Journal of Pharmaceutical and Health Sciences 2017;5(2), 171-179.

Original Article

JPHS

Preparation of ZnO and ZnS nanoparticles and in-vitro study of their antimicrobial effect

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Abstract

Zinc sulfide (ZnS) and zinc oxide (ZnO) nanoparticles were evaluated for their antimicrobial activity against four pathogenic strains. ZnS and ZnO nanoparticles were synthesized by simple aqueous chemical reaction in an aqueous solution. The main advantage of these nanoparticles (size of 10-30 nm) was that they simply could be prepared using cheap precursors in a cost effective and high throughput manner. The structural, morphological and chemical composition of the prepared nanoparticles were investigated by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and energy dispersion X-ray dispersive fluorescence spectroscopy (EDAX). The antimicrobial effects of the ZnS and ZnOnanoparticls were studied by serial dilution technique and also by well diffusion technique, against four pathogenic microorganism strains of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and C. Albicans. Both nanoparticles of ZnS and ZnO showed antimicrobial activity against both gram-positive Staphylococcus aureus and gram-negative Escherichia coli and Pseudomonas aeruginosa and fungi of Candida albicans. The best antimicrobial efficacy (as MIC of 50 μg/ml) was related to the effect of ZnO nanoparticles on Staphylococcus aureus and the most resistant pathogen was Candida barbicans against ZnS nanoparticles with a MIC more than 250 μg/ml.

Keywords: Zinc sulfide, nanoparticles, aqueous chemical synthesis, Zinc Oxide, Antimicrobial activity.

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Introduction

Antibacterial compounds can be described as the local killer of bacteria or as agents to slow down their growth, without being toxic to surrounding healthy tissue. Many available antibacterial agents prepared from pure natural products or via chemical modification of natural compounds like b-lactams and as pure synthetic antibiotics, like sulfonamides (Von Nussbaum et al. 2006). Antibacterial agents can be classified as either bactericidal or bacteriostatic. Although, because of broad use and abuse, the appearance of bacterial resistance to antibacterial drugs has become a problem. Resistance is usually based on evolutionary processes and gene transfer by conjugation, transduction or transformation (Witte, 2004).Thus, due to progressive resistance against many conventional antibacterial agents, infectious diseases continue to be the greatest health challenges in all over the world. In addition to multiple drug resistance, adverse side effects and intolerable toxicity of common antibacterial agents has prompted the discovery of alternative antibacterial treatments (Baker-Austin et al. 2006).

Although antimicrobial agents are often complex chemical compounds, some of the nanosized simple inorganic compounds have also shown antibacterial activity in vitro as well as in animal models (Padmavati et al. 2008; Huh et al. 2011). NPs offer a higher surface area to volume ratio, resulting in the appearance of new mechanical, chemical, magnetic and optical properties that differ from their bulk properties (Huh et al. 2011). Here, NPs have been demonstrated to be interesting in the context of inhibition bacteria (Whitesides, 2005). Bacterial sensitivity to NPs is not only related to the structure of the cell wall in gram-positive and gram-negative bacteria (Ashkarran et al. 2012) but also, characteristics and composition of NPs can influence the susceptibility or tolerance of bacteria to NPs. For example, Escherichia coli (–) is highly susceptible, whereas Staphylococcus aureus (+) and Bacillus subtilis (+) are less susceptible to CuO NPs (BaekandAn, 2011). The antibacterial effect of Ag NPs is higher than Cu NPs against E. coli (–) and S. aureus (+) bacteria (lu et al. 2009). S. aureus (+) and B. subtilis (+) are more susceptible than E. coli (–) to NiO and ZnO NPs (Baek andAn, 2011).Antibacterial activity of zinc compound nanoparticles (i.eZnO-NPs) has received significant interest worldwide recently (Seiland Webster, 2012).

