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Influence of Modified ZnO Quantum Dots and Nanostructures as New Antibacterials

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ABSTRACT

Antibacterial activities of various spherical zinc oxide nanoparticles and nano special morphological structures including quantum dots, nanorod arrays, nanoporous shapes and needle-like crystals had been investigated as new nanomedicine compounds. Also antibacterial activity based on minimal inhibitory concentration and the growth inhibitory zone (well method) was evaluated. ZnO nanostructures were fabricated by novel hydrolysis sol-gel-hydrothermal process followed with rapid quenching as new technique using glycerine, vegetable fatty esters such as coconut, sunflower and Lauric alcohol ethoxylated as organic templates soluble in eco-friendly nanofluids. The results showed that *Bacillus anthracis* and *Pseudomonas aerogenes* were extremely sensitive to treatment with unique ZnO nanostructured. Their growth inhibitory zone presented 30 mm and 25 mm inhibition zone with better inhibitory effect compared to the Gentamicin antibiotic standard. ZnO nanostructures had also been indicated to have a wide range of antibacterial activities against both Gram-positive and Gram-negative bacteria especially more effective on (gr+) species using the growth inhibitory zone. We could design and make significant formulations of fatty acids and esters-capped ZnO quantum dots nanofluids which created high promising agents for controlling Anthrax, Staphylococcus epidermidis and their influences in antimicrobial properties with low cost for future.

Keywords: Nanobiotechnology; Antibacterial Activity; Hydrolysis Sol-Gel-Hydrothermal; ZnO Quantum Dots; MIC and Well Method; Complex Defects

1. Introduction

Strong luminescence material zinc oxide including hexagonal wurtzite crystal is a wide band gap (3.37 eV) semiconductor with a large excitation binding energy and an exciton Bohr radius in the range of 1.4 - 3.5 nm [1] and is a commercially important material used in paints, rubbers, concrete, electronics, lasers, transistors, photodetectors, gas and biosensors, piezoelectric and solar cells, optoelectronics, photocatalysts, cosmetic, biomedicine, food industry, anticorrosive coating, antibacterial and antifungal agents. These wide varieties of prominent applications require the fabrication of special morphological and functionalization of ZnO nanostructures surface [2-4]. In fact, if we are able to modify the surface of ZnO nanoparticles, the most of their excellent properties will wonderful be obvious. Many efforts have been made to synthesize ZnO with various morphologies, including nanorods, nanowires, nanorings, nanoflowers, nanospherical, nanotubes, nanodisks, nanodumbbells, nanoneedles, nanowhiskers, nanonail, nanobelts, nanosheets, nanosprings, nanoribbon and many more by self-assembly of nanoscaled building blocks [5,6]. To achieve these interesting morphologies there are many different preparing techniques for synthesis of ZnO nanostructures such as direct precipitation, spray pyrolysis, microemulsion, the hydrothermal treatment, sol-gel process using surfactant additive as templates (chemical hydrolysis), wet-chemical

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procedure, audible sound method, hydrothermal microwave heating, flame spray pyrolysis (FSP), high-temperature methods, chemical vapor deposition (CVD), molecular beam epitaxy (MBE) [7], and finally the particular rapid cold or hot annealing quenching sol-gel method (cold and thermal shock) for producing of new quantum dots ZnO nanostructures.

Quantum-sized nanodots ZnO represents a unique class of zero-dimensional nanostructures which can generate novel properties that differ from those of their bulk crystals due to their small sizes and large surface-to-volume ratios [8]. In the quantum-size region, the absorption of UV or visible light strongly depends on their size, shape, kind of fabrication conditions and techniques, temperature of calcination, annealing condition and aging time, presence of dopants and on PVP (polyvinylpyrrolidone) or green fatty acids coating. Actually, these key factors are able to produce n-type semiconductor containg the measured of direct band gap in quantum-dots ZnO nanoparticles. On the other hand, the visible emission is mainly attributed to the surface or structural defects of the crystal, resulting in large variations in the emission peaks [8]. The existence of certain defect complexes such as V_{Zn}-H_i, O-H, and Zn-H_O has advantages over the pure semiconductor and quantum dots ZnO nanostructures [9] and a wide visible-emission at the deep level defect in the ZnO nanoparticles (NPs) are due to the existence of cationic Zn vacancies (V_{Zn}), oxygen vacancies (V_O), Zn interstitials (Zn_i), charge defects, surface defects and their complex defects [10].

Semiconductor quantum dots (QDs) have shown unique optical properties, strong photoluminescence (PL) emission and potential applications in biological fluorescent labels like CdSe and CdTe QDs [11] and ZnO as a nontoxic and cheap luminescent material is a promising candidate as antibacterial agent and several mechanisms have been proposed for this evidence [12] which is considered to be due to the induction of intercellular reactive oxygen species, including (H_2O_2) from its surface as a strong oxidizing agent harmful to bacterial cells which can penetrate into the cell membrane [13,14]. The high rate of generation of surface oxygen species from ZnO leads to the death of the bacteria. There are some reports on the considerable antibacterial activity of TiO_2, MgO, CaO, SiO_2 and ZnO [15] which is attributed to the generation of reactive oxygen species on the surface of these inorganic oxides owing to they contain mineral elements essential to humans and exhibit strong activity even when administered in small amounts. Another possible mechanism for ZnO antibacterial activity is the release of Zn^{2+} ions. It is well known that ZnO normally becomes unstable in the solution, and when H_2O_2 is produced, the Zn^{2+} ion concentration is increased as a result of ZnO decomposition [16]. Quantum dots (QDs) ZnO nanoparticles have shown strong activity against some of Gram- positive and Gram-negative bacteria and biocompatibility with colloidal semiconductor luminescent inorganic materials [17] and a promising member of the Cd-free QD family is ZnO nanoparticles. Functionalization and treat- ment of ZnO quantum Dots with polymers, organosilanes and vegetable fatty acids is able to modify the surface of QDs ZnO. Nanospherical, nanorods, nano-porous ZnO and nanowire morphologies could be used as effective bactericidal materials against both Gram-positive and Gram-negative bacteria. ZnO quantum dot nanoparticles containing polymer templates (PVP, PEG, PVA, polystyrene) and oleic acid treatment will be promising candidate as interesting nanodrug-carriers and also new antibacterial agents. ZnO is one of five zinc compounds that are currently listed as generally recognized as safe by the U.S. Food and Drug Administration (21CFR182.8991). Antimicrobial efficacy of zinc oxide quantum dots contain polystyrene and PVP as active antibacterials against some of bacteria was investigated in culture media and liquid egg white [3] and was found that the functionalization and treatment of surface can produce significant results. The availability of ZnO QDs in media was important for antibacterial efficiency. They even have antibacterial activity against spores that are resistant to high temperature and high pressure and parameters such as size of ZnO nanoparticles, larger the surface area, crystalline structure, particle shape, concentration, time, temperature and combination with other bacteriocins (synergistic effect) will be the focus of further study. However, the anticancer property of ZnO nanoparticles is an undeveloped area that is of potential medical interest in future [18-22].

