ISSN: 0970-020 X; CODEN: OJCHEG
Oriental Journal of Chemistry
2011, Vol. 27, No. (3): Pg. 811-822

http://www.orientjchem.org

Synthesis and Antibacterial Properties of Zinc Oxide Combined with Copper oxide Nanocrystales

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(Received: May 14, 2011; Accepted: July 22, 2011)

ABSTRACT

With the emergence of microbial organisms resistant to multiple antibiotics, especially, in the many hospitals, kindergartens and public place; in the present time, many researchers are attempting to develop new. The aim of this research is to comparsion antibacterial activity of mono-metallic with composite nanocrystales, against *Escherichia coli*(PTCC 1533)*, Salmonella galinarum* (PTCC 1510), *Staphylococcus aureus* (PTCC 1113), *Pseudomonas aeroginosa* (PTCC 1310), and *Bacillus subtilis* (PTCC 1023), with and without sonication, for the first time.Mono-metallic with composite nanocrystales, are synthesized via wet method and ensure with oxalate decomposition in high temperature (5000c). FT-IR, XRD, and SEM were used for determination of spectroscopic, structural and morphology of samples, respectively. Also, the nanoparticles were digested and analyzed by ICP-AES for determining the presence of residual chemical element in the nanoparticles, after sonication. Bacterial sensitivity to nanocrystales, with and without sonication, were commonly tested using by disc diffusion test and agar dilution test, also with determination of minimum inhibitory concentration(MIC), and minimum bactericidal concentration(MBC).The particles size were less of 30 nm, approximately. The diameter inhibitory zone showed that all of the strains, have got more Sensitivity against ZnO. Antibacterial activity of CuO nano-crystales were weaker than ZnO and ZnO/CuO. Furthermore, we reported that the least value of MIC, against all strains, dependent to the ZnO nanoparticles, wheras the great value of MIC dependent to the CuO nanoparticles. Also, the great value of bactericidal effects displayed to ZnO nanocomposites.This study shows us that synthesise of mono-metallic and composite nanocrystales, with oxalate decomposition method is simple and so useful. Despite the fact, we confirmed that utilized of the ultrasonic vibration were futile, entirely. It is worthy noting that, the ZnO mono-metallic nanocrystales, had got very effective against all of the strains wheras, the CuO/ZnO nanocrystales had not great antimicrobial agent against all of the strains and combination of zinc oxide and copper oxide nanocrystales, gave decline their bactericidal effect.

Key words: Synthesis; Antibacterial Properties; CuO/ZnO Nanocrystales.

INTRODUCTION

With the appearance of microbial organisms resistant to multiple antibiotics agents, microbial pollution, increase nosocomial infection, antibacterial effects of nanocomposites have attended by the many scientists in the last decade[1,2,3,4]. At the present time, antibacterial properties of nano metal oxides have found as novel of antimicrobial agent. Also, the majority of scientists have proposed that the nano metal oxide ions, such as Zinc or Copper have useful to controlling of hospitals infectious microorganisms[4,5,6]. Reddy, confirmed that Zinc oxide nanocrystales have antibacterial effect against of *Escherichia coli* and *Staphylococcus aureus*[7]. Also, Guogang Ren, achieved so widespread experience on the antibacterial effects of nano Copper oxides against of *Escherichia coli* and *meticillin-resistant Staphylococcus aureus*[8]. Jayesh, showed that Copper nanocrystales have great promise as antimicrobial agent against *Escherichia coli*, *Bacilus subtilis* and *Staphylococcus aureus*[9]. They also, assume that Copper nanoparticles could be have greater affinity to surface active groups of *Bacilus subtilis*, which may be have led to its greater bactericidal effect. However, limited information on the possible antimicrobial activity of CuO and ZnO nanoparticles are available[8] and the mechanism of action of the Copper nanocrystales is not yet fully established[9] but nowadays, we know that the bactericidal effect of metal nanoparticles can be attributed to their small size, photocatalystic of activity and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution[10]. In spite of the fact, they have believed that residual these metal ions may be adversely affect human health[11], but scientists experiments demonstrated selectivity in the toxic nature of ZnO nanocrystales to different bacterial systems and human T lymphocytes. These results suggested that ZnO nanocrystales may potentially prove useful as nanomedicine based antimicrobial agents at selective therapeutic dosing regimens[7]. Also, jayesh, believed that combination of metal oxide nanocrystales may give rise to more complete bactericidal effect against mixed bacterial population[8]. The Purpose of this study was synthesis of ZnO and CuO nanocrystales Monometallic and CuO/ZnO Nanocomposites via thermal decomposition of oxalate precursor method, for first time. In addition, the antibacterial activities to CuO/ZnO nanocomposites, Also, ZnO and CuO nanocrystales monometallic, against strains were procured from the Persian Type Culture Collection (PTCC); such as *Escherichia coli (PTCC 1533), Salmonella galinarum (PTCC 1510), Staphylococcus aureus (PTCC 1113), Pseudomonas aeroginosa (PTCC 1310), and Bacillus subtilis (PTCC 1023)*, were compared and antagonistic effects of them were explored. The antimicrobial effect was determined based on the inhibition zone measured in the disk diffusion tests and in the agar dilution tests conducted in plates also by determining the minimum growth inhibitory concentrations (MIC) and minimum bactericidal concentration(MBC) of nanocrystales in liquid batch cultures. In one comparative study we have also scrutinized antibacterial conducted of nanocrystales before & after to ultrasonic frequency by ultrasonic set.