Zinc is a vital trace element necessary for approximately all aspects of life (Andreini et al. 2006). It is essential for all bacteria in the limited deal but excess amounts of the metal can possess poisonous influence on them (Ellison et al. 2013).Zinc oxide and zinc sulfide are important inorganic materials that have multiple properties, such as semiconducting properties, antibacterial activity and growth promoter. It is widely applied in the field of optoelectronics (Yu et al. 2003; Gao et al. 2005), pharmaceutics (Baldwin et al. 2001), cosmetics (Sheldon et al. 2000; Mitchnick et al. 1999), food science (Daniel et al. 2003)and agriculture (Smith et al. 1997; Carlson et al. 1999).

Combined with nanotechnology, zinc compound nanoparticles can be prepared, which possess some unique characters, such as small particle size and large area surface (Yamamoto, 2001). Therefore, the interactions of nanoparticles with microorganisms have recently attracted more attention (Jones et al. 2008; Reddy et al. 2007). Moreover, zinc oxide nanoparticles have selective toxicity and are generally regarded as a safe reagent to humans and animals (Liu et al. 2009; Berube et al. 2008), which means that these agents could be an ideal potential antibacterial reagent to replace some antibiotics. Preparation of these NPs via a novel mediator and process were implemented here to obtain specific characteristics for Zinc Oxide and Zinc Sulfide NPs. Therefore, our study was

undertaken to prepare ZnOandZnS NPs and to investigate the antibacterial activity of these two NPs against Escherichia coli, Streptococcus aureus, Pseudomonas Earoginosa, and Candida Albicans in vitro.

Material and method

Material

Bacteria and Fungi: A total of 4 pathogens belonging to 2 gram-negative,1 gram-positive strains, and one fungus were tested. These were of human origin, identified as described by Barrow and Feltham and preserved in freeze dried state.

Chemical compounds: Zinc nitrate, Sodium ethoxylated sulfosuccinate, zinc chloride (ZnCl2) and sodium sulfide (Na2S) were purchased from Merck, Germany.

Media: Liquid media used for the study were nutrient broth (NB, Oxoid) and Mueller-Hinton broth (MHB, Oxoid): solid media were nutrient agar (NA, Oxoid).

Synthesis of Zinc Oxide nanoparticle

Synthesis of ZnO nanoparticles was carried out by an aqueous chemical method using ZnNO3 and NaOH as source materials. Sodium ethoxylated sulfosuccinate was used as detergent and viscosity modifier. All the reagents were of analytical grade. The entire process was carried out in distilled water for its inherent advantages of being simple and environment-friendly. All steps of the synthesis were performed at 25 ºC temperature and in ambient conditions. In a typical preparation solution of 0.5 M, NaOH was added drop by drop to 1M ZnNO3 solution which was kept on stirring using a magnetic stirrer for 30 minutes and 800 rpm; this resulted in the formation of ZnOnano colloid. The nanoparticles were collected on Watlman 42 paper further purification was made in an ultrasonic bath. The resultant product was finally dried in Aven at 50 ºC for 2 hours.

Synthesis of Zinc Sulfide nanoparticle

Synthesis of ZnS nanoparticles was carried out by an aqueous chemical method using ZnNO3 and Na2S as source materials. Sodium ethoxylated sulfosuccinate was used as detergent and viscosity modifier.All the reagents were of analytical grade. The entire process was carried out in distilled water, for its inherent advantages of being simple and environment-friendly. All steps of the synthesis were performed at a 28ºC temperature and ambient conditions. In a typical preparation, a solution of 1 M Na2S was added drop by drop to the 1M ZnNO3 solution, which was kept stirring using a magnetic stirrer for 30minutes; this resulted in the formation of ZnS nano colloid. The nanoparticles were collected on Watlman 42 paper further purification was made in an ultrasonic bath. The resultant product was finally dried in Aven at 50 ° C for 2 hours.

Physicochemical characterization of nanoparticles

The prepared sample was subjected to characterization by XRD (Model D8, Bruker) to determine the phase purity and average particle size of the sample. The nanophase was identified by comparing peak positions and intensities (fingerprint method) (Yang et al. 2008). To investigate the morphological structure of sample surfaces, surface textures were examined by energy dispersion X-ray fluorescence spectroscopy (EDAX) (Tokyo, Japan), was also carried out to ascertain the composition.

