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ANTIBACTERAIL ACTIVITY OF ZnO AND Na / ZnO NANOROD ARRAY

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ABSTRACT---*A novel method was used for synthesized Na/ZnO nanorods. Various techniques were used to determine the structural properties of pure and loaded ZnO Nanorods such as X-ray diffraction (XRD) , Scanning electron microscopy (SEM) , Energy dispersive spectra(EDS) and Atomic force microscope (AFM). Also biological activity was studied through bacterial inhibition of nanorods against gram positive (Pseudomonas) and gram negative (Staphylococcus aureus) were measured by total account of bacteria technique The results show that the antibacterial activity was inhibited by using Na/ ZnO nanorods compared to the activity of pure ZnO NPs . The mechanism of microbial inhibition in presence of pure and loaded ZnO nanorods in aqueous solution was suggested.*

***Keyword*:--** loading ZnO , antimicrobial inhibition of nanoparticles and Na loaded ZnO.

I. INTRODUCTION

Nanotechnology deals with the manufacture and application of materials with size of up to 100 nm. They are widely used in a number of processes that include material science, agriculture, cosmetic , medical, food industry and diagnostic applications[1]. Nanosize semiconductors have shown remarkable antibacterial activity at very low concentration due to their unique chemical and physical features and high surface area to volume ratio [2].

Nanoparticles of ZnO are a promising material for the realization and future of nanotechnology ZnO can be utilized for electronic and photonic devices, as well as for high-frequency applications." ZnO is a key technological material and it is a unique material. ZnO a direct band gap semiconductor with the band gap of 3.37eV

[3] that is suitable for short wavelength optoelectronic applications and high exaction binding energy of 60 meV [4] . ZnO has been synthesized using several method as like homogeneous precipitation [5] microwave methods [6] pulsed laser deposition thermal evaporation [7] and sol–gel method[8]. The physical and chemical properties of ZnO nanoparticles such as size, size distribution and crystallity , morphology melting point, band gap, optical and electronic properties strongly dependence to preparation method of synthesis of this material[9]. The co-precipitation method is a popular method because of its low cost, reproducibility, simplicity and reliability conditions of synthesis[10].

ZnO nanoparticles, with their unique properties such as biocompatibility, high selectivity, enhanced cytotoxicity and easy synthesis, may be a promisingantibacterial agent[11]. The various antibacterial mechanisms of nanomaterials are mostly attributed to their high specific surface area-to-volume ratios [12].

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II. EXPERIMENTAL PART

1.1. Materials

Zinc nitrate ($Zn(NO_3)_2$), sodium chloride (NaCl), sodium hydroxide (NaOH), absolute ethanol (C_2H_5OH) and Mueller-Hinton agar.

1.2. Synthesis of pure loaded zinc oxide nanorods .

For the synthesis of zinc oxide nanorods (ZnO NRs), the following procedure was used. The chemical precursors used in reaction $Zn(NO_3)_2$, NaOH and deionized water .5.6 g of Zn $(NO_3)_2$ was dissolved in 250 ml of deionized water , then (1 M) of NaOH was drop wise added to the solution under vigorous magnetic stirring with speed of 1250 rpm at room temperature until pH of the solution reached 13. Afterward the heterogeneous solution was refluxed at about 110 C^0 for 3 h. The white precipitate was separated and washed two times with deionized water and ethanol to remove the unreacted reagents and dried in an oven at 70C^0 for 24 h. Then the product was crushed into powder by using ceramic mortar .The final product was obtained by calcination of the precipitate at 200^{0}C for 1 h.

Na/ZnO NRs were synthesized by using the same procedure of preparing pure ZnO NRs 4 g NaCl was add to the aqueous solution of Zn $(NO_3)_2$ before added (1 M) NaOH .

1.3. Preparation of pure and loaded ZnO NRs for total account of bacteria Method:

Nanorods were weighed as 0.01 g of then dissolved in 5 ml deionized water and treated with ultrasonic for 45 minutes, then 100 µl of bacteria was injected to the suspension solution and culture bacteria after shaking the solution incubator for 1 hour, . bacteria was inoculated on Mueller-Hinton agar medium and incubated at 37°C for 24 hours. the Inhibition percentage of bacteria was calculated by using the following equation[13]:-

$$Inhibition\ percentage = \left(\frac{C_0 - C_t}{C_0}\right) X\ 100 \quad \text{----------(1)}$$

$$\left(\qquad \right)$$

III. ANALYTICAL RESULTS AND DISCUSSION

3.1 X-ray Diffraction Analysis (XRD)

X-ray Powder Diffraction (XRD) studies were carried using X-ray diffractometer with cu kα radiation (λ = 1.5418 Å) in the range of 10–80° to determine their crystal structure and phase. The crystalline phase and purity of ZnO predicted from XRD

analysis and shown in Figure 4. The diffraction peak of pure ZnO appeared at 2

values 31.793°, 34.459°, 36.282°, 47.558° and 56.607° are related to (100), (002),