EXPERIMENTAL

Synthesis of CuO monometallic nanocrystals via oxalate decomposition method

Copper nitrate (Suprapur, MERCK, Germany) was added to ethanol (Suprapur, MERCK, Germany) in a two neck flask giving a 0.3 M blue solution. The temperature was elevated to 50 ^{0}C and after 30 min of continuous stirring oxalic acid (Suprapur, MERCK, Germany) was rapidly added to the solution. The molar ratio Cu:OA was 1. The system was kept at 50 ^{0}C under reflux for 2h and a white precipitate was obtained; then the acetic acid and some of the ethanol were released moisture and the arising viscous gel was dried at 80 ^{0}C over night. The dried copper oxalate was ground and calcined at 500°C for 2h.

Synthesis of ZnO monometallic nanocrystals via oxalate decomposition method

Zinc acetate (Suprapur, MERCK, Germany) was added to ethanol (Suprapur, MERCK, Germany) in a two neck flask giving a 0.3 M white solution. The temperature was elevated to 50 ^{0}C and after 30 min of continuous stirring oxalic acid (Suprapur, MERCK, Germany) was rapidly added to the solution. The molar ratio Zn:OA was 1.

The system was kept at 50 ^{0}C under reflux for 2h and a white precipitate was obtained; then the acetic acid and some of the ethanol were released moisture and the arising viscous gel was dried at 80 ^{0}C over night. The dried Zinc oxalate was ground and calcined at 500 ^{0}C for 2h.

Synthesis of CuO/ZnO nanocomposites via oxalate decomposition method

Zinc chloride (Suprapur, MERCK, Germany) and copper nitrate (Suprapur, MERCK, Germany) were added to ethanol (Suprapur, MERCK, Germany) in a two neck flask giving a 0.3 M blue solution. The temperature was raised to 50 ^{o}C and after 30 min of continuous stirring, oxalic acid (Suprapur, MERCK, Germany) was rapidly added to the solution. The molar ratio Zn/Cu:OA was 1. The system was kept at 50 ^{o}C under reflux for 2h and a blue precipitate was obtained; then the resulting viscous gel was dried at 80 ^{o}C overnight. The dried ZnO/CuO oxalate was ground and calcined at 500 ^{o}C for 2h.

Characterization

Experiences of dependen on the crystallinity of the nanoparticles were carried out using a X-ray Diffractometer set (XRD, Bruker D8-Advance Diffractometer using Cu K± radiation). Also, the nanoparticles were digested and analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, LIBERTY – RL, Varian Australia Co.) for determining the presence of residual chemical element in the nanoparticles. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker spectrophotometer in KBr pellets. Surface morphology of product was characterized by using a scanning electronic microscopy (SEM, Cam Scan MV2300) with an accelerating voltage of 30 kV.

Disk diffusion test

Bacterial sensitivity to antibiotics is commonly inspected by a disk diffusion test, employing antibiotic impregnated disks[12]. A comparable examination with nanocomposites and monometallic nanocrystales loaded disks was utilized in this research. A 10 ml suspension of each nanoparticles (approximately, 16384 µg.ml^{-1}) was prepared into the Muller Hinton Broth medium and then suspension of each nanoparticles was sonicated at room temperature and frequency of 28 KHz, during at the 10 minute, subsequently filtered through a membrane filter (0.2 µm, 15 mm diameter Shimie Rasan Teeb). The nanoparticles laden filter paper were dried in an oven for 1h and small disks of uniform size (6 mm diameter) containing 16384 µgml^{-1} nanoparticles were punched out and stored in a desiccator at room temperature. For each type of the bacterial inoculums (1.5×10^8 CFU.ml^{-1}) were cultured completely on the surface of a Muller Hinton Agar plate before placing the disks on the plate. The plates were incubated at 35 ^{0}C for 24h, after which the average diameter of the inhibition zone enclosing the disks was measured with a ruler with up to 1 mm resolution. The examination were also replicated, without sonication and so the results were compared together. Subsequently, the tests were reported for each type of nanoparticles and with each microbial strain on three replicates.

Agar Dilution test

A 16384 µg.ml^{-1} suspension of each nanoparticles was prepared into the Muller Hinton Broth medium, approximately, and then of each nanoparticles was sonicated at room temperature and frequency of 28 KHz, during at the 10 minute. For each type of the bacterial inoculums (1.5×10^8 CFU.ml^{-1}) were cultured completely on the surface of a Muller Hinton Agar plate before excavating the cavity on the plate. Then, 100 µgml^{-1} from suspension of each nanoparticles was filled into the cavities. The plates were incubated at 35 ^{0}C for 24h, after which the average diameter of the inhibition zone enclosing the cavities was measured with a ruler with up to 1 mm resolution. The examination were also replicated, without sonication and so the results were compared together. Subsequently, the tests were reported for each type of nanoparticles and with each microbial strain on three replicates.

