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Full Length Research Paper

Synergistic antibacterial effects of nano zinc oxide combined with silver nanocrystales

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Antibacterial nanocrystals have attracted great interests in recent years. In fact, with the emergence and increase of microbial organisms resistant to multiple antibiotics, many researchers have tried to develop new antibiotics. The aim of this research is to compare antibacterial activity of mono-metallic with composite nanocrystals, against *Escherichia coli, Salmonella galinarium, Staphylococcus aureus, Pesudomonas aueroginosa,* and *Bacillus subtilis,* with and without sonication, for the first time. Mono-metallic with composite nanocrystals, are synthesized via wet method and ensure with oxalate decomposition in high temperature (500°C). FT-IR, XRD, SEM were used for determination of spectroscopic, structural and morphology of samples, respectively. Also the nanoparticls were digested and analyzed by ICP-AES for determining the presence of residual chemical element in the nanoparticls, after sonication. Bacterial sensitivity to nanocrystals, with and without sonication, were commonly tested using disc diffusion test and agar dilution test, also with determination of minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). The particles size was less of 100 nm, approximately. This study shows that synthesise of mono-metallic and composite nanocrystals, with oxalate decomposition method is simple and so useful. Although, we confirmed that utilized of the ultrasonic vibration were futile, entirely. Also, the Ag/ZnO nanocrystals have great antimicrobial agent against all of the strains and just combination of zinc oxide and silver nanocrystals, give increase their bactericidal effect.

Key words: Antibacterial activity, Ag/ZnO, nanocomposite, synergistic antibacterial activities.

INTRODUCTION

In the last decade, microbial pollution of environments is a major threat to public health. With the appearance of microbial organisms resistant to multiple antibiotics agents and β-Lactam antibiotics, increase nosocomial infection, antibacterial effects of nanocomposites have been also attended by the many researchers in recent years (Ping et al., 2005; Yacoby et al., 2007; Bustos-Martinez et al., 2006). Antibacterial properties of nano metal oxides have been discovered as new generation of antimicrobial agent and researchers have offered the use of silver and zinc ions as superior disinfectants from hospitals infectious microorganisms (Reddy et al., 2007; Lin et al., 2000; Lin et al., 1996). Although, they have believed that residual these metal ions may adversely affect human health (Blanc et al., 2005), but, scientists experiments demonstrated selectivity in the toxic nature of Zn_O nanoparticls to different bacterial systems and human T lymphocytes. These results suggested that Zn_O nanoparticls may potentially prove useful as nanomedicine based antimicrobial agents at selective therapeutic dosing regimens (Reddy et al., 2007). The mechanism of action of the silver and zinc nanoparticls is not yet fully established (Reddy et al., 2007; Jayesh et al., 2007). But nowadays, we know that the bactericidal

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effect of metal nanoparticls has been 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 (Morones et al., 2005). While various hypotheses have been proposed to explain the mechanism of antimicrobial activity of silver nanoparticls, it is widely believed that silver nanoparticls are incorporated in the cell membrane, which causes leakage of intracellular substances and eventually causes cell death (Cho et al., 2005; Sondi et al., 2004). Some of the silver nanoparticls also penetrate into the cells. It is also reported that bactericidal efficiency is affected by the type of microorganism. In studies with Gram negative, *Escherichia coli*, and Gram positive, *Staphylococcus aureus*, Kim reported greater biocidal efficiency of silver nanoparticls for *E. coli*, and attributed it to difference in cell wall structure between Gram negative and Gram positive microorganisms (Kim et al., 2007).

Also recently, jayesh assumed that combination of metal oxide nanoparticls may give rise to more complete bactericidal effect against mixed bacterial population (jayesh et al., 2007). The Purpose of this study was synthesis of Zn_O and Ag Nanocrystals Monometallic and Ag/Zn_O Nanocomposites via thermal decomposition of oxalate precursor method, for first time.

Moreover, the antibacterial activities to Ag/Zn_O nanocomposites, Zn_O and Ag nanocrystals monometallic, against strains were procured from the Persian Type Culture Collection; such as *E. coli* (PTCC 1533) *S. aureus* (PTCC 1113) *Bacillus subtilis* (PTCC 1023) *Salmonella galinarum* (PTCC 1510) *Pseudomonas aeroginosa* (PTCC 1310), have been compared and synergistic effects of them have been 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 nanoparticls in liquid batch cultures. In one comparative study we have also scrutinized antibacterial conducted of nanoparticls before and after to ultrasonic frequency by ultrasonic set.