In the present study, three various groups of ZnO nanostructures such as nanoparticles, quantum dots, and modified surface compounds using polymers, vegetable fatty acids, coconut, sunflower esters have been synthezied and also ZnO nanoparticles doped silica-substrate was made. We believe that they can generate strong nanoporous surfaces liable to do chemical reactions with live bacteria. Also the types of nanospherical, nanorods and nanoporous ZnO morphologies could be characterized. It was also found that the size of the quantum dots could be tailored by controlling the process parameters aligned with additional surfactants, stabilizers, suitable solvents or capping agents. Audible sound method and sol-gel hydrothermal microwave process were carried out for synthesis of ZnO nanostructure and antibacterial activities against both gr+ and gr− bacteria were successfully investigated by well method and MIC test. Potential applications of ZnO QDs and treatment products were observed for most of both strong microorganisms for the first time.

2. Experimental Details

All chemicals are analytical grade and are used as received without further purification.

Preparation of ZnO Nanostructures by Sol-Gel Process, Wet-Chemical Procedure, Audible Sound and Hydrothermal Microwave Method

Sample 1: 0.03 mole zinc acetate dihydrate Zn $(CH_3CO_2)_2{\cdot}2H_2O$ in 15 - 20 ml isopropanol solution was dissolved (solution A). 10 ml coconut fatty glycerine ester-PEG was added and heated. The resultant cold-mixture containg of isopropanol, glycerine and LA7EO (Lauryl alcohol 7 mole ethoxylate) was prepared (solution B). Solution A was added to solution B under the rapid cold quenching and was hydrolyzed with 20% NaOH solution under vigorous stirring and was kept at 0˚C. After the mixing, they were heated to reflux quickly at 70˚C - 85˚C in the hot bath. The product was washed and filtered and finally dried at 100˚C in oven and subsequently calcined at 850˚C.

Sample 2: The sample 1 was washed and filtered several times with H_2O/ethanol (1:1 v/v) solution. The obtained sol was dried in hot air oven at 90˚C for 48 h further the white powder was calcined at 700˚C again for 6 h to form ZnO nanocrystals.

Sample 3: This product is the same as the sample 1, but we used cold 1, 2-propylene oxide (C_3H_6O) nonionic surfactant instead of coconut fatty glycerine ester-PEG and 0.6 gr. hydroxy propyl cellulose (J-type) as stabilizer with 2 gr. starch in white powder before of calcination process.

Sample 4: Audible sound is produced by sound pressure applied to a listener's ear. The pressure is initiated by some mechanical devices like speakers that create a series of pulses of energy which cause air molecules to vibrate. The sol-gel method was made using of zinc acetate dihydrate which hydrolyzed by cold NaOH solution like sample 3, then audible sound method was carried out under the specific frequency of 11,100 Hz and intensity of 115 dB in hot bath at 70˚C - 80˚C during 3 days at sonic condition. This frequency was selected to produce the maximum intensity which is possible for our devices. The audible sound with this condition was performed above the nanoparticles suspension vessel.

Sample 5: 0.0095 mole zinc acetate dihydrate merck company was hydrolyzed by 4.5 gr. KOH solution containg 200 ml mixture of isopropanol, methanol, ethanol and water. Then the solution of PEG-6000 and 1 gr. PVP (polyvinyl pyrrolidone) was added meanwhile the hydrolyzing reaction was occurring. The yellow precipitation appeared after stirring for 2 h at 70˚C - 85˚C. Then the product was evaporated and dried in oven. Final product dissolved in mixture of hexane and alcohols and was kept at 0˚C until the ZnO QDs were fully precipitated and settled. After the removal of the supernatant it was ready to be calcined at 850˚C. The obtained white product was washed with the mixture of alcohols several times and filtered and dried at 85˚C and was annealed at 700˚C again.

Sample 6: This product was fabricated the same as sample 5 using of audible sound method including the frequency of 150 Hz and the intensity of 94 dB This special frequency had been chosen because of its high ability to produce visible vibration at nanoparticles suspension. Finally the suspensions were left for 7 days under sonic exposure.

Sample 7: The surface of ZnO nanoproduct was modified by doped silica-substrate using coprecipitation method. The mixture of 0.2 mole zinc acetate dihydrate and 0.8 mole TEOS (tetraethyl orthosilicate) were dissolved in alcohol solution including oleic acid as stabilizer. The pH was adjusted at 10 - 11 with cold NaOH and ethylene diamine solution by drop wise until the solution reaches a suitable pH. After the hydrothermal process at 90˚C for 4 days, the yellow powder precursor became ready to calcine at 850˚C.

Sample 8: Oleic acid-capped ZnO Q-Dots was fabricated by sol-gel method using zinc acetate dihydrate (1.2 mmole) in ethanol, LA3 (Lauryl alcohol 3 mole ethoxylated) as surfactant was mixed below 50˚C under vigorous stirring. Around the 10 - 20 gr. oleic acid in alcohol was added during the reflux of mixture. The hydrolysis reaction was carried out with TBAH reagent (tetrabutylammonium hydroxide) in ice bath at 0˚C. After dissolving the precipitation, pH value was changed to acidic and supernatant was evaporated at 60˚C, and the soluble product in oleic acid was appeared.