Determination of the Minimum Inhibitory Concentration(MIC) and minimum bactericidal concentration(MBC)

The lowest concentration of material that inhibits the growth of an organism was defined as the minimum inhibitory concentration (MIC)[12]. From the serial dilution method, was employed for determine the MIC of the nanoparticles. Each of the twelve test tubes was filled with 1ml of the liquid

Muller Hinton Broth medium. Into each of the test tubes number 1 and 2, one ml solution containing 16384 μgml^{-1} of nanoparticles that had been sonicated at room temperature and frequency of 28 KHz, during at the 10 minute, already, was added and mixed thoroughly with the culture medium. The concentration of nanoparticles in each test tube become 8192 μgml^{-1}. Then, 1 ml of the content of test tube number 2 was added to test tube number 3 and mixed completely. This process was performed serially to test tube number 16. Consequence, 1 ml content of test tube number 16 was discarded. In order to have equal amounts of material in all the test tubes, 0.9 ml of test tube number 1 was discarded. finally, 0.1 ml of standard microbial suspensions *Escherichia coli* (PTCC 1533), *Salmonella galinarum* (PTCC 1510), *Staphylococcus aureus* (PTCC 1113), *Pseudomonas aeroginosa* (PTCC 1310) containing 1.5×10^8 $CFUml^{-1}$ microorganism, were added to test tubes number 2 to 17, and the test tubes were incubated at 35 °C for 24h. Then, the microbial growth was studied by turbidimetric measurement, using a spectrophotometer (Nano-volum spectrophotometer, Scandrop 250, Analytik jena Co.)[13]. The experiments also included a positive control (test tube containing nanoparticles and Muller Hinton Broth medium, devoid of inoculum) and a negative control (test tube containing inoculum and Muller Hinton Broth medium, devoid of nanoparticles). The negative controls indicated the microbial growth profile in the absence of nanoparticles. All the experiments were carried out in triplicate. The minimum bactericidal concentration (MBC), i.e., the lowest concentration of nanoparticles that kills 99.9% of the bacteria was also determined from the batch culture studies. For growth inhibitory concentration (PMIC) the presence of viable microorganisms was tested and the lowest concentration causing bactericidal effect was reported as MBC as suggested by Avadi et al.[14]. To experiments for bactericidal effect, a loopful from each test tube (Specially, negative and positive test tubes) was inoculated on Muller Hinton Agar and incubated at 35 ^{0}C for 24h. The nanoparticles concentration illustrating bactericidal effect was picked out based on absence of colonies on the agar plate. The release of Cu^+ and Zn^{2+} ions from the nanoparticles into DI water and Muller Hinton broth medium was deliberated by suspending 10 mg of nanoparticles in 100 ml DI water/medium and sonicating with ultrasonic set (PARSONIC 7500s, Pars Nahand ENGG. Co. IRAN) for 10 minute. The suspension was kept in a rotary shaker (Gyrotwister 3-Dshaker , labnet Co. USA) under the same conditions as in the above studies and residual Cu^+ and Zn^{2+} concentration in the aqueous phase was definited by ICP-AES after 24h.

RESULTS AND DISCUSSION

The XRD analysis

The XRD pattern of CuO, ZnO and CuO/ZnO nanoparticles (Fig 1. a, b and c) were compared and interpreted with standard data of International Centre of Diffraction Data (ICDD). The average crystallite sizes (C.S) of the nanoparticles were calculated using the Debye-Scherer Equation from the major diffraction peaks ($C.S = K.\lambda/ ²´.\cos´$). Where K´ is a constant equal to 0.9, l is the wavelength of Cu K± radiation, ² is the full width at half maximum (FWHM) of the diffraction peak in radiant and ´ is the Bragg angles of the main planes. The average crystallite size of the CuO, ZnO and CuO /ZnO were 26.38 nm, 24.75nm and 5.04 nm respectively.

The ICP-AES analysis

By ICP-AES analysis, we were succeed to estimation of residual boron, after digestion of Copper/Zinc oxide nanocomposites. HNO_3 indicated boron levels of 2120 ppm for Cu^+ cation and d"1 ppm for Zn^+ cation, in the Copper/Zinc oxide nanocomposites. However, SEM images of nanoparticles were showed that copper oxide, zinc oxide and Copper/Zinc oxide metal particles were exactly in the shape of spherical and clustered. Also ,SEM images were confirmed that all of nanoparticles were exactly pured.

