Effect of Zinc Oxide nanoparticals preparation from Zinc Sulphate ($ZnSo_4$) against gram negative or gram positive microorganisms *in vitro*

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Summary

This research aims to prepare ZnO NPs by using chemical bath deposition way from $ZnSO_4$ and NaOH as starting materials. It was examined by X-ray diffraction, Scanning Electron Microscopy, Zeta potential and Fourier Transformation Infrared. Scanning Electron Microscopy images showed various morphological changes of ZnO nanoparticles obtained by the above method and the different magnification Scanning Electron Microscopy images of the nanoparticle and confirms that the Nano flowers are grown with well-defined morphology and diameters varying between 60-110 nm. The effect of Zinc oxide nanoparticles against bacteria *staphylococcus aureus*, *E.coli* and *Pseudomous aeruginosa* showed the ability of this substance to inhibit the growth of all types of bacteria in different concentrations. The percentage of survival bacteria was (2, 3.7 and 6%) for *E.coli* bacteria and (1, 1.5 and 5 %) for *Pseudomous aeruginosa* bacteria, while the percentage was (0.8, 1 and 1.5 %) for *staphylococcus aurous* respectively for all concentration.

Keywords: Chemical deposition, ZnO Nanoparticles, Zinc sulfate, *Staphylococcus aureus, E.coli, Pseudomous aeruginosa.*

Introduction

In the previous decade, Nano materials have been created and connected broadly in various diverse (1). Nanoparticles are characterized with width littler than 100 nm and are progressively utilized as a part of various applications, including drug delivery and to pass organ barriers like blood-brain barriers (2).

Nanotechnology is a field of convergence among life sciences, material science and it is sectors. The evolution of nanoparticle for medicinal and nutritional application have been found to exhibit useful properties, because they are different from those particles of micro and macro scale (3). There are a lot of materials to reduce bacterial grouping on outer layer of tissue including molecules possessing positive charged such as Triazin derivatives, Ttriclosan, Chitosan, Copper, or Silver. Zinc oxide is considered the best of these substances and has been decided to use zinc oxide (ZnO) as antibacterial factor, because it is low in toxicity and of low cost (4). ZnO was an II-VI mixes semiconductor with a steady quartzite structure and a direct wide band hole (3.37 eV) (5 and 6); ZnO is economically accessible with favorable circumstances, for example, relatively minimal effort, agreeable non-dangerous nature, high protection from radiation harm, and a high warm and concoction strength (7). It has a very high surface to volume ratio i.e. increased number of atoms per unit volume. It increases the proportion of atoms at the surface and hence increases its relative proportion inside the prescription volume. They can be very easily absorbed from the intestine or stomach and can go everywhere in the human body and interferes with sub cellular mechanisms (3).

For the advancement of antimicrobial materials for employments on an expansive scale, like in prescription and for wearing clothing, it has important to locate the ideal of ZnO focus to adequately battle microbes where as counteracting cytotoxic impacts. In this way, the principle center of our examination is the examination of the relationship between's the ZnO content, the antimicrobial action and the cyto compatibility of ZnO covered materials. Moreover, our examination tends to the change of the covered polyester with respect to the visual semblance and particularly of the perpetual quality of the antimicrobial impact. The antibacterial movement of ZnO Nanoparticls covered materials has been demonstrated utilizing the nosocomial pathogens *Staphylococcus aureus*

and *Klebsiella pneumoniae* (4). There have been numerous techniques to blend nano ZnO. A portion of the broadly utilized strategies are for example, Chemical vapor affidavit (CVD), plunge covering, or mechanical alloying (8). In this experiment, Zinc oxide nanoparticles were synthesized by chemical bath deposition method from Zinc Sulfate with Sodium hydroxide as starting materials. Then, its effect is tested against *staphylococcus aureus* as gram positive bacteria and *E. coli*, *Pseudomous aeruginosa* as gram negative bacteria because the resistances of many types of bacteria to a lot of types of antibiotics.

Materials and Methods

Synthesis of Zinc oxide Nanoparticles: Zinc sulfate heptahydrate with sodium hydroxide were utilized as part of the analyses. Each one of the chemicals used was of systematic reagent survey got from Merck (Mumbai, India), and deionized water is used for the preparation of arrangements. ZnO nano particles prepared from Addition of 0.1 M from solution zinc sulfate with 0.2 M NaOH, and then put solution on hot plate temperature 100℃ under vigorous stirring and PH 12, for 2hr. After dissolving completely NaOH was added drop by drop for 30 min. A colorless solution obtained was sealed in a beaker and allowed to settle overnight. A white precipitate was acquired at the base and mother liquor on top was evacuated. The white precipitate was washed several times with deionized water which is now assumed to be Zn $(OH)_2$ free of impurity. ZnO nanoparticles was obtained by drying at temperature of 400℃ (9).

