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ORIGINAL RESEARCH PAPER

Antibacterial effect assessment of ZnS: Ag nanoparticles

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ABSTRACT

Objective(s): A large ratio of surface to volume of nanoparticles in comparison with bulk ones, will increase the cell penetration and therefore their toxicity.

Materials and Methods: Chemical precipitation method was used in order to synthesis of ZnS:Ag quantum dots. Their Physical properties and characteristics were assessed by X-ray diffraction, Ultra Violet-Visible Spectrophotometer, Transmission Electron Microscope and it was shown that the obtained ZnS:Ag quantum dots are cubic with high-quality. Antibacterial effects of ZnS:Ag nanoparticles against *Pseudomonas aeroginosa, Staphylococcus aureus* and *Salmonella typhi* were investigated. Disc bacteriological tests were used in order to assessment of the antibacterial effects of ZnS:Ag nanoparticles.

Results: The size of inhibition zone was different according to the type of bacteria and the concentrations of ZnS:Ag QDs. The maximum diameter was happened for *S. aureus*. The results of MICs obtained fromBroth Dilution for *Pseudomonas aeruginosa* , *Staphylococcus aureus* and *Salmonella typhi*, are 3.05 , 3.05 and 6.1 mg/ml whereas the amounts of obtained MBCs are 12.2 , 6.1 and 12.2 mg/ml respectively.

Conclusion: In conclusion, by increasing the nanoparticle concentration in wells and discs, the growth inhibition and diameter of inhibition zone has also been increased.

Keywords: *Antibacterial effect, Pseudomonas aeruginosa, Quantum dots, Staphylococci aureus, Salmonella typhi*

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INTRODUCTION

Colloidal semiconductor nanocrystals which generally consist of II-VI or III-V groups of elements table named quantum dots (QDs). Similar to other nanoparticles, QDs Optical and electrical properties assessment show that their characteristics are strongly size dependent [1-5]. They have many useful applications in different field of arias like industry and life science. On the other hand, most of chemical materials used for their production are toxic, expensive, and even explosive [6-7]. Zinc Sulfide (ZnS) is one of the most popular II-VI group semiconductors with the proper physical properties. It has two different structures as solid hexagonal or cubic crystal. The band gap of the cubic form is about 3.68 eV at 300 ^{O}K whereas it is about 3.91 eV for the hexagonal form. The molecular weight of ZnS is 97.474 g/mol which Zn is 67.104 and S is 32.896. Most of the ZnS nanoparticle characteristics are completely different from that of bulk forms. Scientists are concentrating on developing the controlled synthesis of ZnS nanoparticles [6-8]. Recently, it has been assessed extensively due to its high refractive index and wide band gap. ZnS nanoparticles have wide range of applications in industry and life science. Nanoparticles have a wide cell surface due to the large surface to volume ratio in comparison with bulk ones [9]. There are many methods of

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synthesizing ZnS nanoparticles such as photochemical route, wetted chemical method, low-temperature hydrothermal method and solid thermal process [8-10]. A large surface to volume ratio of nanoparticles in comparison with bulk ones, will increase the range of probable interaction with cell surface [1]. The antibacterial effect of nanoparticles such as ZnO, MgO, TiO_2, SiO_2 is considerable and therefore their selective toxicity towards biological systems suggests their potential application as therapeutics, diagnostics, surgical devices and nanomedicine based antimicrobial agents [2]. Silver is a white, shining and soft metal. It has the highest electrical conductivity between all metals. However the size of silver nanoparticles is 1-100 nm and in this range approximately most of its properties will be changed [8]. Silver mechanism against bacteria is not yet fully understood, the silver particles may stick to the surface of the cell membrane and the cell common tasks such as breathing and disrupted transport. It is due to the improved efficiency of nanoparticles, because by increasing the particle surface, the adhesion to the surface of cells increases, resulting in higher permeability and germicidal it [4]. In this study, an attempt was to assess the antimicrobial effects of ZnS:Ag QDs against on the three pathogen bacterias (*Pseudomonas aeroginosa, Staphylococcus aureus, Salmonella typhi*). The effect of different ZnS:Ag concentrations in the zone of inhibition diameter was also studied.