An electronic image of nanoparticles was obtained by Scanning Electron Microscopy to confirm size distribution and morphology of nanoparticles.

Antimicrobial assay

In vitro tests for determination of Minimum Inhibitory Concentration (MIC) of ZnO and ZnS

nanoparticles

To prepare ZnS nanoparticle solution 0.01g of the synthesized ZnS nanoparticles were dissolved in 10 ml of sterile distilled water with the help of a magnetic stirrer. The final concentration of ZnS and ZnO nanoparticles in the solution was 1μg/ml. This solution was applied in the wells bored in the agar plates for the study of antimicrobial activity.

The gram-negative bacteria were grown in MHB and the gram-positive ones in NB for 18h to obtain optimum growth.The above-mentioned solutions of NPs were added to molten nutrient agar at 50ºC in such a manner that the final concentrations were 0(control), 100, 200, 300, 400 μg/ml, thoroughly mixed, final pH adjusted to 7.2 to 7.4 and poured into sterile Petri dishes.The inoculum consisted of the suitably diluted broth culture of a bacterium. The MIC of NPs was determined by spot inoculating one 2mm (internal diameter) loopful of a culture containing 105 colony forming units (CFU) of pathogenic bacteria and fungi, on the plates following the guidelines of CLSI. The plates were incubated at 37 °C. Growth was recorded at 18h as well as after 72 h. (Clinical and Laboratory Standards Institute, 2009).

Determination of antimicrobial action of ZnS and ZnO by well diffusion assay

The in vitro effect of the NPs was determined by well diffusion technique as described by Miles and Amyes (1996). Each 5mm diameter well was cut with the help of sterile borer on the agar surface at suitable distances apart, so that the respective agents would not diffuse into one another to produce a continuous range of concentrations in the initial period of inhibition. This was done by initial well sensitivity test of a microorganism with respect to a particular concentration of an agent and determining the diameters of zone of inhibition.

Results

In Figure 1, scanning electronic images if ZnO and ZnS nanoparticles have been shown. ZnO nanoparticles had a mean size of 20-31 nm after counting a frame of 100 particles diameters. For ZnS nanoparticles, mean size was 10-14 nm. As it can be seen in Figure 1, particles of ZnS in smaller than ZnO nanoparticles when both were prepared by chemical dissolution method.It is seen that the nanoparticles are homogenously dispersed and almost spherically shaped.