MATERIALS AND METHODS

Synthesis of Zno monometallic nanocrystals via oxalate decomposition method

Zinc acetate (Suprapur, MERCK, and 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°C and after 30 min of continuous stirring oxalic acid (Suprapur, MERCK, and Germany) was rapidly added to the solution. The molar ratio Zn:OA was 1. The system was kept at 50°C under reflux for 2 h 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°C over night. The dried Zinc oxalate was ground and calcined at 550°C for 2 h.

Synthesis of Ag monometallic nano-crystales via oxalate decomposition method

Silver nitrate (Suprapur, MERCK, Germany) was added to ethanol (Suprapur, MERCK, Germany) in a two neck flask giving a 0.3 M gray solution. The temperature was elevated to 50°C and after 30 min of continuous stirring, oxalic acid (Suprapur, MERCK, Germany) was rapidly added to the solution. The molar ratio Ag:OA was 1. The system was kept at 50°C under reflux for 2 h 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°C overnight. The dried silver oxalate was ground and calcined at 550°C for 2 h.

Synthesis of Ag/ZnO nanocomposites via oxalate decomposition method

Zinc chloride (Suprapur, MERCK, Germany) and silver nitrate (Suprapur, MERCK, Germany) were added to ethanol (Suprapur, MERCK, Germany) in a two neck flask giving a 0.3 M gray solution. The temperature was raised to 50°C and after 30 min of continuous stirring, oxalic acid (Suprapur, MERCK, Germany) was rapidly added to the solution. The molar ratio Zn/Ag: OA was 1. The system was kept at 50°C under reflux for 2 h and a gray precipitate was obtained; then the resulting viscous gel was dried at 80°C overnight. The dried Zno/Ag oxalate was ground and calcined at 550°C for 2 h.

Characterization

Experiences of dependent on the crystallinity of the nanoparticls were carried out using an X-ray diffractometer set (XRD, Bruker D8-Advance Diffractometer using Cu Kα radiation). Also the nanoparticls 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 nanoparticls. 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 disk (Case and Johnson, 1984). A comparable examination with nanocomposites and mono-metallic nanocrystals loaded disks was utilized in this research. A 10 ml suspension of each nanoparticls (approximately, 16384 μgml^{-1}) was prepared into the Muller Hinton Broth medium and then suspension of each nanoparticls was sonicated at room temperature and frequency of 28 kHz, during at the 10 min, subsequently filtered through a membrane filter (0.2 µm, 15 mm diameter Shimie Rasan Teb). The nanoparticls laden filter paper was dried in an oven for 1 h and small disks of uniform size (6 mm diameter) containing 16384 μgml^{-1} nanoparticls were punched out and stored in a desiccator at room temperature. For each type of the bacterial inoculums (1.5×10^8 $CFUml^{-1}$) 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°C for 24 h, after which the average diameter of the inhibition zone enclosing the discs was measured with a ruler with up to 1 mm resolution. The examination was also replicated without sonication and so the results were compared together. Subsequently, the tests were reported for each type of nanoparticls and with each microbial

strain on three replicates.

Agar dilution test

A 16384 µgml^{-1} suspension of each nanoparticls was prepared into the Muller Hinton Broth medium, approximately, and then each nanoparticls was sonicated at room temperature and frequency of 28 kHz, at 10 min. For each type of the bacterial inoculums (1.5 × 10^{8} CFUml^{-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 nanoparticls was filled into the cavities. The plates were incubated at 3°C for 24 h, 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 was also replicated, without sonication, so the results were compared together. Subsequently, the tests were reported for each type of nanoparticls 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) (Qi et al., 2004). From the serial dilution method, was employed to determine the MIC of the nanoparticls (Dabbagh et al., 2008). Each of the twelve test tubes was filled with 1 ml 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 nanoparticls that had been sonicated at room temperature and frequency of 28 KHz, at 10 min, was already added and mixed thoroughly with the culture medium. The concentration of nanoparticls in each test tube becomes 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 (*S. aureus*, *Pseudomonas aeruginosa, B. subtilis, S. galinarum, E. coli*) 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 24 h. Then, the microbial growth was studied by turbidimetric measurement, using a spectrophotometer (Nano-volum spectrophotomet , Scandrop 250, Analytik jena Co.) (Siva et al., 2004). The experiments also included a positive control (test tube containing nanoparticls and Muller Hinton broth medium, devoid of inoculum) and a negative control (test tube containing inoculum and Muller Hinton broth medium, devoid of nanoparticls). The negative controls indicated the microbial growth profile in the absence of nanoparticls (Jayesh et al., 2007; Williams et al., 2006). All the experiments were carried out in triplicate.