The FT-IR spectra analysis

Fig. 2 shows FT-IR spectra of (a) CuO, (b) ZnO and (c) CuO/ZnO. The supplement of oxalic acid to the ethanol solution of CuO cation was cause to the precipitation of a blue solid of copper oxalate as shown by FT-IR spectrum in Fig. 2.a The broad band at 3420.26 cm^{-1} was allocated to both the $´_s$(O-H) and $´_{as}$(O-H) of hydration water. The extreme band at 1635.51 cm^{-1} was allocated to asymmetric and water tensional tremble ´(H-O-H). The shoulder

Table 1: Disc diffusion Test (µgml^{-1}), Agar dilution Test (µgml^{-1}) , MIC (µgml^{-1}) and MBC (µgml^{-1}) of copper oxide nanoparticles, zinc oxide nanoparticles and copper oxide /zinc oxide nanocomposites for various microorganisms

	Nanoparticls	Disc diffusion Test (DIZ)		Agar dilution Test (DIZ)		MIC	MBC
		Before sonication	After sonication	Before sonication	After sonication		
Pseudomonas aeruginosa	ZnO	10mm	10 mm	10mm	8 mm	256 µg/ml	> 4096 µg/ml
	CuO	Negative	Negative	Negative	Negative	> 4096 µg/ml	> 4096 µg/ml
	CuO /ZnO	6mm	10 mm	10mm	7 mm	2048 µg/ml	> 4096 µg/ml
Bacillus subtilis	ZnO	20mm	15 mm	25mm	20 mm	512 µg/ml	4096 µg/ml
	CuO	Negative	Negative	Negative	Negative	>4096 µg/ml	>4096 µg/ml
	CuO /ZnO	10mm	10 mm	15mm	13 mm	2048 µg/ml	> 4096 µg/ml
Salmonella galinarium	ZnO	20mm	10 mm	24mm	15 mm	128 µg/ml	512 µg/ml
	CuO	Negative	Negative	Negative	Negative	> 4096 µg/ml	> 4096 µg/ml
	CuO /ZnO	15mm	10 mm	18mm	14 mm	2048 µg/ml	> 4096 µg/ml
Escherichia coli	ZnO	18mm	12 mm	20mm	15 mm	64 µg/ml	512 µg/ml
	CuO	Negative	Negative	Negative	Negative	> 4096 µg/ml	>4096 µg/ml
	CuO /ZnO	10mm	10 mm	15mm	12 mm	2048 µg/ml	> 4096 µg/ml
Staphylococcus aureus	ZnO	12mm	12 mm	18mm	15 mm	256 µg/ml	2048 µg/ml
	CuO	Negative	Negative	Negative	Negative	> 4096 µg/ml	> 4096 µg/ml
	CuO /ZnO	15mm	10 mm	15mm	12 mm	2048 µg/ml	> 4096 µg/ml