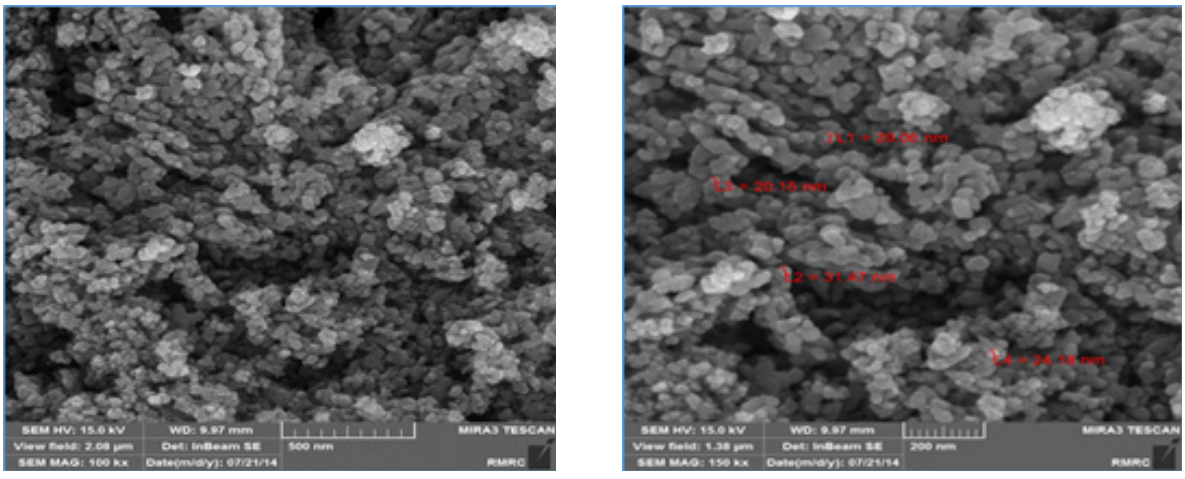


Figure 1: Electro micrographs of ZnS (left image) and ZnO (right image) nanoparticles (100k magnitude)

Energy Dispersion X-ray Fluorescence Spectroscopy (EDAX)

Energy Dispersion X-ray Fluorescence Spectroscopy (Tokyo, Japan) was also carried out to ascertain the composition. Based on observation, purity of ZnO in our reaction was more than 98% and less than 2% impurities were in β form, as is shown in Figure 2. The purity of ZnS nanoparticles based on the crystallinity of the sample was more than 97%. XRD peaks of ZnS and ZnO nanoparticles also confirmed purity and size (less than 100 nm) of these particles. In Figure 3, peaks of ZnS are shown. The successful synthesis of nanoparticles can be concluded based on these findings. From the XRD results, as shown in Figure 3, it is clear that pure ZnS nanoparticles were obtained in powder form.

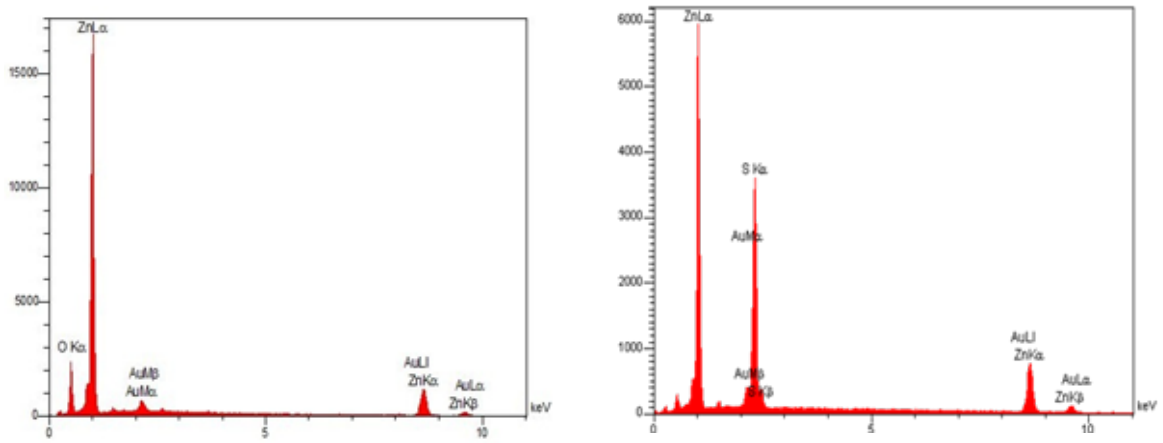


Figure 2: EDAX profile of ZnO nanoparticle (left) and ZnS nanoparticles (right).

The broadened peaks in the XRD pattern indicated the formation of ZnS nanocrystals with small crystallites. The average crystallite size (D) was calculated from the full-width at half-maximum (FWHM) of the most intense peak of the plane of ZnS nanoparticles using the Debye-Scherrer formula for spherical particles [Eq. (1)].

$D = 0.89\lambda / (\beta \cos \theta)$ (1)

Where λ is the wavelength, β is the full width at the half maximum of the ZnS nanoparticles and θ is the diffraction angle. From this equation, the average particle size was estimated to be 12 nm which was also supported by SEM.