(101) (102) and (110) phases of zinc oxide respectively .These results show a high agreement between the pattern of the prepared sample and the phase of standard ZnO (JCPDS Card No. 36-1451), which is a hexagonal wurtzite polycrystalline structure. This demonstrates that Na doping increases the lattice constant as a result of larger ionic radius of Na^{+} (0.102 nm) compared with that of Zn^{2+} (0.074 nm)[14]. The average crystallite size of ZnO calculated by the Debye-Scherrer[15] equation (2):

$$D=\frac{k\lambda}{\beta Cos\theta}\text{----------(2)}$$

Where λ is wave length of X-Ray (0.1541 nm), βi is FWHM (full width at half maximum)in radians θ is the diffraction angle (18.267° and 18.240° for sample A and B respectively) D is particle diameter size. The average crystallite size of the ZnO NRs was estimated to be about 24.381 nm, while the average size crystal of Na/ZnO NRs was increased to 31.15 nm.

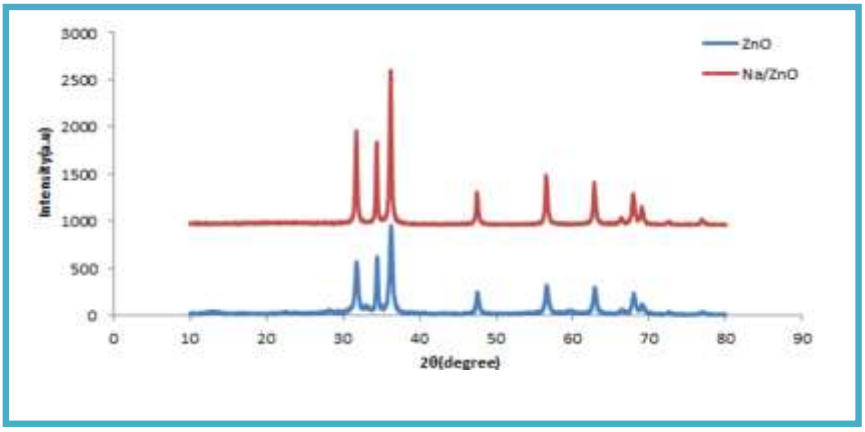


Figure 1: XRD pattern of ZnO NRs and Na/ZnO NR

3.2 ***Scanning Electron Microscopy (SEM) and Energy dispersive spectra EDS***:- *Fig.(2)* shows the Scanning Image Microscopy (SEM) image of pure and sodium loaded ZnO nanostructures. This SEM image revealed the morphology of the nanoparticles, which resemble to agglomerated particles to form a cluster[16]. The results of this analysis showed all samples have an individual rod structure with the grain size ranging between 40-53.