Samples 9, 10 and 11: In this synthesis the sol-gel method was carried out for hydrolysis of 0.2 M zinc acetate dihydrate in isopropyl alcohol with 0.4 M NaOH solution at pH 10 - 11 in appearance of TEA (three ethanolamine), EDA (ethylene diamine) and citric acid as template-assisted. The mixture was heated to reflux at 85˚C for 8 h. The white crystalline ZnO nanoparticles appeared after the filtration, washing, drying and calcination at 850˚C.

Samples 12, 13 and 14: Sample 12 was fabricated using LA3EO (Lauryl Alcohol 3 moles EO) and nonylphenol 10-EO by sol-gel method and further cold quenching. sample 13 was synthesized by sol-gel, hydrothermal process including PEG6000 and PVP as capped polymer templates by fast cold quenching method and calcined at 850˚C only once. Finally, sample 14 was synthesized by cold sol-gel method in alkaline condition using tetra butyl ammonium hydroxide (TBAH) containing NON9EO (nonylphenol 9 moles ethoxylated) contains 1,2-propylene oxide as nonionic surfactant.

3. Results and Discussion

3.1. SEM Pictures of QDs and ZnO Nanoparticles According to the Symbol Samples

For sample 1 in **Figure 1**.

For sample 2 in **Figure 2**.

For samples (a) extended nanoparticles of 3 and (b) nanospherical 4 in **Figure 3**.

For nanorods as nanowhiskers or nanoflowers ZnO QDs samples 5 and 13 in **Figure 4**.

For samples (a) ZnO nanoparticles of sample 6 and (b) nanoparticles of sample 7 in **Figure 5**.

For samples (a) ZnO nanorods which are dispersed in oleic acid for sample 8, (b) sample 10, (c) very homogeneous nanospherical of sample 11 and (d) ZnO QDs of sample 12 in **Figure 6**.

3.2. XRD Patterns of ZnO Nanostructures (Samples 2, 5 and 11)

The XRD patterns of the three products demonstrate that all of the diffraction peaks can be indexed as typical hexagonal phase of ZnO for samples 2 and 5 including space group p63 mc, but the sample 11 showed Wurtzite structure with hexagonal phase according to lattice constant of a = b = 3.249 Å and c = 5.206 Å with JCPDS card no. 36 - 1451. These peaks at scattering angles (2θ) correspond to the reflection from: (100), (002), (101), (102), (110), (103), (200) and finally (112) crystal planes respectively. **Figure 7** shows XRD patterns of ZnO nanoparticles for nanorods of samples 2 and 5 and nanospherical for sample 11 [2,7,23-25].

FTIR Spectroscopy of Three ZnO Nanostructures

FTIR spectrum of ZnO in KBr matrix showed a broad

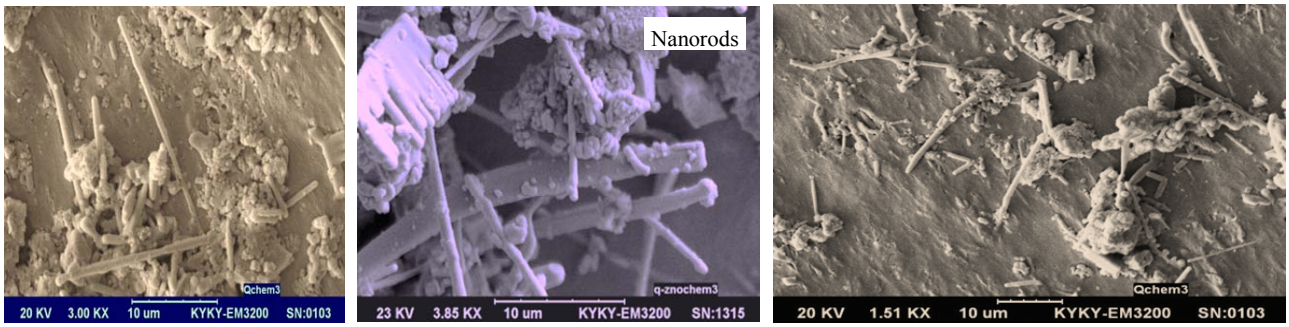


Figure 1. SEM images of QDs ZnO nanorods for sample 1.

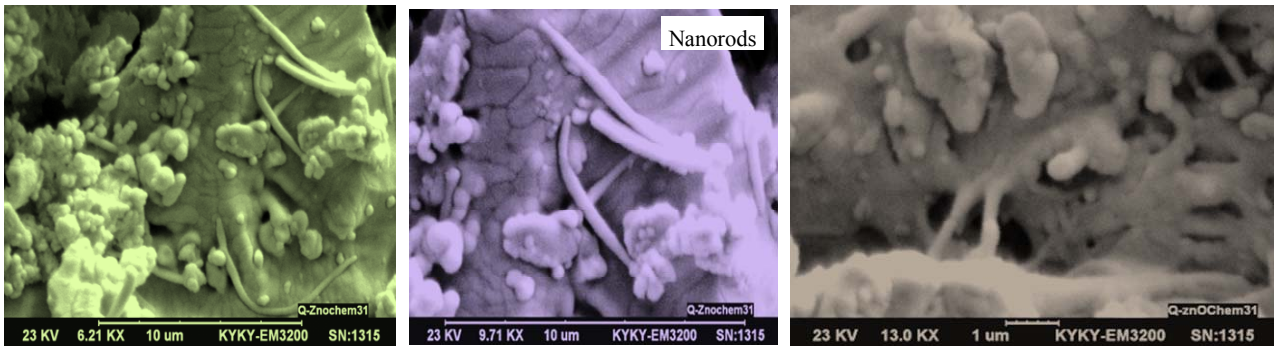
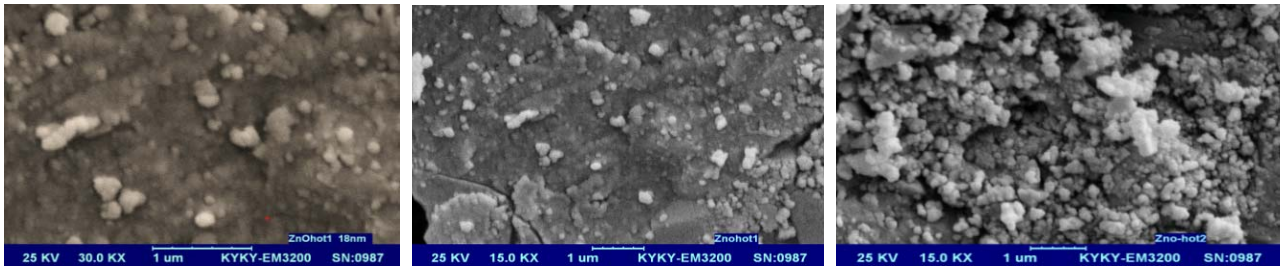
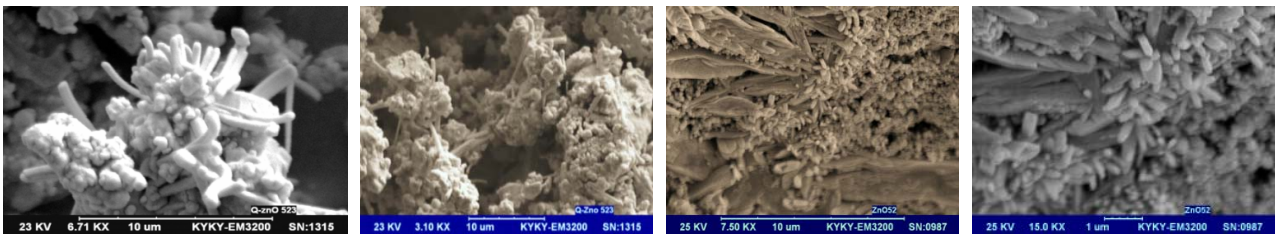


