

RESEARCH PAPER

Antibacterial and Structural, Morphological Properties of Nanosized $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ Prepared by the Co-Precipitation Method

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ABSTRACT

This study investigated the structural, morphological, and antibacterial properties of Nickel - Zinc Spinel Ferrite ($\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$) nanoparticles prepared by the co-precipitation method at different pH values (2.5, 9, 11, and 13) and sintered at 700 °C for 2 h. X-ray diffraction (XRD) analysis confirmed the formation of a cubic spinel ferrite structure, with characteristic reflections corresponding to the (220), (311), (222), (400), (422), (333), (440), and (533) planes. The lattice parameters for samples prepared at pH 9, 11, and 13 were 8.382, 8.391, and 8.373 Å, respectively, while the crystallite size ranged from 18 to 48 nm. The theoretical densities were 5.22, 5.39, and 5.36 g/cm³, respectively. Scanning electron microscopy (SEM) revealed agglomerated nanoparticles with irregular and semi-cubic morphologies. Antibacterial activity was evaluated against *Staphylococcus aureus* and *Escherichia coli* (*S.aures* and *E.coli*) using the agar well-diffusion method. The inhibition zones increased with nanoparticle concentration, reaching 32 and 38 mm, respectively, at the highest concentration.

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INTRODUCTION

In recent years, much spinel ferrites have been synthesized by many researchers and possess various structures, spectra and magnetic properties [1]. Spinel ferrites are widely used in microwave technology [2], drug delivery [3], gas sensor [4-5] and electronics devices [6]. All of these applications are intimately tied to the transition metals that can occupy lattice sites in the spinel ferrite structure [3]. Technological applications require such materials to be more or less free of porosity, have a high density and possess a microstructure with defined composition [4].

For the under the investigation Nano-powders of Ferrite is accomplished that in case of allowing

the ferrite Nano-powder to crystallize spinel formation takes place and tetrahedral sites for trivalent B cations, octahedral sites for divalent A cations are both possible. The context of each of these cations influences this. Based on the cation coordination of M^{2+} and Fe^{3+} spinel ferrites can be classified into two subgroups: normal spinel AB_2O_4 with formula $(\text{A}^{2+})\text{A}[\text{Fe}^{3+}\text{Fe}^{3+}]\text{BO}_4$ and inverse type B (AB) O_4 $\text{A}^{2+}\text{A}(\text{A}^{2+}\text{Fe}^{3+})\text{BO}_4$. These two ferrites are quite similar [6,5]. As shown in the crystallization of spinel ferrite, these two equations can be satisfied at the same time. In this process, since the metal ion is smaller than the oxygen ion, it gets sandwiched between two oxygen ions. The properties of spinel ferrites depend on how cations

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are substituted in (A) and (B) sites. Among many soft magnetic materials, nickel ferrite is one of the most commonly used and important ones [7]. Its key features are low eddy current loss, moderate conductivity, and good electrochemical stability. Among these, nickel-zinc spinel ferrite stands out because of its high permeability, low resistivity, and excellent high-frequency performance [8]. For the preparation of NCC, hydrothermal method is widely applied. [9], co-precipitation, the sol-gel [10], microwave combustion, etc. were used to develop the Ni-Zn Nano ferrite. The sol-gel process, a method having an essential beneficial role of utilization and performance, is one among them. It was possible to prepare the sol with high quality and purity, chemical homogeneity, and a large energy saver on suitable glass substrate [11]. The composition, particle size, dopant concentration and impurities and methods of production and heat treatment are just some microscopic properties of Ni-Zn ferrites.

MATERIALS AND METHODS

To prepare the compounds used in the chemical precipitation experiments, iron nitrate, zinc nitrate, and nickel nitrate were dissolved in 100 ml of distilled water with a magnetic stirrer at 60°C for half an hour until completely dissolved. Then all the solutions were combined in a glass bowl at 60°C for 4.5 hours. In the second phase, 40 g of sodium hydroxide (NaOH) was dissolved in a glass flask with 200 ml of distilled water and stirred repeatedly on a magnetic stirrer at 60°C for 15 minutes. The precipitation is induced using the sodium hydroxide solution, and the pH is measured. In the final step, all the solutions were homogenized in a glass flask for half an hour while stirring with a magnetic stirrer. During stirring, the sodium hydroxide solution was added drop by drop using a burette. The pH was checked at 60°C until all the sodium hydroxide solution had evaporated. Then, the leftover solution was filtered through paper and rinsed several times with distilled water to get rid of any nitrates that wouldn't dissolve. After that, the product was separated and dried in an oven at 100°C to get a dry, water-free compound. Finally, the resulting powder was heated at 700°C for 2 hours.