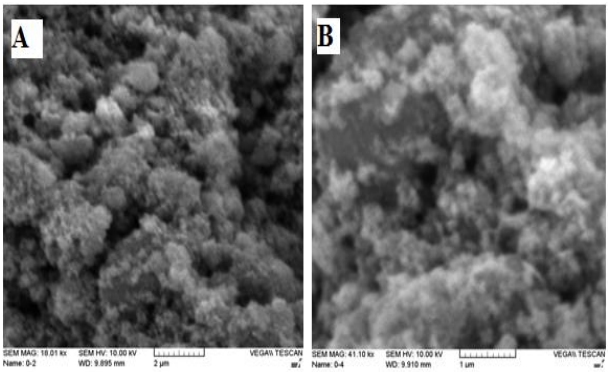
Antibacterial activity of zinc oxide nanoparticles tested against *Staphylococcus aureus* as gram positive bacteria and *E. coli*, *Pseudomous aeruginosa* as gram negative bacteria. This bacteria was cultured on nutrient agar for 18-24 hr. at 37°C and bacterial suspension was prepared by using normal saline (0.9%) to obtain bacterial samples with concentration ~10^7-10^8 CFU/ml by 0.5 McFarland standards. Then one ml of each bacterial suspension was add to 9 ml of normal saline containing zinc oxide nanoparticles in different concentration (0.8, 1.6 and 3.2 mg/ ml) to reach to this concentrations and incubated at 37 °C in incubator shaker at 160 rpm for 24 hr. Then, the mixtures was serially diluted in normal saline and cultured on Molar Hinton Agar (using 100 µl spread out on M.H. agar), incubated at 37°C for 24 hr. Then colonies were counted after the incubation in plates (10). The formula to calculate the bacteria survival rate is as following:

Number of Colonies [(CFU)/ml] = (Number of colonies for each dilution)/ (dilution 10^3 factor)/ sample volume. As well, evaluated the percentage decreasing of bacteria by bacteria survival rate K (11)

$$K = \frac{(A - B)}{A} x100\%$$

Results and Discussion

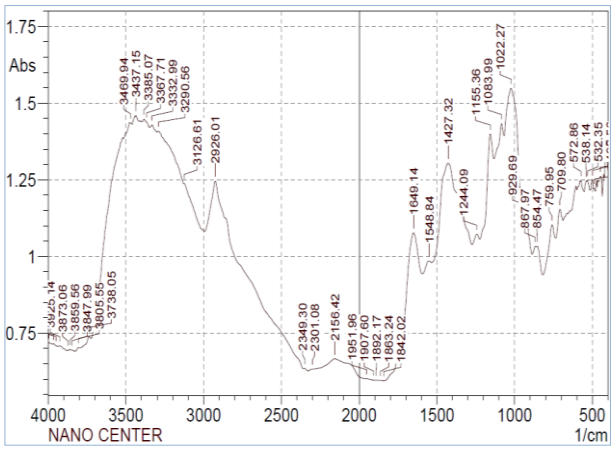
The functionalized particles were characterized by the following techniques (Fig. 1) shows the different magnification SEM images of the nanoparticle and confirms that the Nano flower are grown with well defined morphology, due to the addition of NaOH to the solution thus raising the pH framed in the form of oxide product(PH 12). The results indicated that addition of high amount of NaOH to aqueous solution of solution could greatly affect the morphology and size of ZnO. This procedure of preparation of ZnO NPs, concrete the nanopartical shape and diameters varying between 60-110 nm.



Figure, 1 A and B: Shows the images of SEM of ZnO nanopartical.

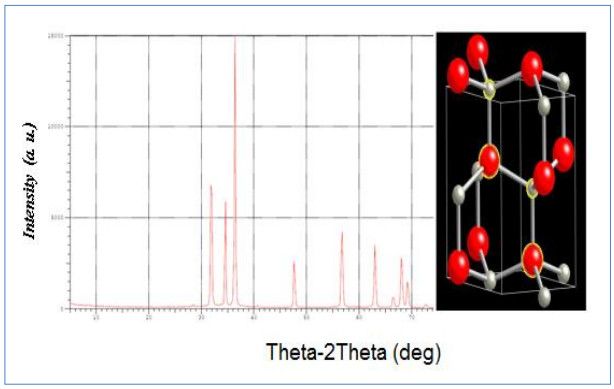
The FTIR spectrum of zinc oxide nanoparticals are shown in (Fig, 2) that was possessed in a chain absorption summit from 400 to 4000 cm^{-1}. Bands of 417, 437 and 740 cm^{-1} spectra of ZnO and 1540.1, 1492.9, 1357, 1043.49, 833.25 a companion with expansion oscillation of crystalline hexagonal ZnO expansion vacillation. The broad absorption

peaks at the range (3200-3600 cm^{-1}) belong to the presence of hydroxyl group of vibration at the surface of ZnO samples.