Figure 2. SEM images of QDs ZnO nanorods for sample 2.



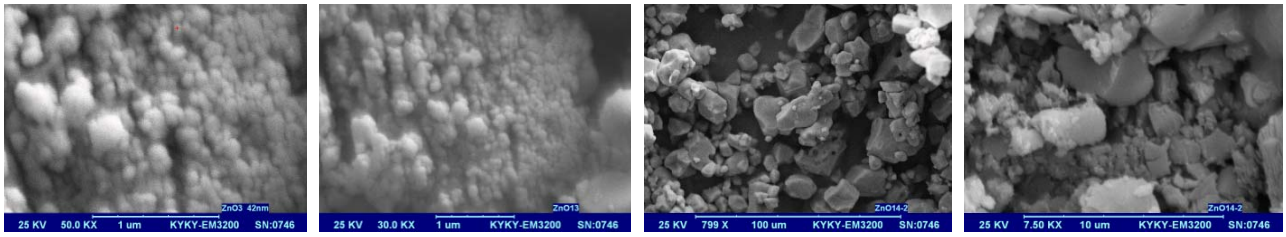
(a) (b)

Figure 3. SEM images of QDs ZnO nanoparticles for (a) sample 3 and (b) sample 4.



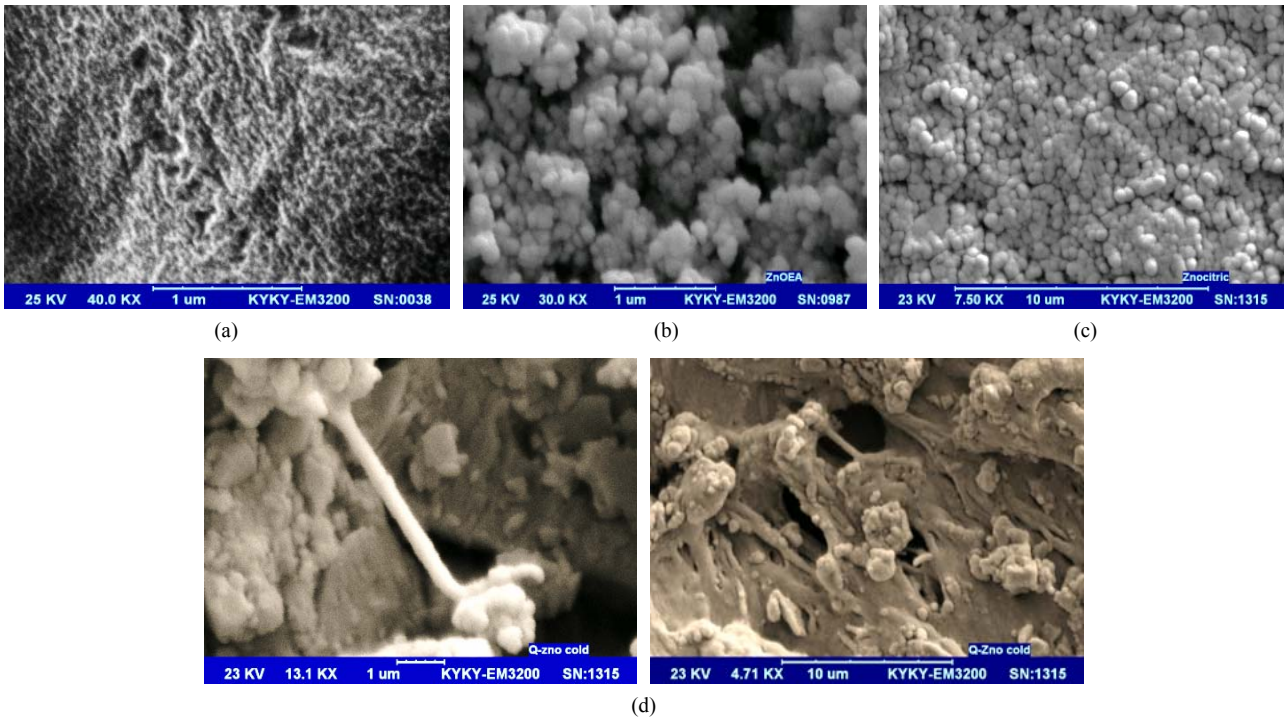
(a) (b)

Figure 4. SEM images of nice formation of QDs ZnO nanorods for (a) sample 5 and (b) nanorods-nanoflowers mixture sample 13.