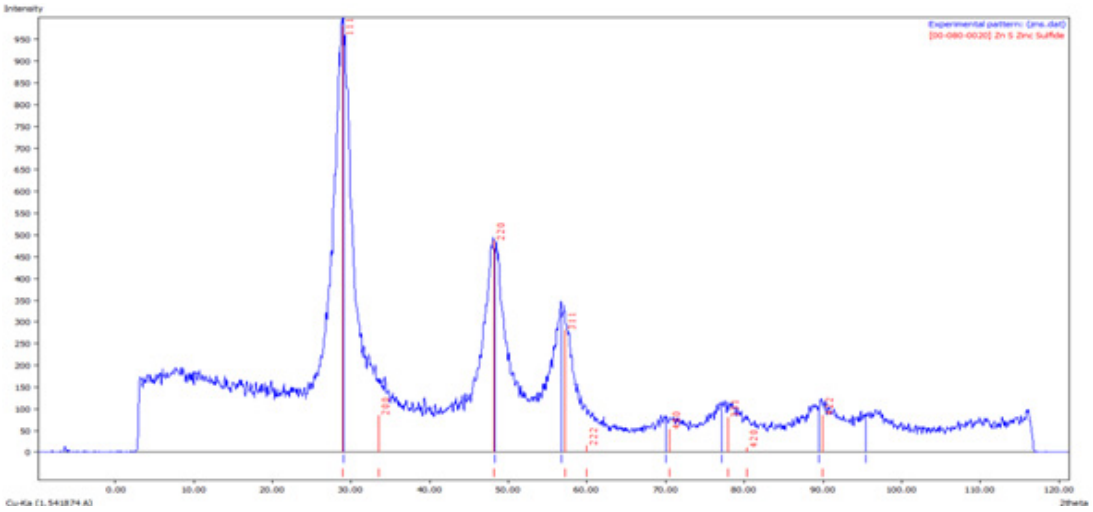


Figure 3: XRD curve of ZnS nanoparticles

In vitro tests for determination of Minimum Inhibitory Concentration (MIC) of ZnO and ZnS nanoparticles by spot inoculation technique

After application of spot inoculation method, results of MIC test were shown in Table 1. Both nanoparticles of ZnO and ZnS showed inhibitory effect on selected microorganisms. Despite our expectations, the overall inhibitory effect of ZnO was more than ZnS. The strongest inhibitory effect was related to ZnO nanoparticles on S. aureus (50 μg/ml), while ZnS nanoparticles had a weak effect on C. albicans (MIC > 250 μg/ml).

Table 1: Minimum Inhibitory Concentration (MIC) of ZnOandZnS nanoparticles

Pathogen name	**Pathogen name**	
	ZnO	ZnS
E. coli	150	200
S. aureus	50	100
P. aeruginosa	100	100
C. albicans	250	250<

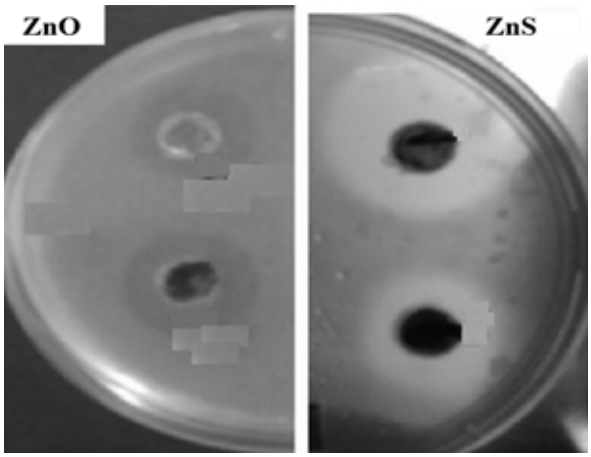
Determination of antimicrobial action of ZnS and ZnO by well diffusion assay

The nanoparticles of ZnS and ZnO produced inhibition zones around the wells that varied from 0 mm to 27 mm when a number of nanoparticles were 100 to 200 μg per well. The diameters of inhibitory circles increased in size, as the amount of ZnO and ZnS nanoparticles increased. The greater sensitivity of gram-positive organisms by ZnS and ZnO nanoparticles was further confirmed by this test. A sample diffusion well has been shown for each nanoparticle in Figure 4; in this case, for exposure of 150 μg of nanoparticles to E. coli culture lead to 23 mm inhibitory diameter for ZnS and 20 mm for ZnO wells. The radius of inhibition correlated with the concentration of nanoparticles. As it can be seen, in C. albicans cases, these nanoparticles have weak inhibition.This can be because of wall nature of fungi that are resistant to metallic nanoparticles.