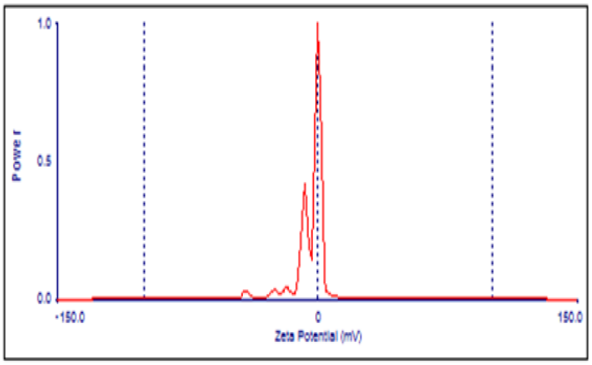
Figure, 2: Show the FTIR of ZnO nanopartical.

Figure (3) shows XRD pattern of synthesized ZnO. The pattern shows that all the diffraction peaks indexed to the hexagonal phase of pure ZnO nanoparticles with a quartzite structure there were Seven pronounced ZnO nanoparticles diffraction peaks appearing to scattering angles (2θ) = 31.76°, 34.21°, 37.2°, 48.1°, 57.49,° and 71.08 correspond to the reflection from: (100), (002), (101),(102), (110), (103) and (112) crystal planes respectively. A strong peak corresponding to (101) plane with a full width at half maximum of approximately 0.28879 is experience. Moreover were no diffraction peaks belong to free Zn or any other impurities observed in the spectrum, this result indicates the excellent nature of the synthesized materials. As well as, the reasonable and sharp pinnacles likewise uncovered that the ZnO nano partical have a high crystalline quality.



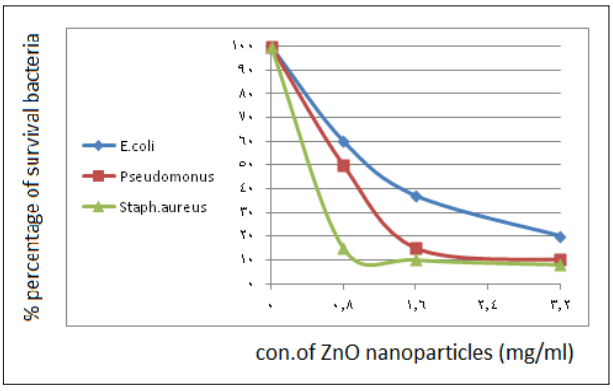
Figure, 3: XRD patterns of ZnO nanopartical.

ZnO nanopartical was resolved at pH 12 with zeta potential esteems - 35.65 mV and a normal molecule size of 60.nm the ZnO NPs were found to in part disaggregate because of surface charge shock disaggregate because of surface charge aversion (12-14), as shown in (Fig. 4).



Figure, 4: Show the Zeta potential of ZnO nanopartical.

Effect of Zno nanopartical on Bacteria: The results of using zinc oxide nanoparticles preparation from zinc sulfate by using sol-gel method explain ability of this material to effect on both gram positive and negative bacteria. It used different concentrations (0.8, 1.6 and 3.2 mg/ml) of zinc oxide nanoparticles. The percentage of survival bacteria was (2, 3.7 and 6%) for *E.coli* bacteria and (1, 1.5 and 5%) for *Pseudomonas* bacteria, while the percentage was (0.8, 1 and 1.5%) for *staphylococcus aureus* respectively for all concentration (Fig. 5 and 6).