RESULTS AND DISCUSSION

X-ray Diffraction (XRD)

The X-ray diffraction patterns of the prepared

$\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles synthesized at different pH values (2.5, 9, 11, and 13) and sintered at 700 °C for 2 h are shown in Fig. 1. The diffraction patterns exhibit the characteristic reflections of a cubic spinel ferrite structure. The observed reflections can be indexed to the (220), (311), (222), (400), (422), (333), (440), and (533) crystallographic planes, which are characteristic of spinel ferrites [12,13]. The most intense diffraction peak was observed near $2\theta = 35.7^\circ$, corresponding to the (311) reflection. The presence of the characteristic spinel reflections indicates the formation of the ferrite phase after the applied precipitation and heat-treatment process [12,13].

The XRD patterns obtained at different pH values show variations in the relative diffraction-peak intensities and peak widths. Such changes may be associated with differences in the development of the crystalline phase during precipitation and subsequent heat treatment. Previous studies have reported that variation of the synthesis pH can influence the structural properties and crystallization behavior of ferrite nanoparticles [13,14]. However, the present XRD data do not provide sufficient evidence to attribute these changes specifically to magnetic ordering, nucleation, crystal growth, or aggregation. Therefore, these effects are discussed only in relation to the observed structural changes. No additional distinct diffraction peaks attributable to detectable crystalline secondary phases were observed within the measured diffraction range, supporting the formation of the $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ spinel phase under the investigated preparation conditions [12,13].

The principal (311) reflection was used for comparison of the structural parameters of the prepared samples. The calculated lattice parameter values for pH 9, 11, and 13 were 8.382, 8.391, and 8.373 Å, respectively. Variations in lattice parameter with preparation conditions have also been reported for Ni–Zn ferrite systems and may be related to changes in cation distribution within the spinel lattice [15,16].

The pH 2.5 sample was not included in the lattice-parameter table because the available diffraction data were not considered sufficiently reliable for assigning a numerical lattice parameter. Therefore, no unsupported value was introduced for this sample. The crystallite size was reported within the range of 18–48 nm based on the available XRD analysis. Changes in crystallite size

with preparation conditions are consistent with the reported influence of synthesis parameters on ferrite nanoparticle structure [13,14,16].

Lattice constant

The lattice parameter (a) of the cubic spinel $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ structure was evaluated from the principal (311) diffraction reflection. The calculated lattice-parameter values for the samples prepared at pH 9, 11, and 13 were 8.382, 8.391, and 8.373 Å, respectively. The results show that the lattice parameter does not exhibit a simple monotonic increase or decrease with increasing pH. A slight

increase was observed from pH 9 to pH 11, followed by a decrease at pH 13. Such variation indicates that the synthesis pH may affect the structural characteristics of the ferrite lattice, including the arrangement and distribution of the constituent cations within the spinel structure [15,16].

The variation in lattice parameter may be associated with changes in cation distribution between the tetrahedral and octahedral sites of the spinel lattice during precipitation and subsequent heat treatment. Similar structural variations associated with synthesis conditions