(a) (b)

Figure 5. SEM images of two ZnO nanoparticles for (a) homogeneous of sample 6 and (b) sample 7.



(a) (b) (c)

(d)

Figure 6. SEM images of various ZnO nanostructures for (a) ZnO QDs of sample 8; (b) sample 10; (c) sample 11 and (d) ZnO QDs as nanorods (nanotubes) for sample 12.

band with very low intensity at 3326.83 cm^{-1} corresponding to the vibration mode of water –OH group, the band at 1653.19 cm^{-1} is due to the OH bending of water. A strong band at 689 cm^{-1} is attributed to the Zn-O stretching band which is indicated in **Figure 8**.

Two sharp peaks at 920 and 956 cm^{-1} showed OH twisting vibrations and lattice Zn-H_O for samples 2 and 5. These sharp peaks are related to substitutional hydrogen at oxygen site H_O bond to the lattice Zn site (Zn-H_O) [9]. The strong peak at 1374 cm^{-1} is also visible, indicating that -COO groups are not completely removed in sample 11 [26]. The low frequency at 600 - 800 cm^{-1} is attributed to hydride Zn-H bending modes for sample 11 (ZnO citric acid).

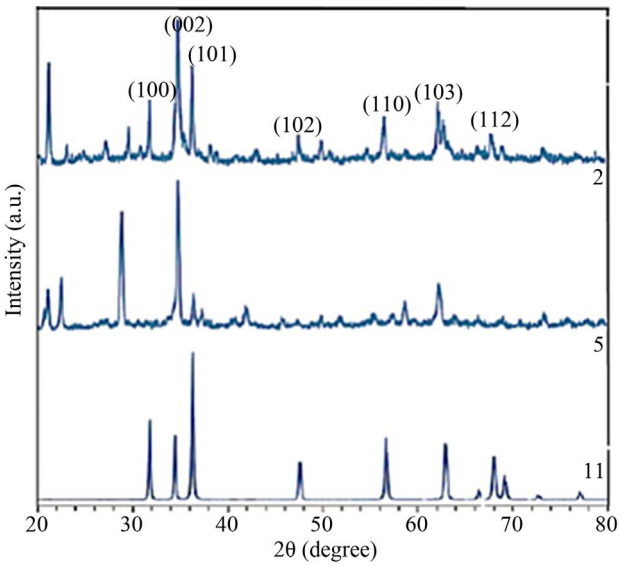


Figure 7. XRD patterns of three ZnO nanoparticles for nanorods of samples 2 and 5 and nanospherical of sample 11.

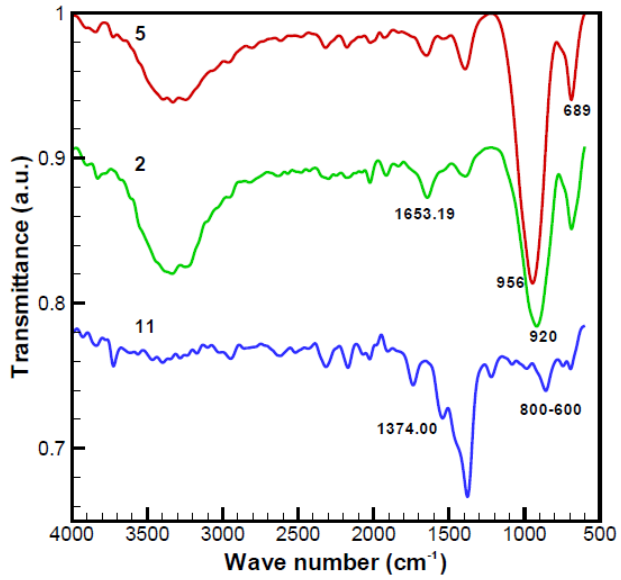


Figure 8. FTIR spectra of three ZnO nanoparticles for nanorods of samples 5 and 2 and nanospherical of sample 11 respectively.

3.3. UV-Vis Absorbance Spectrum for Several of ZnO Nanoparticles

Optical absorption studies of the prepared crystalline series of new nanoparticles colloids were carried out as very intense peaks and expanded portion of them at interval of 350 - 372 nm and their unique trapping states at 400 - 700 nm which are usually attributed to the point defects similar to complex or structural defects for instance of singly ionized oxygen vacancies, zinc vacancies, and surface defects in especial structures of QDs-ZnO (**Figure 9**) [8,23,27].

It seems that surface trapping states were occupied by positively charged oxygen vacancy defects during high annealing temperatures. Therefore, both absorption spectra and emission shows a distinct blue shift under reaction conditions (increase of calcination temperatures during prolonged aging time) [28]. **Figure 10** was shown the typical analysis and feature enlargement of UV-Vis absorption spectrum for four QDs ZnO NPs at limited wavelength of 635 - 665 nm individually. In such circumstance the excitation of electrons from valance band to conduction band happened through these trapping states.

3.4. Room Temperature Photoluminescence Including Distinct Broad and Deep Level Blue-Shift Spectrum

Figures 11 and **12** show photoluminescence spectra and unique ZnO QDTs nanostructures area.

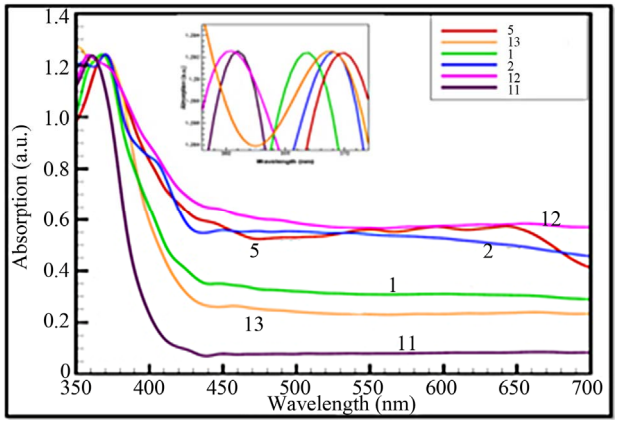


Figure 9. Optical absorption spectra of various QDs ZnO nanorods (samples 5, 13, 2, 12) and nanospherical (samples 3, 11).