The minimum bactericidal concentration (MBC), that is, the lowest concentration of nanoparticls 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 (Avadi et al., 2004). To experiments for bactericidal effect, loopful from each test tube (especially, negative and positive test tubes) was inoculated on Muller Hinton agar and incubated at 35°C for 24 h. The nanoparticls concentration illustrating bactericidal effect was picked out based on absence of colonies on the agar plate.

The release of Ag^{+} and Zn^{2+} ions from the nanoparticls into DI water and Muller Hinton broth medium was deliberated by suspending 10 mg of nanoparticls in 100 ml DI water/medium and sonicating with ultrasonic set (PARSONIC 7500s, Pars Nahand ENGG. Co. IRAN) for 10 min. 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 Ag^{+} and Zn^{2+} concentration in the aqueous phase was definite by ICP-AES after 24 h.

RESULTS

The FT-IR spectra analysis

Figure 1 shows FT-IR spectra of (a) Ag, (b) Zn_O and (c) Ag/Zn_O. The supplement of oxalic acid to the ethanol solution of Ag cation was cause to the precipitation of a gray solid of silver oxalate as shown by FT-IR spectrum in Figure 1a. The broad band at 3427.33 cm^{-1} was allocated to both the v_s(O-H) and v_{as}(O-H) of hydration water. The extreme band at 1634.68 cm^{-1} was allocated to asymmetric and water tensional tremble δ(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 875.31 cm^{-1} and 577.35 cm^{-1} are presents in the spectrum evidence of (O-C-O) tensional tremble and (M-O) tremble, respectively.

Figure 1b depended to ZnO FT-IR spectrum. The broad band at 3445.05 cm^{-1} was allocated to both the v_s(O-H) and v_{as}(O-H) of hydration water. The extreme band at 1629.57 cm^{-1} was allocated to asymmetric and water tensional tremble δ(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} and 551.12 cm^{-1} are presents in the spectrum evidence of (O-C-O) tensional tremble and (Zn-O) tensional tremble, respectively. Also, Figure 1c conclude Ag/ZnO FT-IR spectrum.

The broad band at 3426.47 cm^{-1} was allocated to both the v_s(O-H) and v_{as}(O-H) of hydration water. The extreme band at 1628.14 cm^{-1} was allocated to asymmetric and water tensional tremble δ(H-O-H). The shoulder at 1458.22 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands 625.36 cm^{-1} are presents in the spectrum evidence of (Ag/ZnO) tensional tremble, respectively.

The XRD spectra analysis

The XRD pattern of Ag, Zn_O and Ag/Zn_O nanoparticls (Figures 2a, 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 nanocrystals were calculated using the Debye-Scherer Equation from the major diffraction peaks. ($C.S = \frac{K.\lambda}{\beta.\cos\theta}$ FQUOTE K.λ / β.cosθ). Where K is a constant equal to 0.9, λ is the wavelength of Cu Kα radiation, β is the full width at half maximum (FWHM) of the diffraction peak in radiant and θ is the Bragg angles of the main