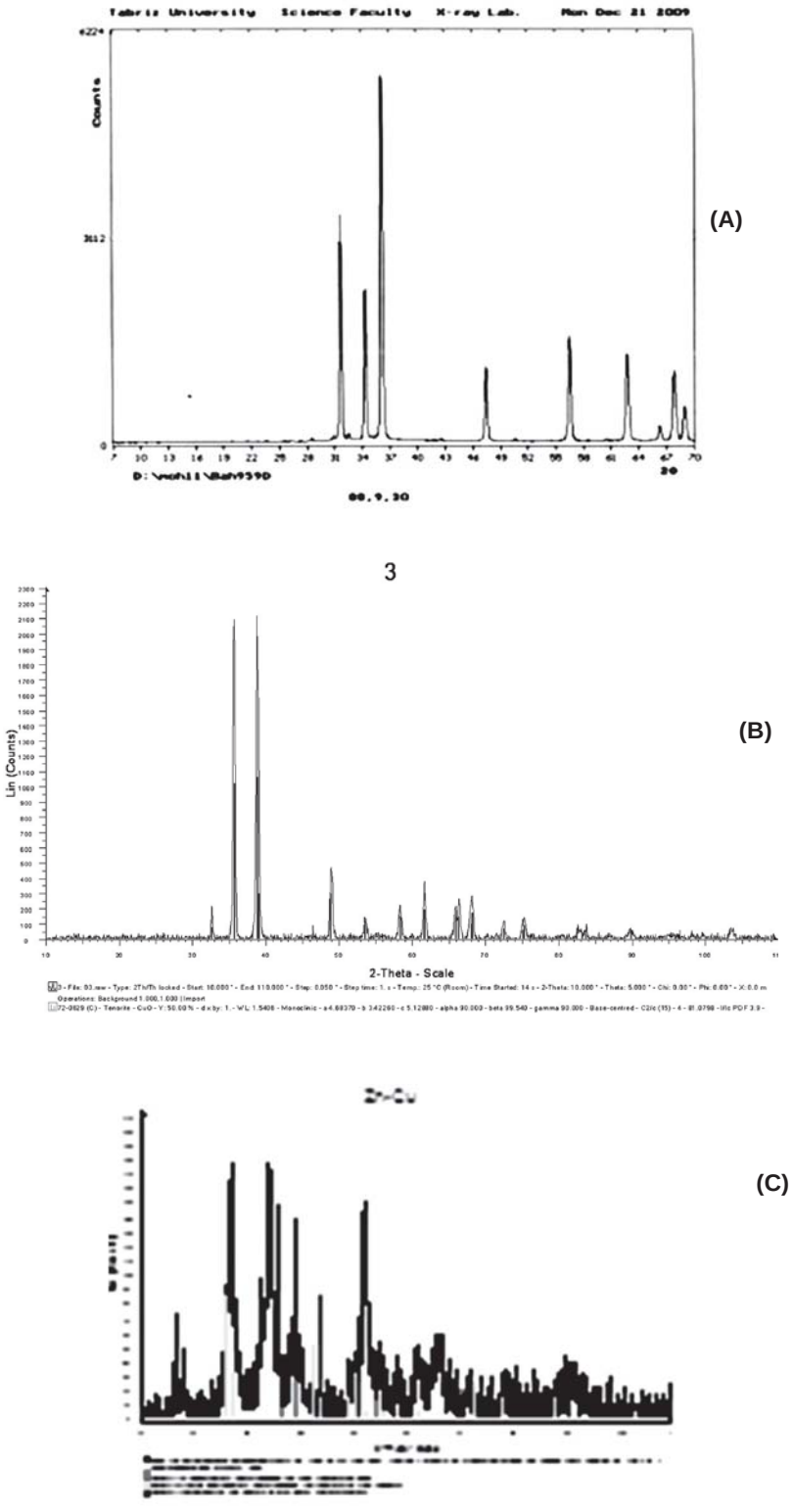


Fig. 1: XRD pattern of (a) CuO, (b) ZnO and (c) CuO/ZnO Nanoparticles

at 1384.45 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands at 535.05 cm^{-1} are presents in the spectrum evidence of (M-O) tremble respectively. Fig. 2.b depended to ZnO FT-IR spectrum. The broad band at 3445.05 cm^{-1} was allocated to both the $\acute{}_s$(O-H) and $\acute{}_{as}$(O-H) of hydration water. The extreme band at 1629.57$cm^{-1}$was allocated to asymmetric and water tensional tremble ´(H-O-H). The shoulder at 1428.89 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands at 876.14 cm^{-1}, 551.12 cm^{-1} and 519.76 cm^{-1} are presents in the spectrum evidence of (O-C-O) tensional tremble and (Zn-O) tensional tremble respectively. Also, Fig. 2.c conclude CuO/ZnO FT-IR spectrum. The broad band at 3861.61 cm^{-1} was allocated to both the $\acute{}_s$(O-H) and $\acute{}_{as}$(O-H) of hydration water. The extreme band at 1624.77 cm^{-1} was allocated to asymmetric and water tensional tremble ´(H-O-H). The shoulder at 1458.93 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands at 985.71 cm^{-1} and 535.34 cm^{-1} are presents in the spectrum evidence of (O-C-O) tensional tremble and CuO/ZnO tensional tremble respectively.

The SEM images analysis

SEM images of nanoparticles were showed that Copper oxide, Zinc oxide and Copper/Zinc oxide metal particles were exactly in the shape of spherical and clustered. Also, SEM images were confirmed that all of nanoparticles were exactly pure.

The antibacterial activity analysis

The antibacterial activity of Copper oxide, Zinc oxide and Copper/Zinc oxide nanoparticles was compared for *Escherichia coli* (PTCC 1533), *Salmonella galinarum* (PTCC 1510), *Staphylococcus aureus* (PTCC 1113), *Pseudomonas aeroginosa* (PTCC 1310), and *Bacillus subtilis* (PTCC 1023) using the diameter of inhibition zones in disk diffusion test and Agar dilution test. In fact, the diameter of inhibition zone (DIZ) reflects dimension of impressionability of the bacteria. We knew, the strains susceptible to disinfectants demonstrate larger DIZ, while resistant strains exhibit smaller DIZ. The disks load with Copper and Zinc oxide nanoparticles were compared to the Copper/Zinc oxide nanoparticles for all strains selected for this study.