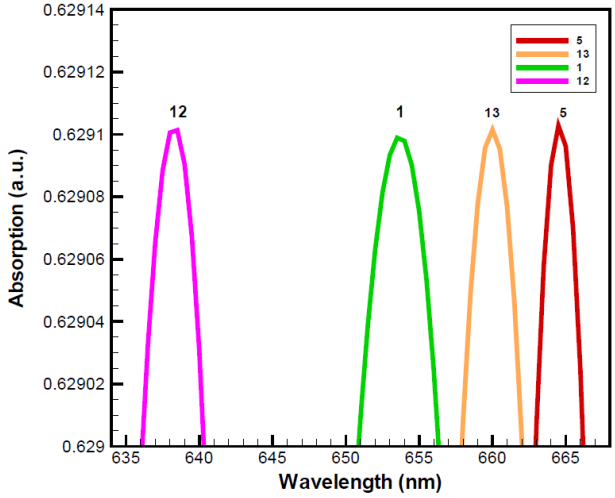


Figure 10. Expansion and analysis of UV-Vis absorption spectra for some QDs ZnO NPs.

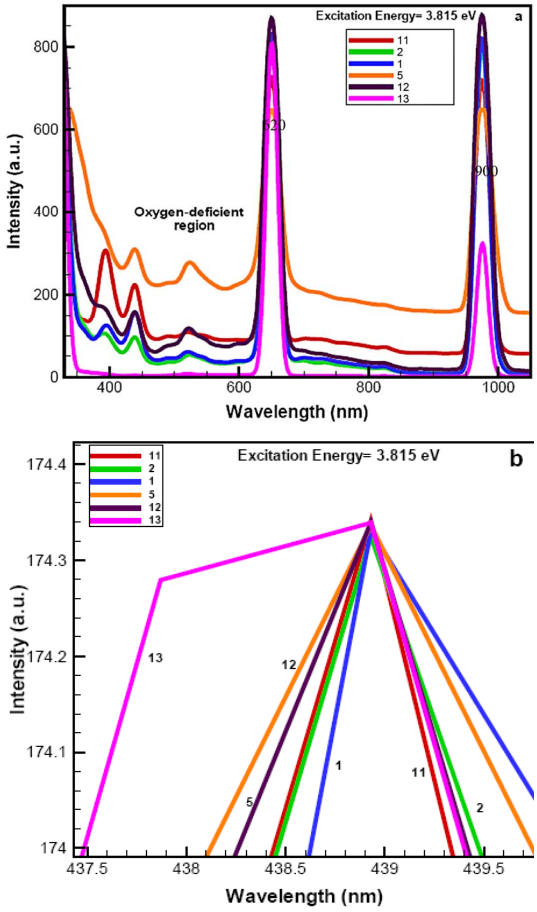


Figure 11. Photoluminescence spectra of (a) various QDs ZnO NPs and (b) the inset shows expansion of limited 437 - 439 nm wavelengths for illustration of broad, ladder and wave-like peaks of especial point defects. All of the products have 620 and 900 nm wavelength in (a) curve.

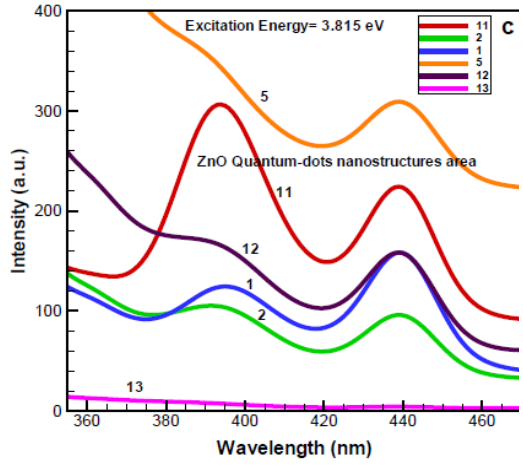


Figure 12. Photoluminescence spectroscopy of (c) some QDs ZnO nanoparticles (No. 11, 2, 1, 5, 12 and 13 from up to down respectively) between especial 360 - 460 nm wavelengths involving complex defects and expansion deep levels blue shift of products. They showed two different max peaks in two divers regions.

In **Figure 12**, it was shown photoluminescence spectroscopy in range 360 - 460 nm expansion for 6 prepared samples.

The room temperature photoluminescence (PL) spectra with 3.815 eV of excitation energy showed distinct visible emission over a wide span of wavelengths which belongs to the oxygen-deficient region and trapping states from around 350 to 600 nm for new QDs ZnO nanoparticles (**Figure 11(a)**). We could expanded especial deep level emissions from 437 to 439 nm and show them in **Figure 11(b)**. They are signs and causes for influence of complex defects and the kind of their structures [29] on optical blue-shift properties at 850˚C - 900˚C annealing temperature [23,25,30,31].

4. Typical Antibacterial Activity for Evaluating of Various ZnO Nanostructures Introduction to the Gram-Negative and Gram-Positive Bacteria Used in This Study

For antibacterial tests we could prepare several gram-negative bacteria such as *Escherichia coli* (RTCC1330), *Klebsiella pneumonia* (RTCC1249), *Pseudomonas aeruginosa* (RTCC1547), *Enterobacter aerogenes* (RTCC1145), Klebsiella pneumonia (RTCC1249) and various gram-positive bacteria like: Staphylococcus aureus (RTCC1885), Listeria monocytogenes (RTCC1293), Enterobacter aerogenes (RTCC1145), Bacillus anthracis (RTCC1036), Bacillus anthracis (RTCC1036), Enterococcus faecalis (RTCC2121), Bacillus cereus (RTCC1040) and Staphylococcus epidermidis (RTCC1898).

4.1. Antibacterial Activity Methods

Microbial strains: The bacterial strains were cultured in brain heart infusion (BHI, merck) under aerobic condition in 37˚C for 24 hours on a reciprocal shaker and subculturing was done twice weekly. Suspensions of the organisms were prepared by picking colonies from appropriately incubated agar cultures to sterile broth, to match a McFarland (barium sulfate standard 0.5) turbidity standard (approximately 1.5×10^8 CFU/ml) (McFarland 1907) [3,13,19,32-35].