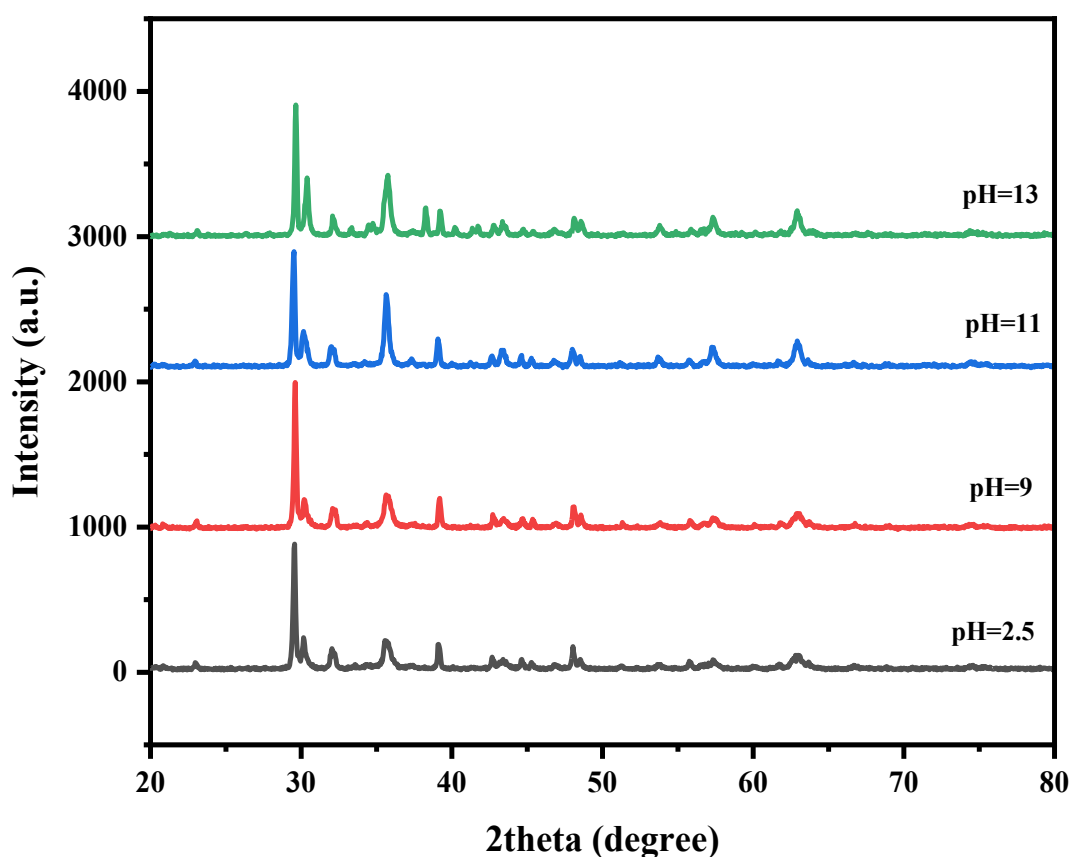


Fig. 1. XRD of $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ with pH value 2.5,9,11,13.

Table 1. The value of lattice constant with pH for the (311) peak.

pH value	Lattice constant a (Å)
9	8.382
11	8.391
13	8.373

and cation distribution have been reported for Ni-Zn ferrite nanoparticles [15,16]. The pH 2.5 sample was not included in the lattice-parameter, because the available XRD data did not provide a

sufficiently reliable basis for determining its lattice parameter (Table 1). Therefore, no numerical value was assigned to this sample without adequate experimental support.