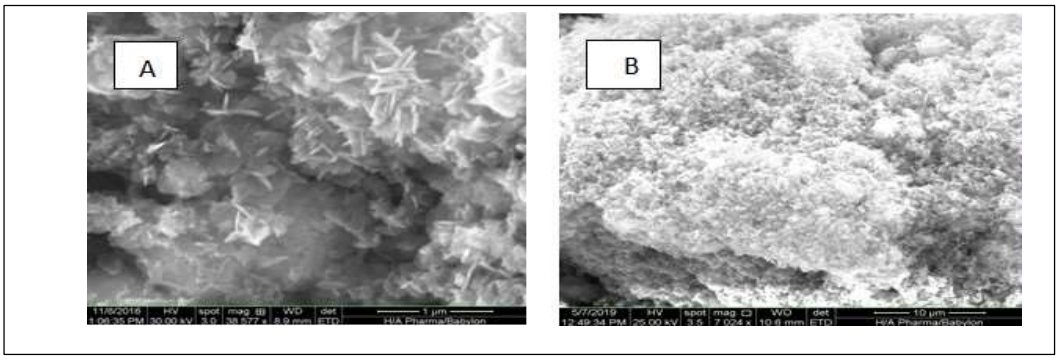
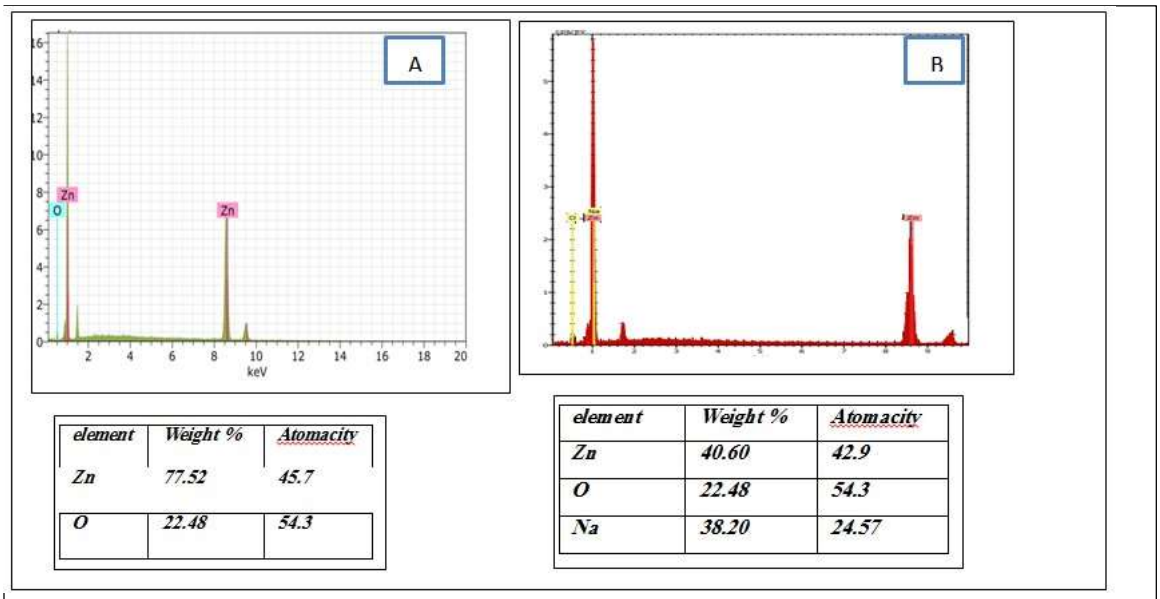


Figure 2: SEM images of pure ZnO(A) and Na/ZnO NRs(B) respectively**.**

Fig.(3) shows the Energy Dispersive Spectroscopy (EDS) spectrum of 39.45 wt % Na loaded ZnO NRs. The results of EDS analysis of ZnO nanoparticles show 52% of zinc and 48% of Oxides in which confirms the elemental composition of ZnO nanoparticles. Sodium was appeared in the Na/ZnO NRs.



element	Weight %	Atomacity
Zn	77.52	45.7
O	22.48	54.3

element	Weight %	Atomacity
Zn	40.60	42.9
O	22.48	54.3
Na	38.20	24.57

Figure 3: EDX of pure ZnO_{NPs}(A) ,Na/ZnO NRs(B) respectively.

3.3 Atomic Force Microscopy (AFM):

The size of the nanoparticles is obtained directly from tip-corrected AFM measurements, and the shape of the nanoparticle is estimated on the basis of AFM images and line scans. and the average grain size for pure ZnO NPs was found about 35.89 nm this average size was increased to 40.01 nm **.**AFM images were also used for roughness,

porosity and fractal dimension[17] Figure (4) shows the AFM (2-D) images of pure ZnO_{NPs} and loaded ZnO NPs. AFM images prove that the

grains are distributed homogeneously within roughness average (Sa) around to(7.74 ,0.73)nm.

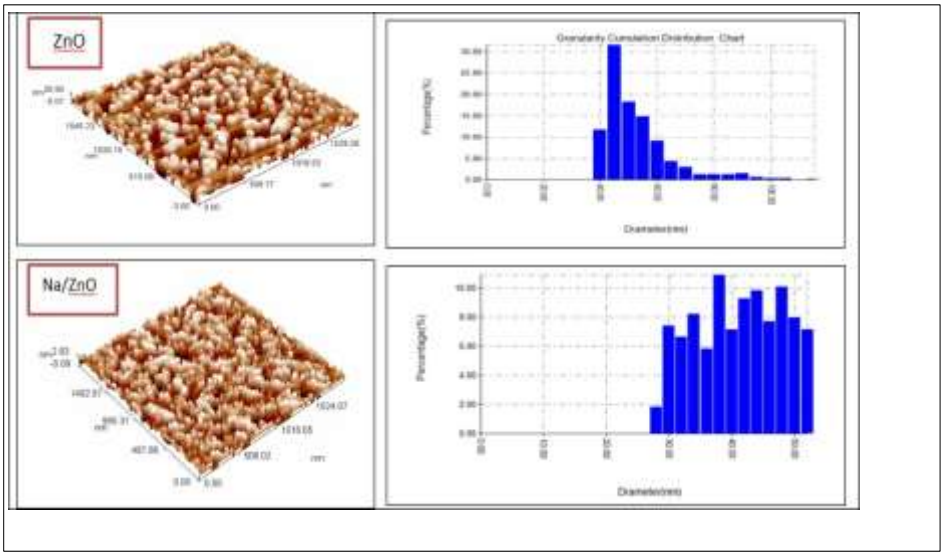


Figure 4: AFM of pure ZnO_{NPs} (A) ,Na/ZnO NRs(B) respectively.