Well method or agar well diffusion test: The agar-well method was performed as prescribed by NCCLS well. First muller Hinton agar plates were cultured by bacterial suspension. Wells of 5 mm in diameter were punched the MH agar using a sterile cork-borer about 2 cm apart. Approximately 100 µl of the material suspensions were dropped into each well which filled them respectively to fullness. After incubation at 37˚C a clear zone around the wells is an evidence for antimicrobial activity. All of these investigations repeated for 24, 48 and 72 h.

Determination of minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of the extracts was determined according to methods described by CLSI 2006. ZnO suspensions were diluted to concentrations ranging from 100 to 0.78 mg/ml in Mueller Hinton broth. To each dilution tubes, 0.1 ml of the bacterial inoculum was seeded. Control tubes with no bacterial inoculation were simultaneously maintained. Tubes were incubated aerobically at 37˚C for 24 hours. The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the MIC (CLSI, 2006). To estimate the MIC of the bacteria suspensions more precisely and for confirmation of the results, a more precise concentration in agar dilution method was used [3,16,20,21,36].

4.2. Evaluation of Methodologies to Determine Antibacterial Activity for Six Q-Dots ZnO Nanoparticles by W. M and MIC Methods Compared with Gentamicin Antibiotic Standard

Tables 1 and **2** show performance of five and seven various QDs ZnO nanoparticles including nanorods and nanospherical structutres against examined Gram negative bacterial strains and the efficiency was compared with standard antibiotic using well diffusion method.

Figure 13 indicates the representation of some agar plates in well method test for sample 5 (Q-ZnO523) against B. cer. (25 mm zone diameter) and sample 8 (ZnO19) against (b) B. ant (30 mm), (c) St. epi (40 mm) and finally (d) St. au (30 mm). The presence of an inhibition zone clearly exhibited the significant antibacterial effect of new QDs ZnO nanostructures on these microbes.

Sample 1 shows strong antibacterial activity against *E. coli*. Sample 3 and sample 5 shows excellent biological activity against *Klebsiella pneumonia* and also sample 3 is very effective agent against *Pseudomonas aeruginosa* and all of gr-bacterial strains in **Table 1**. The results in **Table 2** indicates that sample 2 and 3 in the first row shows excellent activity against *Bacillus anthracis*, and sample 4 illustrates high biological activity against *Staphylococcus aureus*, and sample 3 showed high activity on the *Bacillus cereus*. Sample 13 is sensitive to *Staphylococcus aureus* but sample 5 does not show any influence

Table 1. Effect of five QDs ZnO NPs samples against four Gram negative bacteria with gentamicin standard.

Gram Negative Bacteria	Method	Samples / Reference	1	2	3	4	5
Escherichia coli	W.M	**15 mm**	**20**	10	15	15	-
	MIC		**25**	100	50	50	-
Enterobacter aerogenes	W.M	**10**	-	-	20	**20**	20
	MIC		-	-	25	**25**	25
Klebsiella pneumonia	W.M	**10**	-	-	**25**	15	**25**
	MIC		-	-	**12.5**	50	**12.5**
Pseudomonas aeruginosa	W.M	**10**	15	15	**25**	NT*	NT*
	MIC		50	50	**12.5**	NT	NT

NT* = was not done.

Table 2. Illustration of seven QDs ZnO NP against six Gram positive bacteria.

Gram Positive Bacteria	Method	Samples / Reference	1	2	3	4	5	13	14
Bacillus anthracis	W.M	**15 mm**	**20**	**30**	**30**	15	15	-	15
	MIC		**25**	**6.25**	**6.25**	50	50	-	50
Staphylococcus aureus	W.M	**20**	20	-	15	**25**	10	**20**	**20**
	MIC		25	-	50	**12.5**	100	**25**	**25**
Listeria monocytogenes	W.M	**20**	-	-	-	-	-	-	-
	MIC		-	-	-	-	-	-	-
Enterococcus faecalis	W.M	**10**	-	**20**	NT*	-	-	NT*	-
	MIC		-	**25**	NT	-	-	NT	-
Bacillus cereus	W.M	**20**	20	10	**25**	-	**25**	10	-
	MIC		25	100	**12.5**	-	**12.5**	100	-
Staphylococcus epidermidis	W.M	**20**	-	-	20	15	-	-	-
	MIC		-	-	25	50	-	-	-

NT* = was not done.

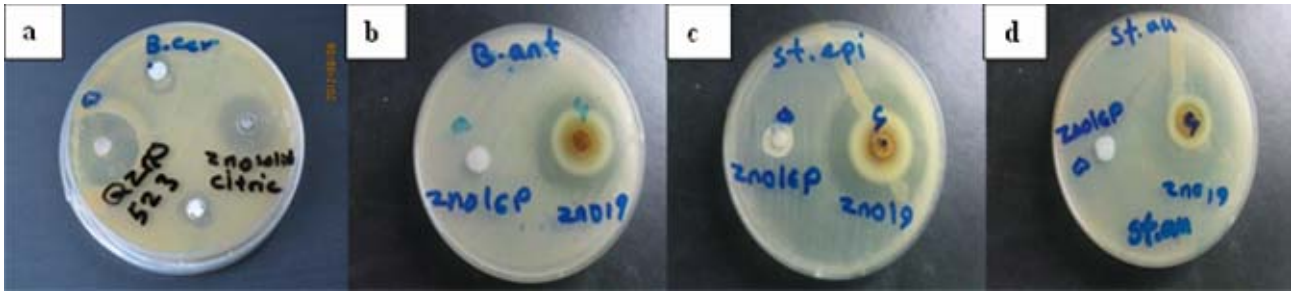


Figure 13. Agar plate test (well method) for (a) sample 5 (with symbol QZnO523) and (b)-(d) sample 8 (with symbol QZnO19) films showing excellent inhibition zone around the films. ZnO16P symbol is the same as sample 8 with polystyrene-capped which it was not successful during the growth process in well test.