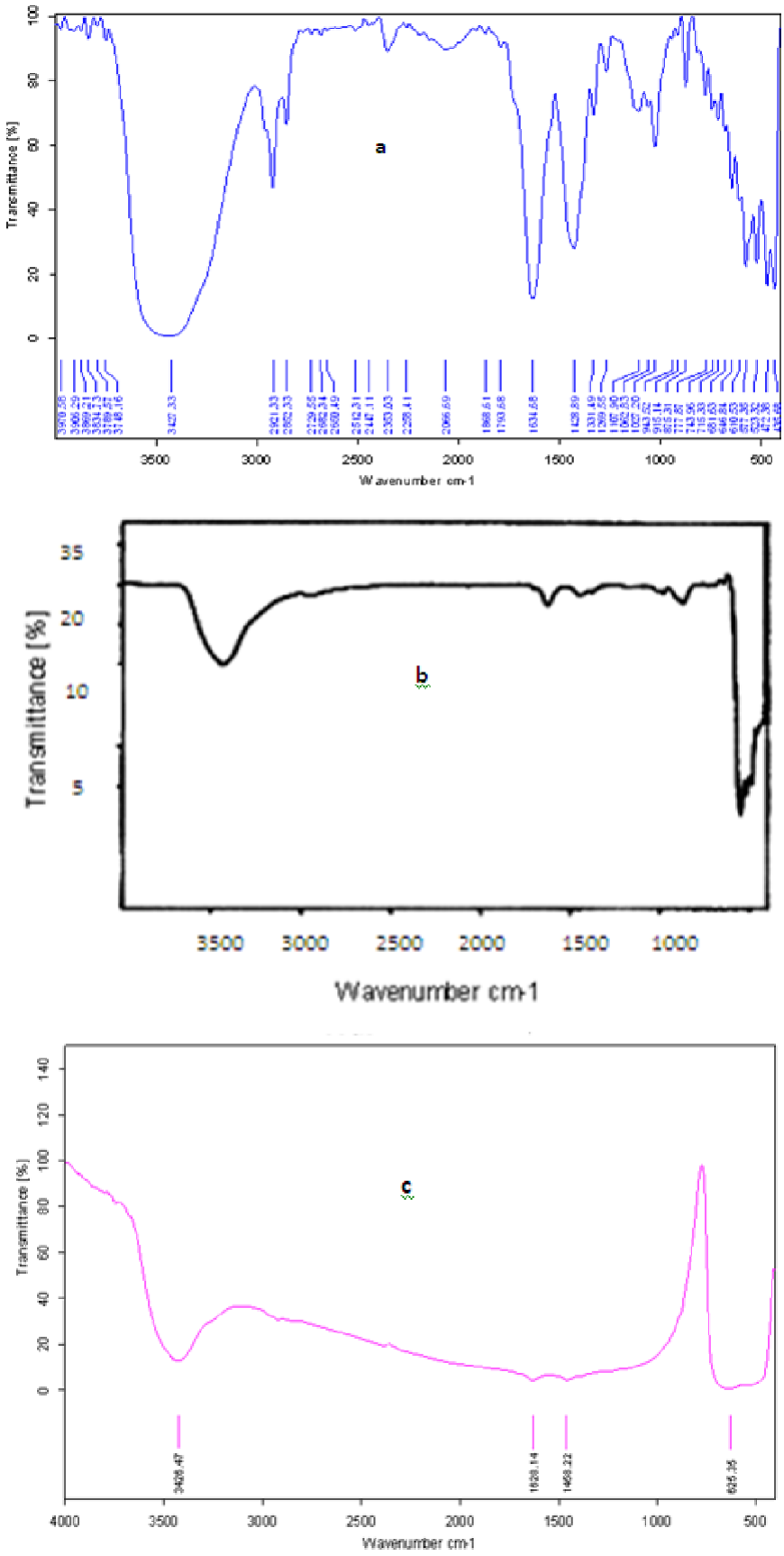

Figure 1. FT-IR pattern of (a) Ag, (b) ZnO and (c) Ag/ZnO nanoparticls.