The DIZ for Zinc oxide and Copper/Zinc oxide nanoparticles impregnated disks was undoubtedly greater than that studied with the Copper nanoparticles impregnated disks for all the strains selected for this study. In fact, the Copper nanoparticles had not any antimicrobial effect against all of the strains selected for this study. Correspondingly, the Zinc oxide impregnated disks for all of the strains were found to be more effective compared to Copper oxide and Copper oxide/Zinc oxide composite nanoparticles impregnated disks. However, the difference in the DIZ was merely 10–15%. In contrast, for *Salmonella galinarium*, *Escherichia coli* and *Bacillus subtilis*, the disks impregnated with Zinc oxide nanoparticles showed a significantly larger DIZ, almost greater compared to that observed with copper oxide/zinc oxide composite nanoparticles. Interestingly, for *Staphylococcus aureus* and *Pseudomonas aeruginosa* the disks impregnated with Zinc oxide nanoparticles showed a weaker DIZ, compared to that observed with other bacteria. Since DIZ was measured on agar plates using a ruler with 1 mm resolution, the possibility of measurement errors exist. Also, by contrast of size DIZ was measured on agar plates, before and after of sonication, were discovered that ulterasonic waves have not any efficacy on antibacterial feature of nanoparticles. The results in depended of the antibacterial effects of nanoparticles against different of bacterial via the disc diffusion test, the agar dilution test the MIC and the MBC are summarized in Table 1. Greater lag phase and lower maximum absorbance (at 600 nm) were observed as the concentration of nanoparticls increased. Similar observation was reported by Sondi and Salopek-Sondi[15]. We analysed effectivity of Copper oxide and Zinc oxide mono-metalic nanoparticles, also, Copper oxide/ Zinc oxide nanocomposites against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis, Salmonella galinarum*, *Pseudomonas aeroginosa*. The bactericidal effect of nanoparticles is dependent on the concentration of nanoparticles and the initial bacterial concentration. In this study, the initial bacterial concentration was constant at 1.5×10^8 CFU ml^{-1} regardless of nanoparticle concentration and microbial strain. Our research shown that zinc oxide nanoparticles have got strong antibacterial effects against all of bacteria chosen for this study, specially *Escherichia coli* . The MIC observed for

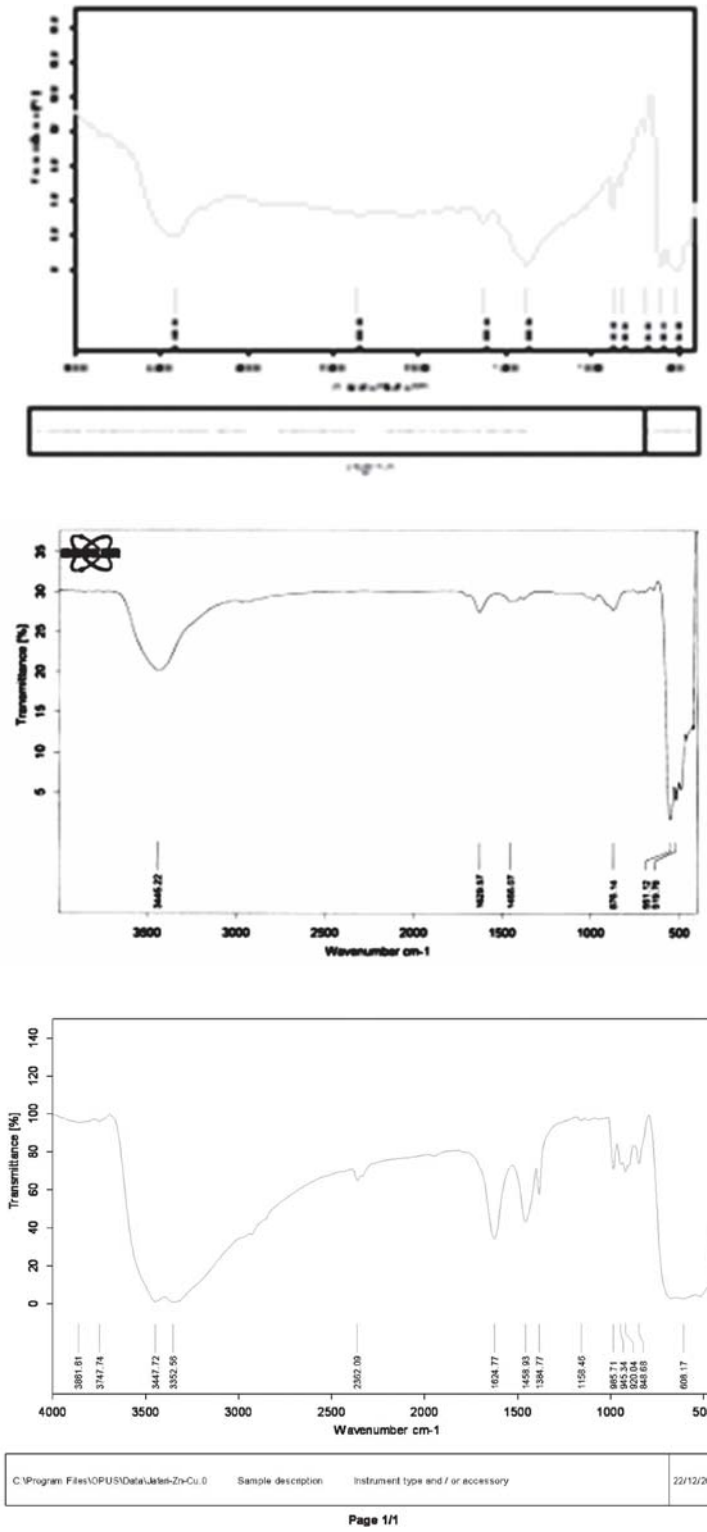
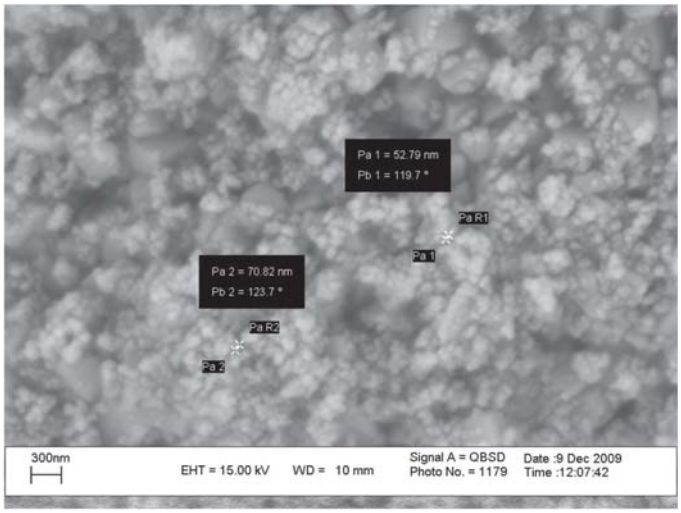


