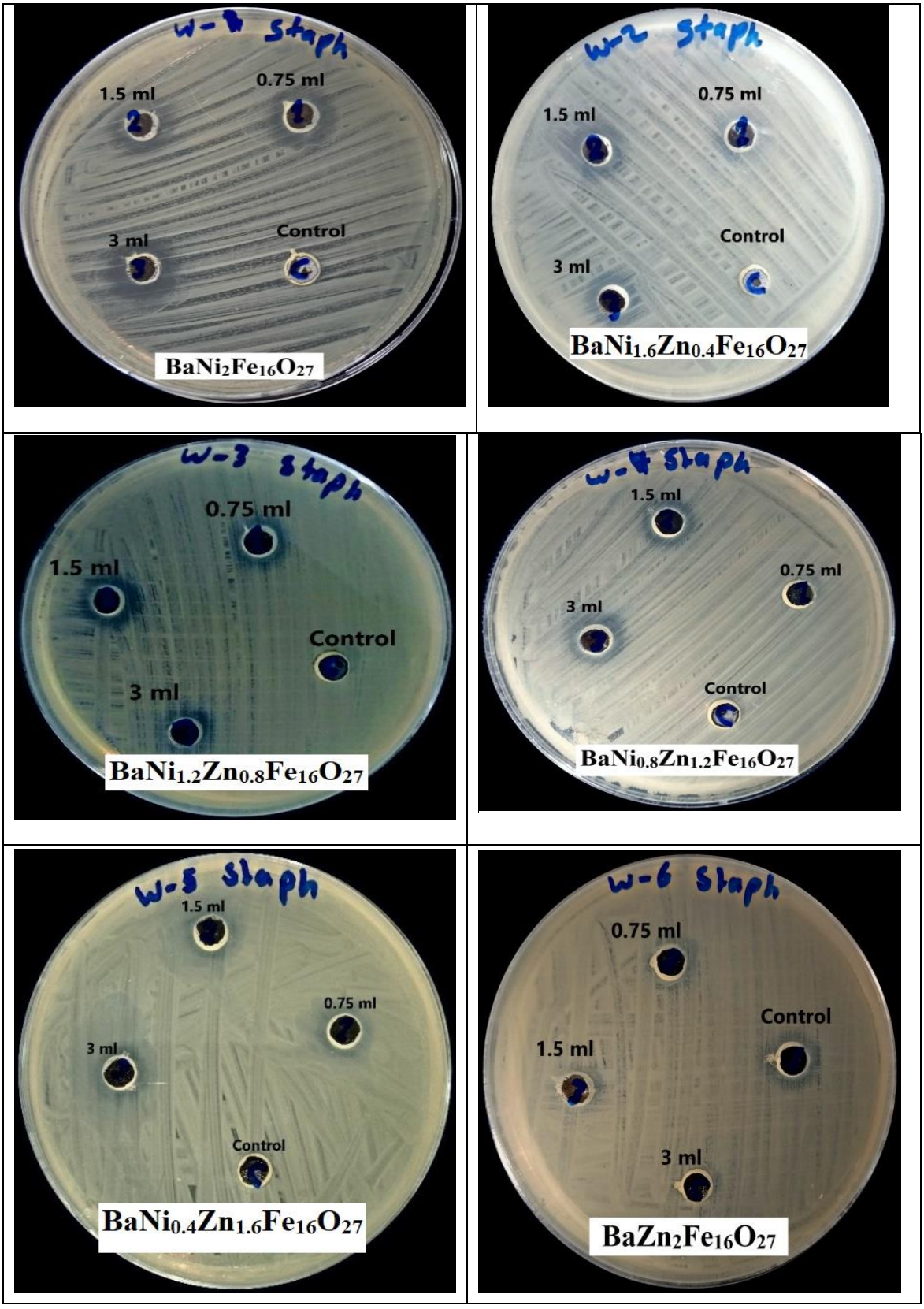

Figure 3. Antibacterial activity of $BaNi_{2-x}Zn_xFe_{16}O_{27}$ (x=0.0, 0.4, 0.8, 1.2, 1.6, and 2) Staphylococcus bacteria