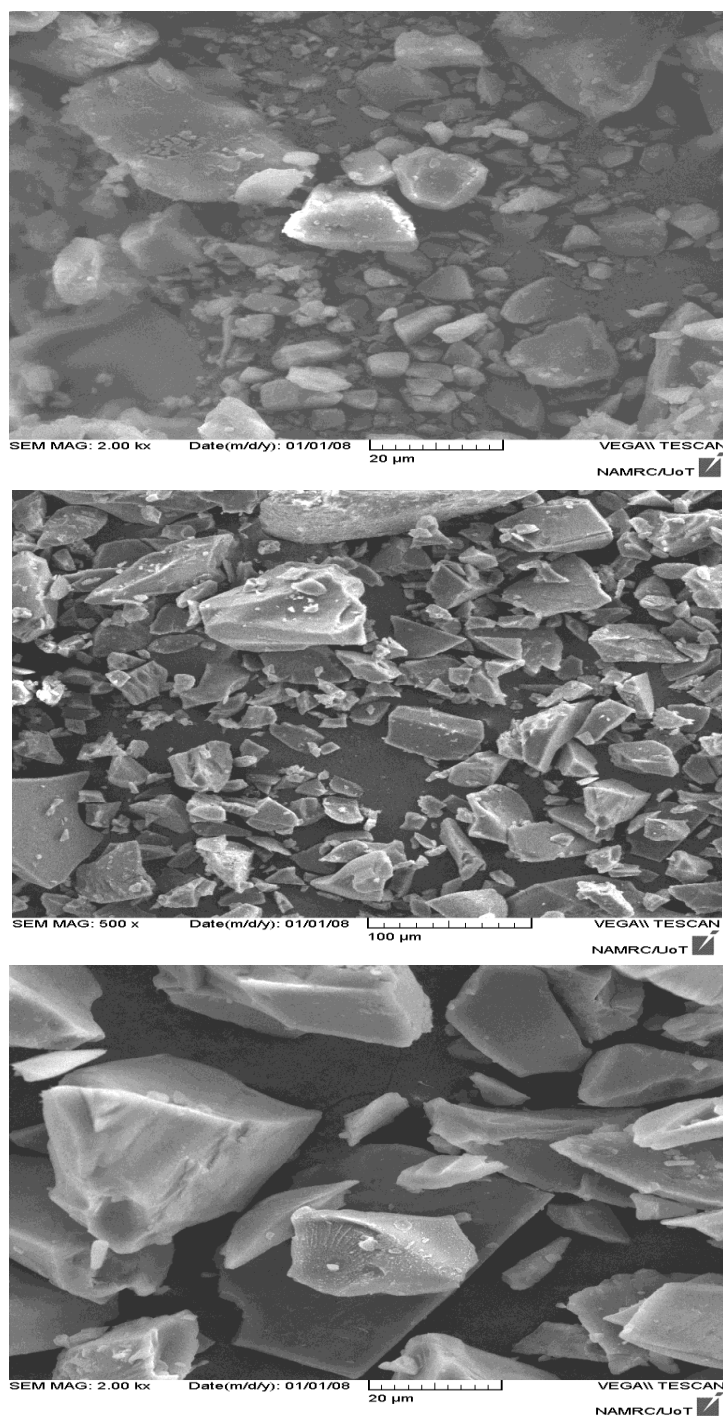


Fig. 2. (a-c): SEM images of $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles prepared at pH 9, 11, 13.

Theoretical Density

The theoretical X-ray density (ρ_x) of the prepared $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles was calculated for the samples prepared at different pH values. The calculated density values were 5.22, 5.39, and 5.36 g/cm³ for pH 9, 11, and 13, respectively (Table 2).

The results show that the theoretical density does not follow a monotonic trend with increasing pH. The density increased from pH 9 to pH 11 and then decreased slightly at pH 13. This variation may be associated with changes in the lattice parameter and the structural arrangement of the constituent cations within the spinel lattice. Variations in structural parameters with synthesis conditions and cation distribution have been reported for ferrite materials [15,16]. The calculated theoretical density is related to the crystallographic unit-cell volume and the molecular weight of the ferrite phase. Therefore, changes in the lattice parameter can contribute to the observed variation in the calculated density. The present results indicate that the preparation pH influences the structural characteristics of the synthesized ferrite, although the available data do not establish a single direct mechanism responsible for the observed density variation [15,16].

SEM analysis for $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles

The surface morphology of the prepared $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles was investigated using scanning electron microscopy (SEM). The SEM micrographs of the samples prepared at pH 9, 11, and 13 and sintered at 700 °C for 2 h are presented in Fig. 2. The SEM images show that the prepared nanoparticles exhibit an agglomerated morphology, with irregular and semi-cubic particle features. Differences in particle appearance and the degree of agglomeration can be observed among the samples prepared at different pH values. These morphological variations may be associated with changes in the precipitation and subsequent particle-growth processes under different synthesis conditions. The observed agglomeration

is commonly associated with the high surface energy of nanoparticles and the tendency of fine particles to form aggregates during drying and heat treatment. Similar morphological features have been reported for Ni–Zn ferrite nanoparticles prepared by different synthesis routes [19].

The SEM observations in the present study demonstrate that the preparation pH is associated with observable differences in the surface morphology and degree of agglomeration of the synthesized $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles. However, the SEM images alone do not establish a specific mechanism responsible for these morphological changes.

Prepare of Mueller Hinton agar

Mueller–Hinton agar was made by dissolving 38 g of the dry medium in 1 L of distilled water. The mixture was heated and stirred well until it completely dissolved. Then, the medium was sterilized by autoclaving at 121 °C for 15 minutes. After that, it was cooled to around 50 °C and poured into sterile Petri dishes under aseptic conditions. The plates were left to solidify and then stored at 4 °C until needed.

Antibacterial activity

The antibacterial activity of the $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles we prepared was tested against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli* using the agar well-diffusion method [20,21]. This test was done in a specialized lab using Mueller–Hinton agar.