2.7.Antibacterial activity of ZnO and Na/ZnO NRs.

The changes of the Inhibition percentage against gram-positive and gram-negative bacteria in presence of ZnO and Na/ZnO were show in Figure 5 and 6. The results show that pure ZnO nanorods had some antibacterial effects on Pseudomonas and staphylococcus area ,while the presence of Na loaded on the surface of ZnO NRs produced small decreases of bacterial inhibition due to increasing of size particles of Na loaded on ZnO nanorods compared with the pure ZnO nanorodes [18].

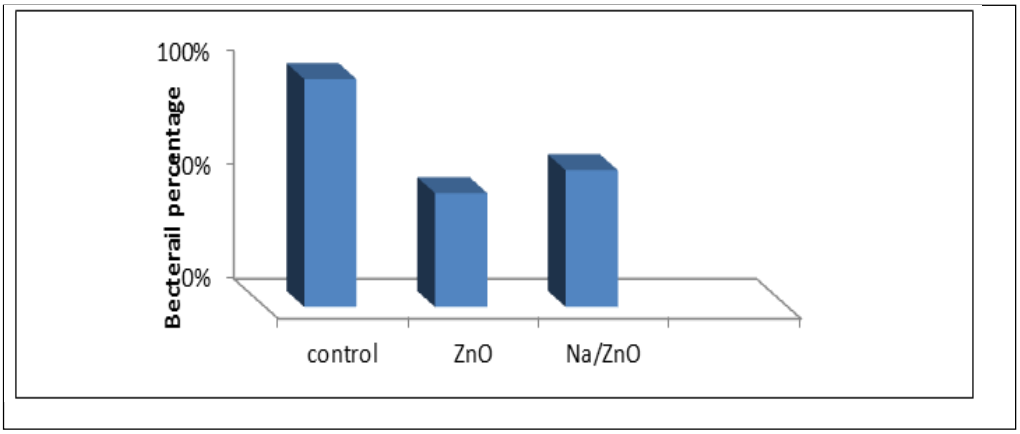


Figure 5: Inhibition percentage for gram positive bacterial by using of pure ZnO_{NPs}, and Na/ZnO NRs respectively

Figure (7) shows that ZnO NPs were more toxic toward Gram-positive bacteria more that Gram-negative bacteria These bacterial strains have chemical composition and different structures of their cell wall. The outer

membrane of Gram-positive bacteria is different from Gram- negative bacteria. Gram negative bacteria have peptidoglycan in its outer layer so they show staining and also helpful for protection from outer substances while this layer was absent in Gram- positive bacteria[19]. The metallic ions of nanometer range attached to the cell via trans membrane protein. After attaching to bacterial cells, producing structural changes in the cell membrane and blocking the transport channels[20] , NPs may be internalized, produce ionization within the cell, and damage intracellular structures resulting in cell death[21] .

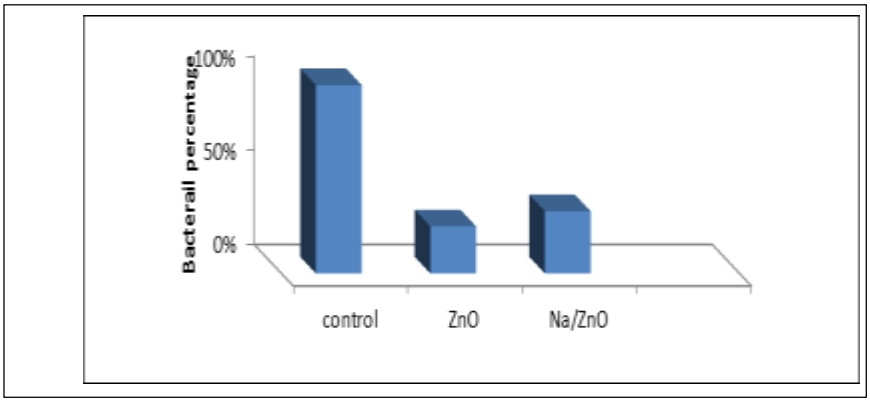


Figure 6: Inhibition percentage for gram negative bacterial by using of pure ZnO_{NPs}, and Na/ZnO NRs respectively.