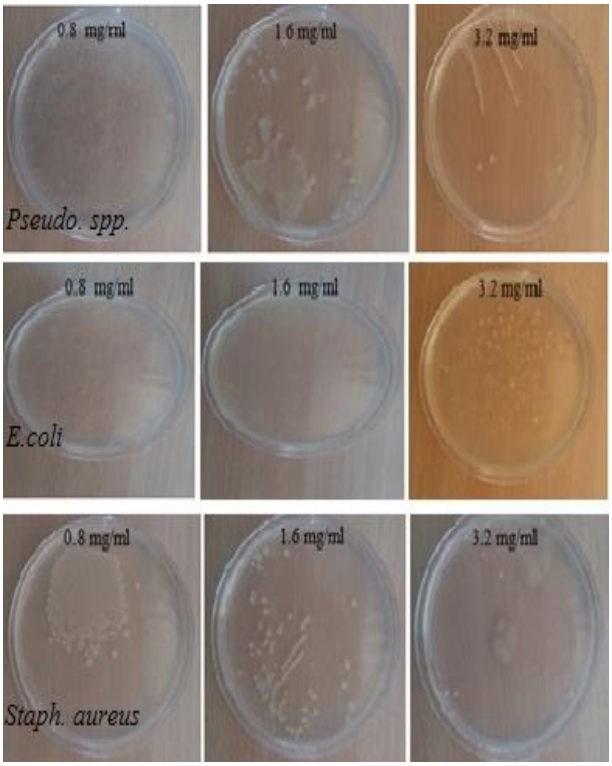


Figure, 5: Percentage of survival rate of bacteria in different concentration of ZnO nanoparticles.

The results showed the ability of ZnO nano particles to affect on bacteria when used in different concentrations and showed the ability of high concentration as compared to little amount. This result agrees with (15) that clarified the vital parts of Particle size and

centralization of ZnO NPs in the antimicrobial action. ZnO NPs antibacterial movement specifically relates with their focus as announced by a few examinations, in a similar manner, the action is estimate subordinate. In any case, this reliance is additionally impacted by convergence of NPs. Bigger surface region and higher fixation are responsible for ZnO NPs antibacterial action.

Also it agreed with (16) who explained the ability of this Nano material to interact with organic compound of surface wall bacteria and destroy it. That led to destroy the cell wall and death of bacteria, and with (17) who also suggested that NPs interact with the plasma membrane of the bacterial cell, this affects the surface chemistry and function and reduces the levels of adenosine triphosphate and the primary energy molecules, which affects the stability of the cell membrane.



Figure, 6: Antibacterial activity of Zinc oxide Nano particles on Gram (-ve and +ve) bacteria in different concentration.

It has demonstrated the synthesis of ZnO NPs by chemical bath deposition method. XRD design demonstrated that all the diffraction tops in the example can be effectively ordered as the unadulterated hexagonal period of ZnO NPs with a quartzite structure. The SEM images of NPs with increase pH depended on the concentration of NaOH, where zeta potential values -165.65 mV for pH 12 with an average particle size of 60 nm, also the results of NPs ability of this substance on both types of bacteria in different concentrations.

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تأثير جسيمات أوكسيد الزنك النانوية المحضرة من كبريتات الزنك ضد البكتريا الموجبة والسالبة لصبغة غرام في الزجاج

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الخلاصة

يهدف البحث إلى تصنيع جزيئات أوكسيد الزنك باستعمال طريقة ترسب حمام كيميائي من كبريتات الزنك وهيدروكسيد الصوديوم كمواد أولية لبدء التفاعل. وقد درست خصائصها باستعمال جهاز حيود الأشعة السينية والمجهر الإلكتروني الماسح، وجهد زيتا المحتملة، تحولات فورييه الأشعة تحت الحمراء وقد أظهرت صور المجهر الإلكتروني الماسح التغيرات المورفولوجية المختلفة من أوكسيد الزنك التي حُصل عليها بالطريقة المذكورة أعلاه. كما اظهرت الصور أشكال الجسيمات النانوية بشكل يشبه الازهار وأقطار تتراوح بين 60-110 نانومتر. دُرس تأثير جزيئات أوكسيد الزنك النانوية على بكتريا المكورات العنقودية الذهبية والإيشريكيا القولونية والزائفة الزنجارية مختبرياً وقد اظهرت تلك المادة قدرة كبيرة في التأثير في كل انواع البكتريا عند استعمالها بجميع التراكيز المختلفة، فكانت النسبة المئوية لبقاء بكتريا الإيشريكيا القولونية هي (2 و3.7 و6 %) ولبكتريا الزائفة الزنجارية كانت(1 و1.5 و5 %) بينما لبكتريا المكورات العنقودية الذهبية كانت (0.8 و1 و1.5 %) بالترتيب وعند استعمال جميع التراكيز السابقة.

الكلمات المفتاحية: الترسيب الكيميائي، أوكسيد الزنك النانوي، كبريتات الزنك، البكتريا العنقودية الذهبية، الإيشريكيا القولونية، الزائفة الزنجارية .