About 20 mL of sterile Mueller–Hinton agar was poured into Petri dishes. After the agar solidified, wells of 6 mm in diameter were made, and the nanoparticle samples were placed in these wells at the concentrations we wanted to test. The bacteria used in the test came from a bacterial source according to the lab's procedure [22]. The plates with bacteria were then incubated overnight at 37 °C. After incubation, we measured the inhibition zones around the wells in millimeters [23–25].

Table 2. Value of Theoretical density at different pH.

pH value	Theoretical density ($\rho_{x\text{-rd}}$) (gram/cm ³)
9	5.22
11	5.39
13	5.36

The antibacterial activity was evaluated at the tested concentrations designated as 12.5%, 25%, 50%, and 100%. The corresponding inhibition-zone measurements are presented in Table 3 and Figs. 3 and 4.

The results showed an increase in the inhibition-zone diameter with increasing nanoparticle concentration. For *S. aureus*, the inhibition zones were 22, 26, 30, and 32 mm at 12.5%, 25%, 50%, and 100%, respectively. For *E. coli*, the corresponding inhibition zones were 32, 34, 36, and 38 mm, respectively.

These results demonstrate concentration-dependent antibacterial activity of the prepared $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles against both tested bacterial species under the experimental conditions used in the assay.

Result of antibacterial activity

The antibacterial activity of the prepared

$\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles was tested against *Staphylococcus aureus* and *Escherichia coli* using the agar well-diffusion method. The inhibition-zone sizes at different nanoparticle concentrations are summarized in Table 3 and shown in Figs. 3 and 4 [23–25]. For *Staphylococcus aureus*, the inhibition-zone diameter grew from 22 mm at 12.5% to 26, 30, and 32 mm at 25%, 50%, and 100%, respectively. For *Escherichia coli*, the inhibition-zone diameters were 32, 34, 36, and 38 mm, respectively. These results show that the inhibition-zone diameter increased as the nanoparticle concentration went up for both types of bacteria. Under the conditions tested, *E. coli* had bigger inhibition zones than *S. aureus* at all nanoparticle concentrations. The concentration-dependent inhibition observed proves that the prepared $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ Nanoparticles showed antibacterial activity against both types of bacteria tested. But this study didn’t directly look into

Table 3. Explain the antibacterial activity of nanoparticles.

Bacterial strain	Control	12.5%	25%	50%	100%
<i>S. aureus</i>	6	22	26	30	32
<i>E. coli</i>	6	32	34	36	38

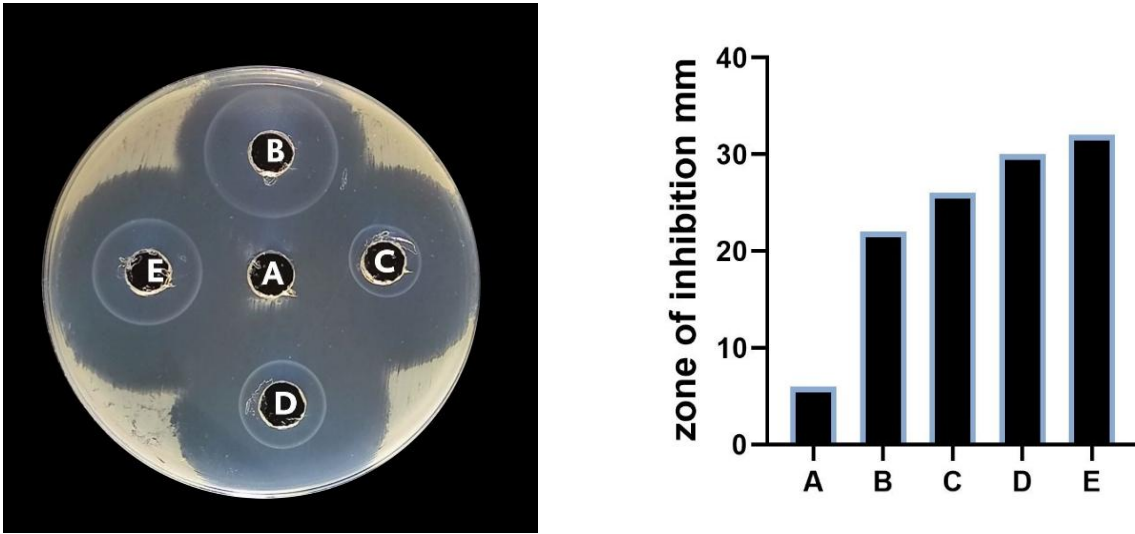


Fig. 3. Antibacterial activity against *S. aureus*: A, Control. B, 12.5%. C, 25%. D, 50%. E, 100%.