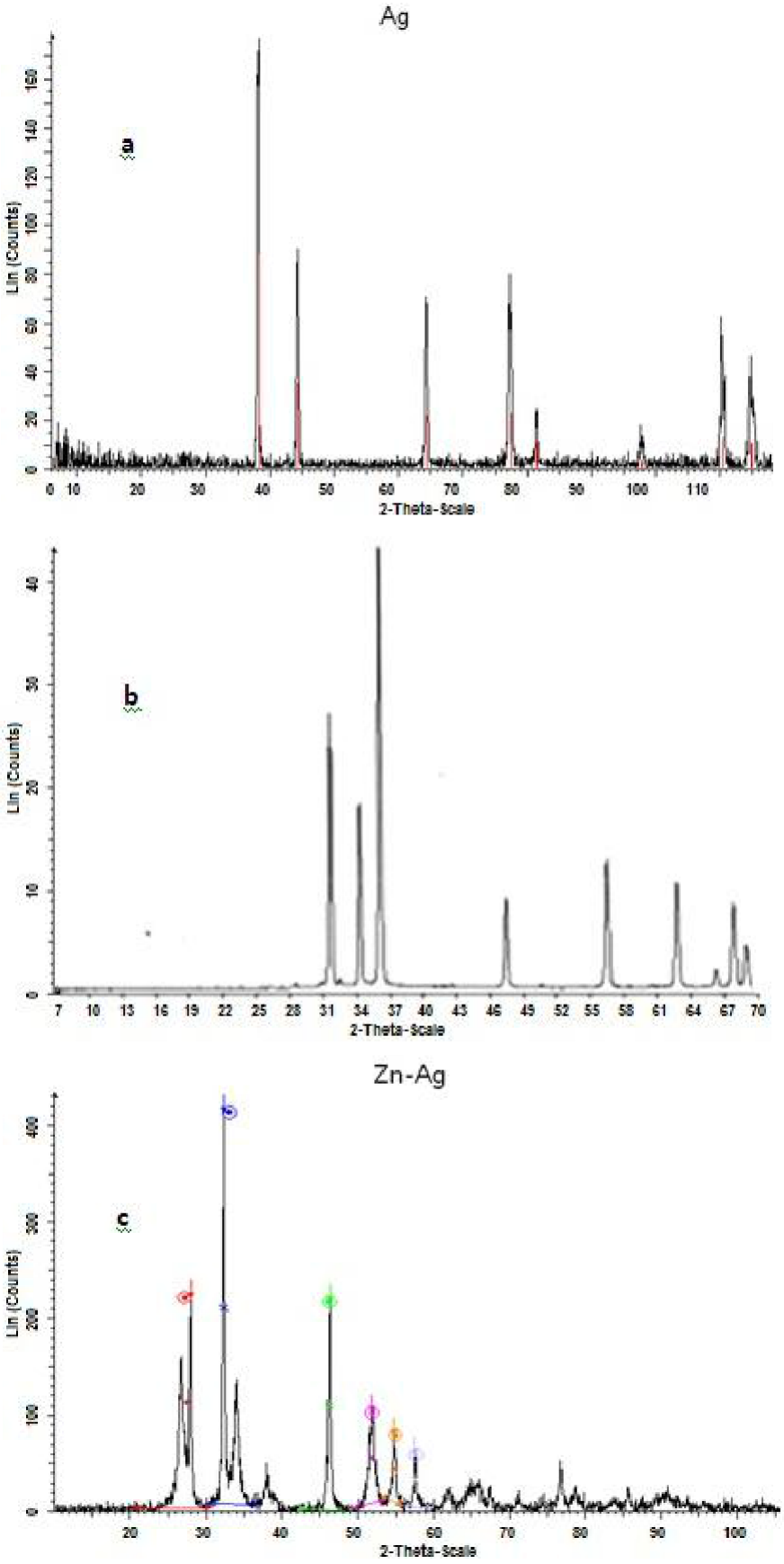

Figure 2. XRD pattern of (a) Ag (b) ZnO and (c) Ag/ZnO nanoparticls.

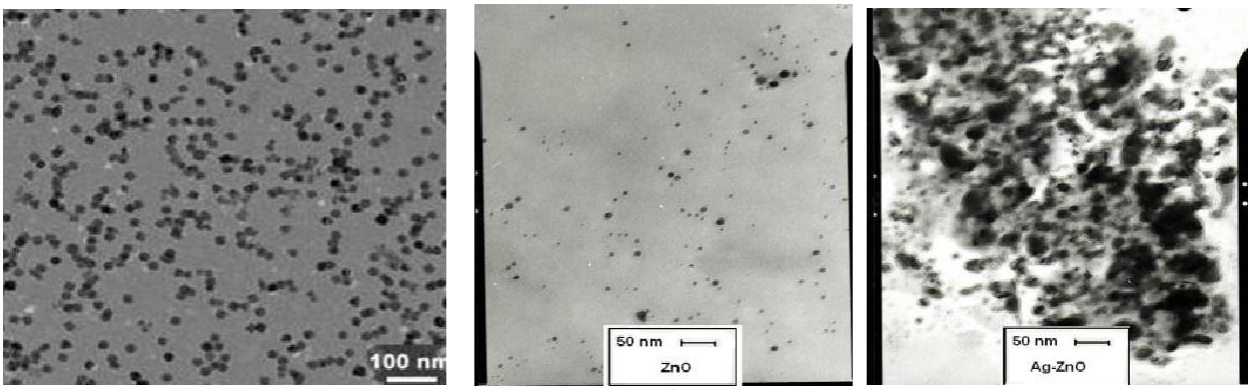

Figure 3. TEM images of Figure(a)Ag, 3(b). ZnTEMoandimages(c)Ag/Znof(a)onanoparticlsAg,(b)ZnO· and (c) Ag/ZnO nanoparticls