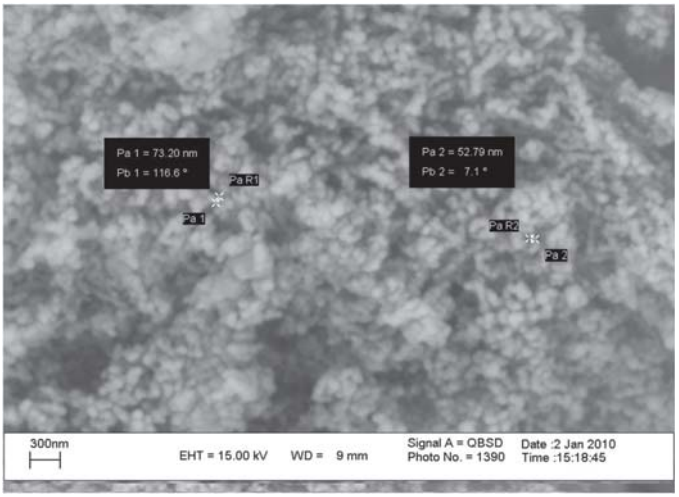
Fig. 2: FT-IR pattern of (a) CuO, (b) ZnO and (c) CuO/ZnO nanoparticles

zinc oxide nanoparticles were 512 µgml^{-1} for *Bacillus subtilis,* 256 µgml^{-1} for *Staphylococcus aureus, 256* µgml^{-1} for *Pseudomonas aeruginosa,* 128 µgml^{-1} for *Salmonella galinarium* and 64 µgml^{-1} for *Escherichia coli.* Our results observed that the *Salmonella galinarium* and strains of *Escherichia coli* , were most sensitivity against of zinc oxide nanoparticles. Surprisingly, antibacterial effect of the copper nanoparticles were so weaker and probably these nanoparticles, had not any antibacterial effect to concentration of chosen for this study. The MIC observed for Copper oxide/Zinc oxide nanocomposites were 2048 µgml^{-1} for all of the strains that chosen for this study. In contrast with all of the nanoparticles that picked out for this study, the most antibacterial effect was seen to Zinc oxide nanoparticles. Gan X[16], believed that colloidal and agglomerated nanoparticles may affect its ability in inhibiting or destroying bacteria and also influence the degree of MIC and MBC. Regarding this theory, Guogang[8] and Jayesh[9] exposed the suspension of nanoparticles in liquid medium to the ulteraviolet waves for 10 minutes to let them out of agglomeration and being dispersed and suspended. After 10 minutes of sonification, release of Cu ions into the liquid medium and their concentration rate in liquid phase determined, using inductively coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) after 24 h of incubation in shaker incubator. Up to now, no comparison reported on the antibacterial rate of metal nanoparticles, in both agglomerated and dispersed states. One of the aims of our study would be the examining and comparing of the rate of antibacterial effects Copper oxide nanoparticles, Zinc oxide nanoparticles and Copper oxide/Zinc oxide nanocomposites of understudy - pre and post - exposed with ultrasonic waves, against standard strains of bacteria, using disc diffusion and agar dilution methods by sonicator machine. The antibacterial effects of metal oxide nanoparticles against 5 standard strains of bacteria in colloidal or agglomerated phase showed no meaningful difference with unagglumerated phase and also the diameter of inhibition zone (DIZ) in plate was not significant. In the current study, after synthesis of nanoparticles of metal oxides CuO, ZnO and combined nanoparticles of CuO/ZnO, their antibacterial effects compared. Jayesh[9] and Guogang[8] performed extensive experiments in determination of microbial sensitivity of various bacteria to Copper nanoparticles, using disc diffusion method.