against it, whereas they are similar to each other in production stages but are different in purification and calcination process. It was found that, very homogenous nanospherical sample 14 exhibited proper sensitivity related to both *Bacillus anthracis* and *Staphylococcus aureus* bacteria. Sample1displaces identical permanent sensitivity responses of the *Bacillus anthracis*, *Staphylococcus aureus* and *Bacillus cereus*. It is promising that we will be able to do treatment and coating its surface owing to bind more strongly to microorganisms. Therefore, by synthesis of these antimicrobial QDs ZnO nanoparticles, we found ideal candidates as antimicrobial agents. Also mechanism of action of ZnO nanoparticles highlighted that they are capable to kill bacteria through various mechanisms, such as by binding to intracellular proteins and inactivating them, generation of radical and reactive oxygen species including hydrogen peroxide (H_2O_2), electrostatic interaction between the ZnO NPs surface and bacteria membranes (cell surfaces) and penetration in and finally via direct damage to cell wall and membrane. Moreover, the key factor is the formation of desired structure and manufacturing of ZnO NPs involve active surfaces with much greater surface area to volume ratio (high BET) to generate defect nanoporous surface (like-nanocatalyst) because imperfection nanopores can create active radicals with enough activation energy without dependence upon the crystal size of ZnO NPs and this is very important and notable point in typical present investigation.

In **Tables 3** and **4**, performance of ZnO NPs groups including audible sound, pretreatments with assistant agents and oleic acid-capped ZnO Q-Dots products which were tested with gr− and gr+ bacteria is presented.

It is remarkable that three samples are effective against *Enterobacter aerogenes* in comparison with gentamicin antibiotic reagent in **Table 3**. Sample 8 illustrated very activity against *Klebsiella pneumonia* that was modified by oleic acid. Nanospherical of ZnO which was treated with silica nanoparticles like-square plates morphology in sample 7 showed excellent inhibiting effect against the growth of *Pseudomonas aeruginosa* and others are sensitive comparing to standard antibiotic. In fact this modification method could increase the surface activity of ZnO nanoparticles to induce proper interactions with microorganisms. **Table 4** is capable to report the performance of modified ZnO NPs by different conditions.

Among all samples in **Table 4**, sample 8 has high prevent effect on life of Staphylococcus *epidermidis* and showed the most impressive antibacterial property against mostly Gram Positive bacteria and increased the inhibition zone diameters against all microbes compared with standard antibiotic (W.M = 20 mm for reference and 40 mm for sample 8) and good biocompatibility. It seems that the appearance of oleic acid as a development of reliable processes using polymer template type could improve the activity of large surface area available for interaction, binding and diffusion in organ of microbes better resulting into cell death [34]. In addition, sample 7 also showed much activity against the *Enterococcus faecalis* and *Staphylococcus epidermidis* and demonstrated excellent activity as an antibacterial agent. Sample 6 is strong antibacterial against *Staphylococcus epidermidis*. Overall, the results of ZnO nanoparticles containg organic templates such as TEA, EDA and citric acid were appeared which were not highly against some of the tested pathogens and did not undergo obvious modification on the surface of the ZnO nanostructures.

5. Conclusion

In this study, ZnO nanoparticles and QDs nanostructures as nanospherical and nanorods arrays were fabricated by sol-gel, wet-chemical, and hydrothermal methods. Audible sound including microwave process as unreported and new procedure for biosynthesis of zinc oxide nanoparticles (ZnO NPs) was used as novel method. PVP and oleic acid as capping agent showed that they could modify and activate the wide surface of nanoZnO structures. In this case, they can have more contact with bacteria and the efficiency will enhance. These studies demonstrate that the especial ZnO QDs nanoparticles including blue shifts spectrums and complex defects on surfaces exhibit

Table 3. Presentation of activity for ZnO NPs aiding of assisted agents and pretreatment.

Gram Negative Bacteria	Method	Samples / Reference	6	7	8**	9	10
Escherichia coli	W.M	**15 mm**	-	-	-	-	-
	MIC		-	-	-	-	-
Enterobacter aerogenes	W.M	**10**	**20**	**15**	-	-	**20**
	MIC		**25**	**50**	-	-	**25**
Klebsiella pneumonia	W.M	**10**	-	-	**20**	-	-
	MIC		-	-	**25**	-	-
Pseudomonas aeruginosa	W.M	**10**	10	**25**	-	**15**	NT*
	MIC		100	**12.5**	-	**50**	NT

NT* = was not done.

Table 4. Investigation of antimicrobial activity of the various ZnO NPs against Gram positive bacteria.

Gram Positive Bacteria	Method	Samples / Reference	**6**	**7**	**8****
Bacillus anthracis	W.M	**15 mm**	-	-	**30**
	MIC		-	-	**6.25**
Staphylococcus aureus	W.M	**20**	-	-	**30**
	MIC		-	-	**6.25**
Listeria monocytogenes	W.M	**20**	15	15	-
	MIC		50	50	-
Enterococcus faecalis	W.M	**10**	-	**20**	-
	MIC		-	**25**	-
Bacillus cereus	W.M	**20**	-	-	**20**
	MIC		-	-	**25**
Staphylococcus epidermidis	W.M	**20 mm**	**25**	**25**	**40**
	MIC		**12.5**	**12.5**	**1.5**

a wide range of antibacterial activities toward various microorganisms compared to standard antibiotic. Besides, our finding confirmed that the shape, structure, morphology and the kind of fabrication products (carry a positive charge) were effective in high performance of microorganisms with negative charge which can create an electromagnetic attraction between the microbe and treated QDs ZnO surface and eventually causing the cellular death. Furthermore, the QDs link to a photosensitizer is used for photodynamic cancer therapy. Our observation confirmed that sample 11 contains citric acid showed prominent activity as antibacterial against *Bacillus cereus* (RTCC1040 gr+), but none of our samples had sensitive stress responses to *Listeria monocytogenes* (RITCC1293 gr+). In addition, the efficacy of antibacterial activity of significant nanorods of sample 12 (cold quenching) is not known up to now for us and needs to detect many alternative bacteria tests.

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