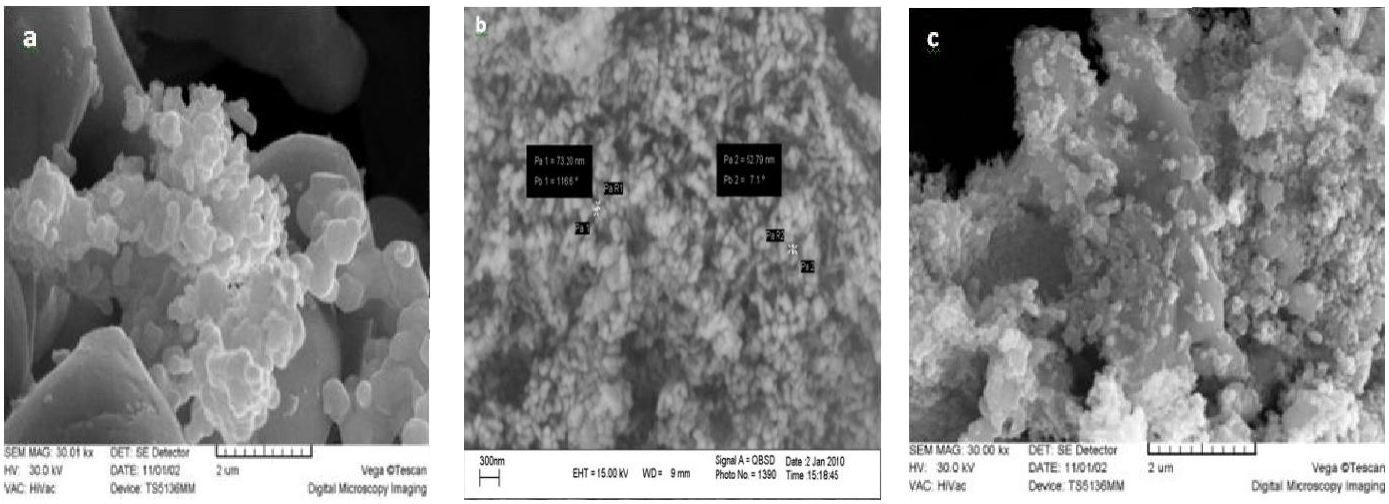

Figure 4. EM images (a) Ag, (b) Zno and (c) Ag/Zno nanoparticls.

planes. The average crystallite size of the Ag, Zn_O and Ag/Zn_O were 8.66 nm, 24.75 nm and 12.15 nm, respectively.

The ICP-AES spectra analysis

By ICP-AES analysis, we were succeeding to estimate of residual ions, after digestion of nanoparticls by sonication. They indicated ions levels of 120 ppm to silver and ≤ 1 ppm to zinc oxide, in the silver/Zinc Oxide nanoparticls, respectively.

The TEM and SEM images analysis

TEM image of silver nanoparticls were taken (Figure 3) and approved that the metal particles were in the nano range, approximately. However, SEM images (Figure 4) of nanoparticls were showed that silver; zinc oxide and silver/zinc oxide metal particles were exactly in the shape of spherical and clustered.

The antibacterial activity analysis

The antibacterial activity of silver, zinc Oxide and silver/ zinc oxide nanoparticls was compared for *S. aureus*, *P. aeruginosa, B. subtilis, S. galinarum, E. coli* 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 demon-strate larger DIZ, while resistant strains exhibit smaller DIZ. The disks with silver and Zinc oxide nanoparticls were compared to the silver/zinc oxide nanoparticls for all

Table 1. Disc diffusion Test (µgml^{-1}), Agar dilution Test (µgml^{-1}), MIC (µgml^{-1}) and MBC (µgml^{-1}) of silver, zinc oxide and silver/zinc oxide nanoparticls for various microorganisms.

	Nano particles	Disc diffusion Test(DIZ) (mm)	Agar dilution Test (DIZ)	MIC (µg/ml)	MBC (µg/ml)
	Zn	10	8 mm	256	4096 >
P. aeruginosa	Ag	12	10 mm	> 4096	> 4096
	Ag/Zn	12	10 mm	64	1024
	Zn	15	20 mm	512	4096
B. subtilis	Ag	10	Negative	>4096	>4096
	Ag/Zn	10	Negative	128	2048
	Zn	10	15 mm	128	512
S. galinarium	Ag	10	10 mm	> 4096	> 4096
	Ag/Zn	12	10 mm	32	512
	Zn	12	15 mm	64	512
E. coli	Ag	8	10 mm	2048	>4096
	Ag/Zn	10	10 mm	32	512
	Zn	12	15 mm	256	2048
S. aureus	Ag	8	10 mm	1024	4096
	Ag/Zn	12	10 mm	128	2048

strains selected for this study. The DIZ for zinc oxide and silver/zinc oxide nanoparticls impregnated disks was almost greater than that studied with the silver nano-particls impregnated disks for all the strains selected for this study. Correspondingly, for *S. aureus*, *P. aeruginosa, S. galinarum,* and *E. coli* the zinc oxide and silver/zinc oxide nanoparticls impregnated found tobe more effective compared to silver nano-particls impregnated disks, however the difference in the DIZ was merely 10 to 15%. In contrast, for *P. aeruginosa*, the disks impregnated with silver/zinc oxide nanoparticls showed a significantly larger DIZ, almost greater compared to that observed with silver nanoparticls. Interestingly, for *S. aureus* and *S. galinarum* the disks impregnated with silver nanoparticls showed a weaker DIZ, compared to that observed with other nanoparticls. Since DIZ was measured on agar plates using a ruler with 1 mm resolution, the possibility of measurement errors exists. 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 nanoparticls.