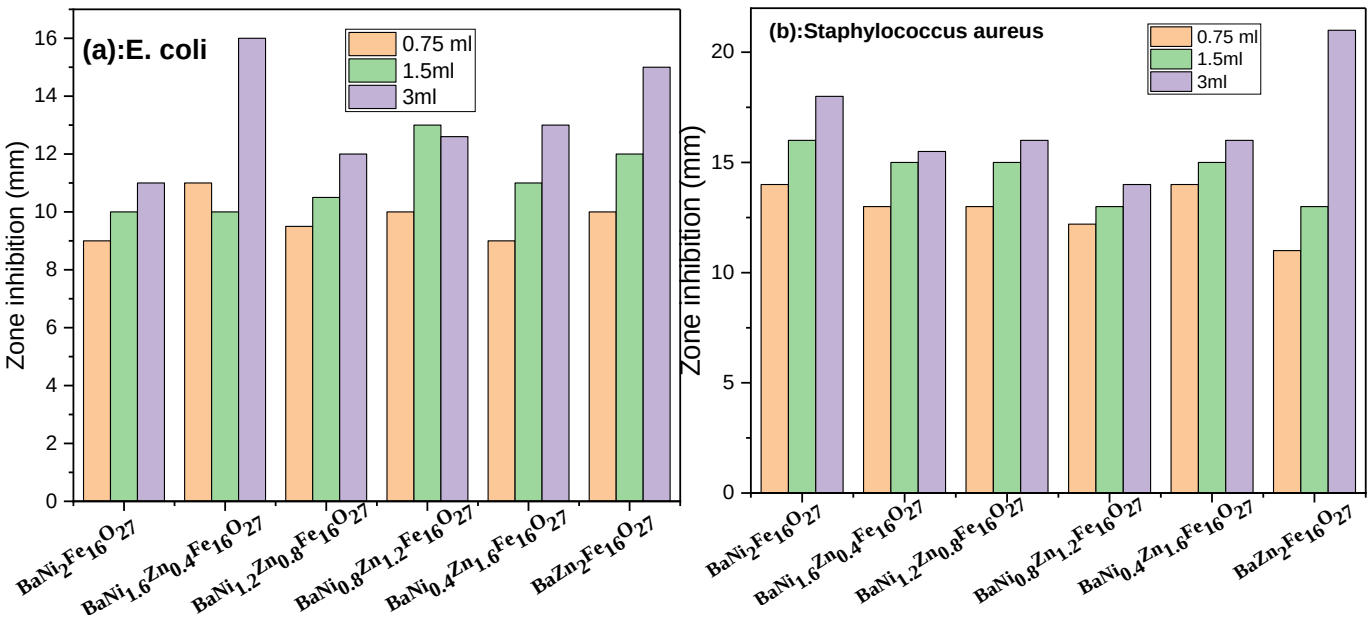

Figure 4. Average diameter of inhibition zones of bacterial versus the concentrations of the nanoparticle's ferrites.

Conclusion

In summary, $BaNi_{2-x}Zn_xFe_{16}O_{27}$(BNZF) was successfully synthesized via ceramic technique. The challenge test was used in this study to assess the relationship between antibacterial activity and the structural parameters of BNZF. The choice of the bacteria was based on the common infection affecting the community. For instance, the presence of E. coli causes nausea, diarrhea while, the effect of Staphylococcus aureus is noticeable and also a causative bacterial in boil and causes complication in wounds.

The samples of NPFs were characterized in terms of crystal size, surface area, porosity and in Muller Hinton broth. The antimicrobial activity was evaluated by disc diffusion and MIC measurements with the NPFs for both microorganism tested. The zone of inhibition was observed, and its diameter was used to measure the antibacterial activity of BNZF NPs.

The results showed that the best activity was observed for smaller BNZF crystals size and the antimicrobial activity was dependent on the surface area. The antibacterial activity effect was more dependent on the crystallite size than the specific surface area. This shows that the improvement of activity does not depend solely on the dopant but also on the structural characteristics of BNFPs. Therefore, it is important to control the reaction in order to produce doped nanoparticles with large quantities of oxygen vacancies and with small sizes. Doped ZnO NPs with small sizes showed the best antibacterial activity.