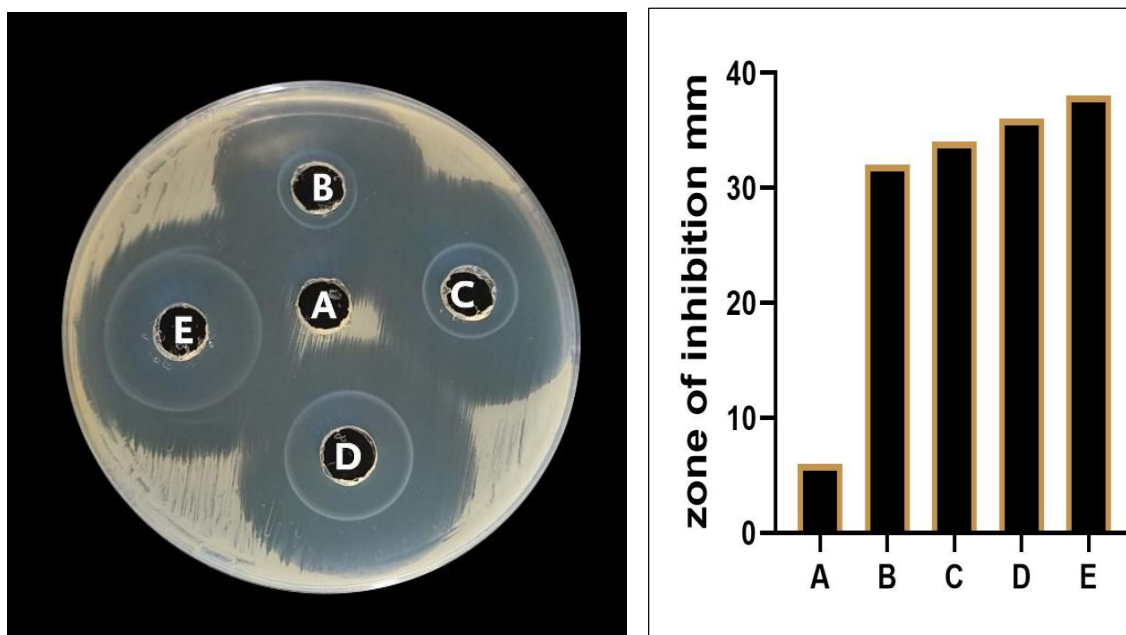


Fig. 4. Antibacterial activity against *E. coli*: A, Control. B, 12.5%. C, 25%. D, 50%. E, 100%.

how they actually kill the bacteria. So, things like generating reactive oxygen species, damaging the cell membrane, or releasing metal ions are just potential reasons, not confirmed ones.

CONCLUSION

$\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles were successfully made using the co-precipitation method at different pH levels and then sintered at 700 °C for 2 hours. X-ray diffraction analysis showed that they formed a cubic spinel ferrite structure, with diffraction peaks typical of the spinel phase. Changes in peak intensity and width were noticed depending on the pH used during preparation. The lattice parameter calculated from the main (311) reflection was 8.382 Å, 8.391 Å, and 8.373 Å for samples prepared at pH 9, 11, and 13, respectively. So, the results suggest that the lattice parameter changed with preparation pH rather than just steadily increasing. The calculated theoretical densities were 5.22, 5.39, and 5.36 g/cm³ for pH 9, 11, and 13, respectively. SEM images showed clumped nanoparticles with irregular and semi-cubic shapes, and the level of clumping varied between the samples prepared at different pH values.

The antibacterial assay demonstrated concentration-dependent inhibition against both

Staphylococcus aureus and *Escherichia coli*. At the highest tested concentration, inhibition zones of 32 mm and 38 mm were obtained against *S. aureus* and *E. coli*, respectively.

The present study demonstrates that the preparation pH is associated with measurable changes in the structural, morphological, and antibacterial characteristics of the prepared $\text{Ni}_{0.6}\text{Zn}_{0.4}\text{Fe}_2\text{O}_4$ nanoparticles. The antibacterial mechanism was not directly investigated in this study; therefore, mechanisms such as reactive oxygen species generation, membrane disruption, and metal-ion release are considered possible explanations rather than experimentally confirmed mechanisms.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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