Table2: Antimicrobial activity of ZnS and ZnO nanoparticles (mm inhibition) in diffusion well method

NP type	Pathogen name	**(Amount of NPs per well (μg**		
		100	150	200
ZnO	E. coli	17	23	26
	S. aureus	15	23	26
	P. aeruginosa	2	10	15
	C. albicans	1	10	13
ZnS	E. coli	15	20	22
	S. aureus	16	22	27
	P. aeruginosa	1	10	12
	C. albicans	0	8	11

Table2: Antimicrobial activity of ZnS and ZnO nanoparticles (mm inhibition) in diffusion well method



Discussion

Results obtained by nanoparticle synthesis using aqueous chemical method showed that this method could produce suitable and homogeneous nanoparticles with good purity and throughput. Results were according to precipitation methods in some other work (MonsefKhoshhesab et al. 2015).

Due to the problem of drug resistance among bacterial pathogens, the search for antimicrobials has now been extended to a class of compounds named "non-antibiotics", which are employed for the therapy of non-infectious pathology and demonstrate significant antimicrobial activity against some of the most pathogenic infectious agents (Dasgupta et al. 2008; Jeyaseeli et al. 2012).

Inhibitory effect of ZnO was more than ZnS nanoparticles, owing to the electronic density on the surface of oxygen atoms in the structure of ZnO. Probably, the interaction of ZnO nanoparticles with a surface protein of pathogens and production of free radicals (ROS) are more frequent to disturb the integrity of microbial wall and membrane. Although, the difference between ZnO and ZnS nanoparticle is not significant generally (P-value = 0.067). The magnitude of inhibitory effect of Zinc nanoparticle is comparable with some of usual like cephalosporins and macrolides (TillotsonandTheriault, 2013).

It may be pointed out here that ZnS and ZnO nanoparticles demonstrated a pronounced inhibitory action against S.aureus, a known multidrug sensitive pathogen. This is in agreement with some other investigation that showed ZnS nanoparticles are bacteriostatic in vitro against both gram positive and gram negative bacteria (Banoee et al. 2010).

Distinctive mechanisms that have been put forward in the literature are listed as following: direct contact of ZnO-NPs with cell walls, resulting in destructing bacterial cell integrity(Zhang et al. 2007), the liberation of antimicrobial ions, mainly Zn cations and ROS formation (Lipovsky et al. 2011). However, the toxicity mechanism varies in various media as the nature of dissolved Zn may change according to the medium components beside the physicochemical properties of ZnO and ZnS nanoparticles (Sirelkhatim et al. 2015).

The difference of inhibition between bacteria and fungi can be explained via variation of their cell walls and cell membrane. Antimicrobial effect of metallic nanoparticles revealed and confirmed in many surveys (Ciobanu et al. 2013), which is in agreement with our findings. Albeit, it should be noticed that ZnO microparticles hasn't shown any antimicro-

bial effect and is an inert excipient in many medical and cosmetic preparations.

Conclusion

This study revealed the sensitivity of ZnO and ZnS nanoparticles toward the microorganisms that are of threatening concern.This study concludes that the toxicity differs from one species to another. Additional research is required to investigate the exact toxicity mechanisms to deeply elucidate the sensitivity of bacteria to the nanoparticle. More emphasis should be given to the correlation between ZnS and ZnO nanoparticle structural, optical, electrical, and chemical properties, and their bacterial toxicity.

Acknowledgment

The research described in this paper was financially supported by research center of Kashan University of Medical Sciences.
Conflict of Interest: There is no conflict of interest in this article.

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