Regarding that the diameter of inhibition zone (DIZ), reflects the sensitivity of the organism, strains of sensitive, show larger DIZ and the resistant strains show smaller DIZ. Investigators proved antibacterial properties of copper oxide nanoparticles. While So far has been reported only few studies on the antibacterial properties of copper nanoparticles. Cioffi[17], researches showed that copper oxide nanoparticles are considered as an important antibacterial agent. Also during research Guogang in 2009 proved Antibacterial properties of copper nanoparticles against *Escherichia coli* and *methicilin resistant Staphylococcus aureus*. It also Guogang proved that Copper oxide nanoparticles, is effective on a wide range of nosocomial infection. The Copper oxide nanoparticles used in this study, by the disc diffution test and Agar dilution test, in 16384 ¼g/ml concentration, did not show any antibacterial properties against five standard strains of the bacteria; *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella galinarium* and *Pseudomonas aeruginosa*. Reddy and their colleagues were among the few scholars that worked on the toxicity of zinc oxide nanoparticles in gram negative and Gram positive bacteria. Reddy research showed that nano zinc oxide had selective toxicity in front of eukaryotes and prokaryotes cells. In fact, T cells resist to toxic effects of nanoparticles, completely[7]. Wang, for first time processed combined copper - zinc metal oxide nanoparticles[18] and subsequent for them Guogang Ren reported Ag-Cu combined nanoparticles as a strong antibacterial agents against *methicillin resistant Staphylococcus aureus.* According to Guogang Ren studies, some strains of gram negative bacteria, have shown high relatively sensitivity to nano copper oxide combined whith nano silver oxide [9]. Also, Jayesh in 2009 proposed combining Silver and Copper nanoparticles may increase bactericidal effects[7] that in fact, encouraged us to this research. So far no complete and comprehensive study have been reported about the combining antibacterial nanoparticles and the comparing their antibacterial effects on the Widespread range of bacteria. Among the few studies, Linng YANG and colleagues in 2006, combining silver nitrate to zinc nanoparticles, for this reason that of enhancing antibacterial activity



(A)



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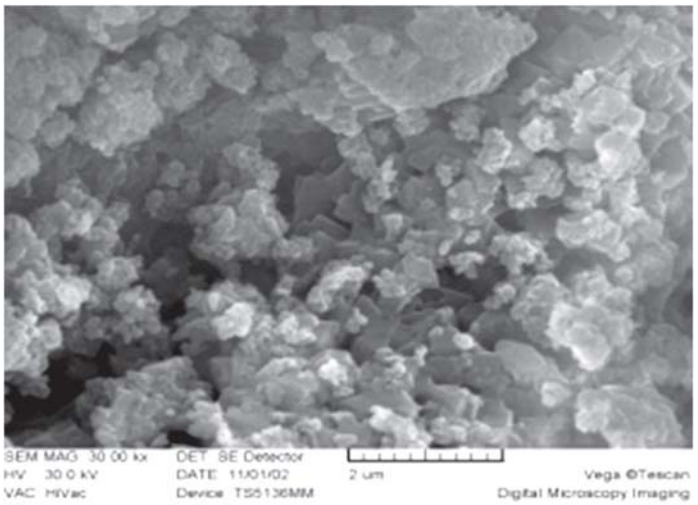

(C)

Fig. 3: SEM images of (a) CuO, (b) ZnO and (c) CuO/ZnO nanoparticles

on the nanoparticles, so achieved remarkable results. Surely, Linng Yang and his colleagues believed that the ability photocatalytic zinc oxide nanoparticles with silver nanoparticles improve and raise ability of its oxidation and reduction and due to stop bacteria growing. It is also stated that the silver oxide which coated on surface of Zing nanoparicles, can trap zing electrons produced by the reaction photocatalytic nanoparticles that increase the separation of electrons and create holes in cell membrane and thus increase the antimicrobial activity[18].

CONCLUSION

According to the research done, Zinc nanoparticles showed the most antibacterial property against all strains. *Escherichia coli*(PTCC 1533) and then *salmonella galinariuom*(PTCC 1510) had the greatest sensitivity to Zinc oxide nanoparticles, in other word, Zinc oxide nanoparticles had the most inhibitory and killing of bacteria properties against *Escherichia coli* and *salmonella galinariuom*. Interesting point was here that unlike the research done by scientists nano Copper oxide showed no antibacterial properties against all bacterial strains studied, also combined Copper/Zinc oxide nanoparticles showed weak bacterial inhibitory and antibacterial effects against all of strains used in our investigation.

ACKNOWLEDGMENTS

We are indebted to research Vice Chancellor of Islamic Azad University Ardabil Branch, for supporting this research. Also, we gratefully acknowledge to University of Industrial Sahand, Tabriz, Department of Nanotechnology, for the XRD and SEM analysis. The authors would like to acknowledge to Iranian Mineral Processing Research Center (IMPRC) for ICP-AES analysis. We gratefully acknowledge the Islamic Azad University Ardabil Branch, Department of chemistry, for FTIR analysis, also specific thanks with Executive Director of Iran-Nanotechnology Organization (Govt. of Iran). The anonymous reviewers are acknowledged for providing valuable comments and insights for improving the manuscript.

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