The results is depended on the antibacterial effects of nanoparticls against different of bacterial via the disc diffusion test, the agar dilution test the MIC and the MBC are summarized in Table 1. A 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 (Sondi et al., 2004). We analysed effectivity of silver, Zinc oxide nanoparticls and silver/zinc oxide nanocomposite against *E. coli*, *S. aureus*, *B. subtilis, S. galinarum*, *P. aeroginosa*. The bactericidal effect of nanoparticls is dependent on the concentration of nanoparticls and the initial bacterial concentration (Pal et al., 2007). In this study, the initial bacterial concentration was constant at 1.5×10^8 CFU ml^{-1} regardless of nanoparticls concentration and microbial strain. Our research shows that zinc oxide nanoparticls have got antibacterial effects against all of bacteria chosen for this study, especially *E coli.*

The MIC observed for zinc oxide nanoparticls were 512 µgml^{-1} for *B. subtilis,* 256 µgml^{-1} for *S. aureus, 256* µgml^{-1} for *P. aeruginosa,* 128 µgml^{-1} for *S. galinarium* and 64 µgml^{-1} for *E. coli.* Our results observed that the *S. galinarium* and strains of *E. coli*, were most sensitivity against zinc oxide nanoparticls. Surprisingly, anti-bacterial effect of the silver nanoparticls was so weaker. These nanoparticls had not even antibacterial effective against *B. subtilis, S. galinarium* and *P. aeruginosa.* The MIC observed for silver nanoparticls were 1024 µgml^{-1} for *S. aureus* and 2048 µgml^{-1} for *E. coli.* In contrast with all of the nanoparticls that picked out for this study, the most antibacterial effect was seen to silver/zinc oxide nanocomposite. Interestingly, the *S. galinarium* and *E. coli* were most sensitivity against of silver/zinc oxide nanocomposite. The MIC observed in this study for silver/zinc oxide nanocomposite were 128 µgml^{-1} for *B. subtilis,* 128 µgml^{-1} for *S. aureus*, 64 µgml^{-1} for *P. aeruginosa*, 32 µgml^{-1} for *S. galinarium* and also 32 µgml^{-1} for *E. coli.*

DISCUSSION

Gan believed that colloidal and agglomerated nano-particls may affect its ability in inhibiting or destroying bacteria and also influence the degree of MIC and MBC (Gan et al., 2004). Regarding this theory, Guogang and Jayesh exposed the suspension of nanoparticles in liquid medium to the ulteraviolet waves for 10 min to let them out of agglomeration and being dispersed and suspended (Guogang et al., 2009; Jayesh et al., 2007). Several studies performed by many authors on the antibacterial properties of Ag nanoparticls in colloidal phase (Guogang et al., 2009; Jayesh et al., 2007; Kim et al., 2007; Sondi et al., 2004; Avadi et al., 2004;). However, 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 of understudy nanoparticls - pre and post - exposed with ultrasonic waves, against standard strains of bacteria, using disc diffusion and agar dilution methods by sonicator machine. Data of the study indicated that in spite of the theories of authors like Gan, Guogang and Jayesh, the antibacterial effects of metal oxide nanoparticls 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 (Guogang et al., 2009; Jayesh et al., 2007; Gan et al., 2004).

Studies of Jayesh indicated that in aqua medium, no systematic change in the size of nanoparticles was observed after 24 h. In the current study, after synthesis of nanoparticls of metal oxides Ag, Zn_O and combined nanoparticls of Ag/Zn_O, their anti-bacterial effects compared. Though, studies of several authors in recent years, confirmed the antibacterial effects of Ag nanoparticles (Lok et al., 2006; Sondi et al., 2004; Batarseh et al., 2004). In the current study, disc diffusion and agar dilution methods was used for deter-mining the antibacterial effects of nanoparticls. Jayesh performed extensive experiments in determi-nation of microbial sensitivity of various bacteria to silver and copper nanoparticls, using disc diffusion method (Jayesh et al., 2007). 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.