Thus, the authors concluded that the most important parameters to explain antimicrobial activity are a small crystal size and high porosity with large pores. The relation between antibacterial activity and concentration of BNZF NPs against both gram-negative and a gram-positive bacteria. The results showed that as the concentration increased, the inhibition zone was increased at higher concentration for both

bacteria. The Barium nickel ferrites show inhibition activity effects against Staphylococcus aurous bacteria, higher than Escherichia coli. bacteria.

Hence, the developed barium nickel ferrites doped by zinc nanoparticle shows high antibacterial effect on (Staphylococcus aurous) and Escherichia coli which makes it a potential material for biomedical application. The small particle size and the high surface area of NPs can enhance antimicrobial activity, causing an improvement in surface reactivity. As we can see, the sample $BaNi_{1.6}Zn_{0.4}Fe_{16}O_{27}$ the most relevant parameter for excellent activity was the small size of the NPs. And $BaZn_2Fe_{16}O_{27}$ due to higher concentrations of ROS. It was found that ZnO NPs with a smaller size are found at a higher inhabitation zone against antibacterial activity. In the same way, the better activity of smaller sized NPs may be attributed the need for more smaller particles to cover the bacterial colony, which results in the generation of higher concentrations of ROS released on the surface of the bacteria, promoting bacterial death more efficiently. In addition, smaller NPs can penetrate the bacterial membrane more easily due to the high surface area. Therefore, the mechanism of NPs toxicity is dependent on modification of NPs properties, as well as particle size, since smaller particles have s larger surface area in relation to volume and can penetrate into the bacterial cell. Furthermore, the increase in oxygen vacancies was responsible for positively loading BNZF NPs, which enabled interactions with the bacterial cell wall, which has a negative charge.

Conflict of interest

No conflict of interest is associated with this work.

Acknowledgment

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تأثير تطعيم أيونات Zn2+ على النشاط المضاد للبكتيريا لجسيمات نانوية من فيريت الباريوم-نيكل.

جذبت مواد الفيريت النانوية (NPFs) مؤخراً اهتماماً متزايداً بسبب خصائصها المثيرة وتطبيقاتها الطبية الواسعة مثل النشاط المضاد للسرطان والفطريات والبكتيريا. في العمل الحالي، تم تصنيع فيريت الباريوم-نيكل المطعم بالزنك بتكوين BaNi2-xZnxFe16O27 (x=0.0، 0.4، 0.8، 1.2، 1.6، و2) لدراسة نشاطه المضاد للبكتيريا باستخدام تقنية السيراميك. تم توصيف مسحوق الفيريت BNZF بواسطة حيود الأشعة السينية. تم استخدام صيغة ديباي-شيرار لحساب متوسط حجم البلورات الذي يتراوح بين 43 و56 نانومتر. يمكن أن يعزز الحجم البلوري الصغير والمساحة السطحية الكبيرة لجسيمات الفيريت النانوية النشاط المضاد للميكروبات، مما يؤدي إلى تحسين التفاعل السطحي. أيضاً، تعتمد قيمة ROS الأعلى عادةً على مساحة سطحية أكبر وحجم بلوري. تم اختبار العينات المحضرة لنشاطها المضاد للبكتيريا ضد البكتيريا سالبة الجرام الإشريكية القولونية والبكتيريا موجبة الجرام المكورات العنقودية الذهبية باستخدام طريقة انتشار الآجار. كانت نتائج النشاط المضاد للبكتيريا لمسحوق الفيريت النيكلي المطعم بالزنك ضد كل من البكتيريا سالبة الجرام وموجبة الجرام مقبولة. أظهرت نتائج النشاط المضاد للبكتيريا لجسيمات الفيريت النيكلي المطعمة بالزنك تأثيراً مثبطاً لنمو المكورات العنقودية الذهبية والإشريكية القولونية مع تأثير مضاد للبكتيريا عالي على المكورات العنقودية الذهبية بقطر منطقة تثبيط يبلغ 21 ملم. لذا، فإن فيريت الباريوم-نيكل المطعم بجسيمات الزنك النانوية يظهر تأثيراً عالياً مضاداً للبكتيريا على المكورات العنقودية الذهبية والإشريكية القولونية مما يجعله مادة محتملة للتطبيقات الطبية الحيوية.