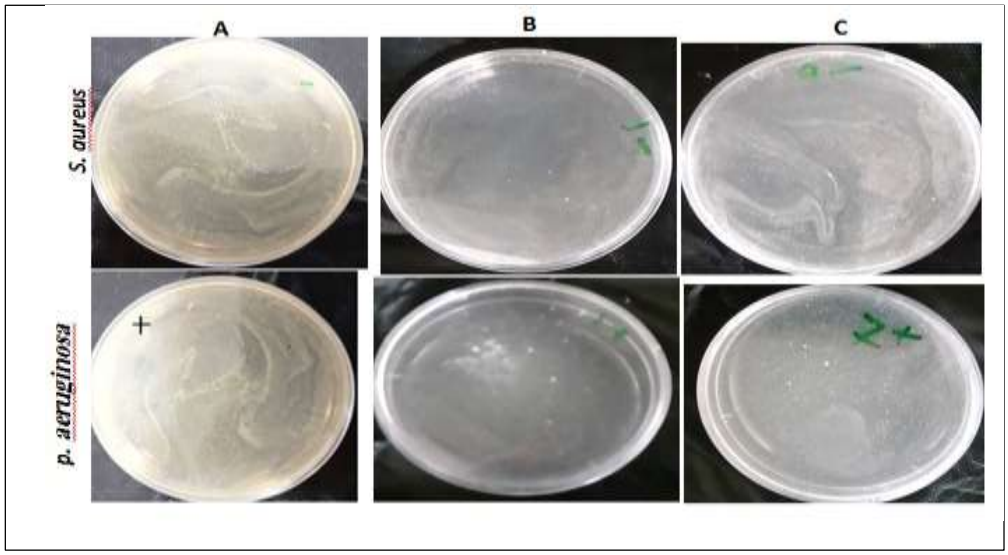


Figure 7: S. aureus and p. aeruginosa plating for colony count (A)control ,(B)pure ZnO and (C)Na/ZnO NRs respectively.

IV. MECHANISM OF BACTERIAL INHIBITION

The mechanism of antibacterial activity of ZnO nanoparticles is not completely illuminated and still controversial, as there are some queries within the spectrum of antibacterial activity requiring deep explanations. Distinctive mechanisms that have been put forward in the literature are listed as following: direct contact of ZnO-NPs with cell walls, resulting in destructing bacterial cell integrity , liberation of antimicrobial ions mainly Zn^{2+} ions[22]. The morphology of ZnO nanoparticles dependent release of Zn^{2+} ions on spherical structures that had the

highest increase in the release of Zn^{2+} ions than rod structures. It was elucidated on the fact that smaller surface curvature of sphere causes high equilibrium solubility. Also, Wang et al. [23]studied the morphology-dependent dissolution of metal ions. The free ions of Zn^{2+} immediately bind with the biomolecules such as proteins and carbohydrates, and all vital functions of bacteria cease to continue. Ions of Zn^{2+} are attached to the biomolecules in the bacterial cell by electrostatic forces. These ions actually coordinated with the protein molecules through the lone pair of electrons on the nitrogen atom of protein par. Antibacterial influence of metal oxide nanoparticles includes its diffusion into the bacterial cell, followed by release of metal ions and DNA damage leading to cell death[24] .

Particle size of ZnO-NPs are play important roles in the antibacterial activity. ZnO- NPs of smaller sizes can easily penetrate into bacterial membranes due to their large interfacial area, thus enhancing their antibacterial efficiency. A large number of studies investigated on the considerable impact of particle size on the antibacterial activity, and the researchers found that controlling ZnO-NPs size was crucial to achieve best bactericidal response, and ZnO-NPs with smaller size (higher specific surface areas) showed highest antibacterial activit[25]. ionic radius of Na^{+} was larger than of Zn^{2+} and this leads to increase size particles of Na/ZnO NPs compared to the size of pure ZnO NPs. so that The antibacterial efficiency decreased with increasing particle size.

V. CONCLUSIONS

- Pure ZnO and Na /ZnO NRs were synthesized by one-pot refluxing method in water at about 110 C^0.
- nanorods were characterized by various techniques, including XRD, SEM, EDX and AFM.
- Antibacteral activity of ZnO NRs was decreased when Na loaded on ZnO NRs due to the increasing of size particles of Na/ZnO NRs .

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