Results of the disc diffusion with Ag nanoparticls, by Thirumurugan against strains of pathogens *E. coli, salmonella typhi, B. subtilis, S. aureus*, indicated higher sensitivity to silver nanoparticls which is in contrast with the results of our study (Thirumurugan et al., 2009). Cho studies the MIC of Ag nanoparticls against *P. aeruginosa* bacteria in which its growth in concentration of 7.5 μgml^{-1} completely inhibited (Cho et al., 2005). However, the growth of strain 1310 of *P. aeruginosa* was used in the current study, was not inhibited in 4096 μgml^{-1}. Lowest MIC in *P. aeruginosa* observed in combined nanoparticls of Ag/ZnO with 64 μgml^{-1},that is, it has the highest inhibitory effect on *P. aeruginosa*. Following their research project, cho reported the MIC rate of silver nanoparticls for *S. aureus* as 12.6 μgml^{-1}, but the MIC for this bacteria affected by silver nanoparticle reported 1024 μgml^{-1} (Cho et al., 2005). The *S. aureus*, used in the current study, showed the least and the most sensitivity to silver and Zn nanoparticls, respectively. Actually, the least degree of MIC in *S. aureus* was related to combined nanoparticls of silver and zinc with concentration of 128 μgml^{-1}, that is, this nanoparticls had the most growth inhibitory effect in *S. aureus*. Lock concluded that the most the dimension of silver nanoparticls, the better would be the MIC and their antibacterial effect would decrease (Lock et al., 2006). Jayesh recorded the MIC rate ranged 40 to 180 μgml^{-1} using tests of determining the sensitivity of silver nanoparticls against different strains of *E. coli* (Jayesh et al., 2007). The aim was to indicate that the sensitivity of different strains of one bacteria to silver nanoparticls shows meaningful difference. Kim studied the Gram negative bacteria *E. coli* and Gram positive *S. aureus,* also reported that the antibacterial silver nanoparticls mostly affects the *E. coli,* which is due to the difference between cell wall of Gram negative and positive microorganisms (Kim et al., 2007). Rate of MIC obtained by Ping Li for silver nanoparticls and against *E. coli* was 40 μgml^{-1}, but the MIC of silver nanoparticls against *S. typhi, B. subtilis* and *S. aureus* were 0.157 μgml^{-1}, 0.625 μgml^{-1}, respectively (Ping Li et al., 2005). In our study, the lowest MIC in *S. galinarum* observed for combined nanoparticls of Ag/ZnO with concentration of 32 μgml^{-1} which showed the most inhibitory effect in *S. galinarum*. Silver nanoparticle had no inhibitory effect against *S. galinarioum*. Considering their studies Kim claimed that *S. aureus* is more resistant to silver nanoparticls compared to *E. coli (*Kim et al., 2007). However, limited studies performed on the antibacterial properties of Zn_O. Reddy were amongst few authors worked on the toxicity of the ZnO nanoparticls in Gram negative and positive bacteria (Reddy et al., 2007). They found that this nanoparticls are able to completely inhibit the growth of *E. coli.* Another idea presented in the study was the processing of combined metal oxides of nanoparticls with antibacterial effects and the examining and comparing their antimicrobial effects. As already mentioned, for the first time, processed the Ag/Zn_O combined metal oxide nanoparticls and following them, Guogang presented the theory of using Zn_O combined metal nanoparticle for obtaining a more resistant antibiotic against methicillin resistant *S. aureus* (MRSA) (Guogang et al., 2009). According to their studies, strains of Gram negative bacteria showed higher resistance to copper oxide nanoparticls combined with silver. However, Jayesh, suggested that combining of copper and silver nano-particls may lead to the increased bactericidal effects (Jayesh et al., 2007). Framework of the idea formed performing the scientific study in the formating of a project. Up to now, no complete and comprehensive

study reported in the field of combining antibacterial nanoparticls and the comparison of their antibacterial properties on a wide spectrum of bacteria. Amongst few studies, Ling Yang, combined silver nanoparticles with Zn to improve antibacterial activity of Zn nanoparticls and investigate the antibacterial effect of Zinc oxide and silver nanoparticls and also comparing them with Ag/Zn_O nanoparticls (Yang et al., 2006). They obtained interesting results. According to the findings of Kawashita and Pak-soo silver significantly increased antimicrobial activity (Kawashita et al., 2000; Pak-soo and Jang Yu, 2003). Actually, Ling Yang believed that photocatalytic ability of Zn_O nanoparticls plus silver nanoparticle, improves and also increases its oxidation and reduction abilities, while suppressing bacteria growth (Yang et al., 2006). How-ever, silver ions, eventually release during sterilization and kill bacteria due to their high antibacterial activation. They theorized that silver ions release following bacteria death and colloid with other bacteria and repeat their sterilization behavior. It was also mentioned that silver covered in the surface of Zn nanoparticls has the ability to involve the electrons produced through photocatalytic reactions of Zn nanoparticls which increases electron isolation and makes gaps in cell membrane, so increase its antimicrobial activity. Regarding studies of these authors, antibacterial property of silver and zinc oxide nanoparticls improves with their combination. In fact, our study confirmed that the Gram negative strains of bacteria had most sensitive to silver/zinc oxide nano-composite. Further out study approved that combination of zinc oxide and silver nanoparticls, increased their bactericidal effect.

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