MATERIALS AND METHODS

Synthesis and assessment of properties

Chemical precipitation method was used in order to synthesis ZnS:Ag nanoparticles. At first, three solutions of Zinc Chloride ($ZnCl_2$), Mercaptoethanol (ME) and Sodium Sulfide ($Na_2S.H_2o$) and silver nitrate ($AgNo_3$) (all from Merck Company, Table 1) were prepared in the distilled water, under vigorous stirring. After that, Zinc Chloride solution was poured into a three spout balloon container. Then, silver nitrate solution and after that mercaptoethanol solution was added to the same balloon (one droplet every 3 seconds). Finally, Na_2S was added to the balloon by the same way. The ZnS:Ag solution was obtained by the explained process[5]. In order to extract any impurity aggregate the final solution was washed by deionized water several times and then was centrifuged. The precipitated sample was dried. All processes were done at room temperature [5].

Table 1. Details of the used materials for synthesizing ZnS:Ag nanoparticles

Material name	Purrity (%)	Used mass or volume
$AgNo_3$	99%	169.87 gr
$ZnCl_2$	99%	136.29 gr
$Na_2S.H_2O$	99%	96 gr
C_2H_5OSH	99%	400µl

XRD (X-Ray Diffraction, Bruker D8 ADVANCE λ=0.154nm Cu Kα radiation) and UV-Vis spectrophotometer (Ultra Violet – Visible, UV-2600 Shimadzu, Japan) were used to investigation of ZnS QDs optical and structural properties. Particle size distribution was assessed by TEM (Transmission Electron Microscope).

Antibacterial activity assay

The antibacterial effect of ZnS:Ag nanoparticles against *Pseudomonas aeroginosa*, *Staphylococcus aureus* and *Salmonella typhi* were studied by the culture method.They were grown aerobically in nutrient broth culture environment for 24 h at 37 °C before using as target organisms. Then 1cc of each bacterium was planted to a plate. The plates were previously prepared by Mueller Hinton Agar culture environment. After that, a well was created in the plates by the Pasteur pipette.

These bacteria-rich environment was inoculated with different concentrations of ZnS:Ag nanoparticles. Just one of the environment was not inoculated (control pipe). All pipes covered with sterile cotton. They incubated at 37 °C for 14 hours. For determination of MIC, 100 ml of each tube were taken with sampler. The solutions were inoculated and spreaded on Mueller Hinton Agar medium. The plates incubated in the same conditions. MIC is the tube which has the minimum growth in comparison with other tubes and MBC is the tube with minimum concentration which has just 1% of remained bacteria.

RESULTS AND DISCUSSION

Fig. 1 indicated the XRD pattern of the ZnS:Ag QDs. As can be seen, the sample had the cubic crystal structure and also was single phase. These findings were according to the standard JCPDS (Joint Committee on Powder Diffraction Standards) card No. 05-0566 and the diffraction peaks corresponded to the (111), (220) and (311) were the crystal plane. The mean size of the particles was calculated 2-3 nm by Debye-Scherer formula. The TEM photo of the sample

was shown in Fig. 2. The size was determined around 5-7 nm from TEM photograph. Generally, the electronic state was an important property of semiconductor. It was described in terms of conductivity and valence bands and also a band gap between them. However, the wavelength of the electrons was closer to the range of the particle sizes as the particles size becomes lower. It means the laws of classical physics have to be substituted by quantum confinement or quantum size effect (QSE). Moreover, many studies have reported the QSE in direct-gap semiconductors such as a shift of the optical absorption edge to higher energies with decreasing size [11].

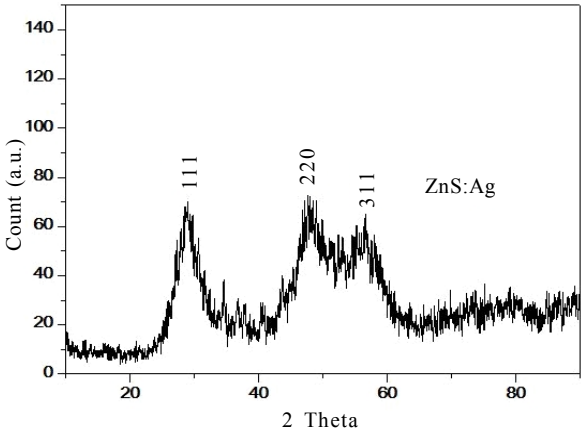


Fig. 1. XRD patterns of the ZnS:Ag QDs

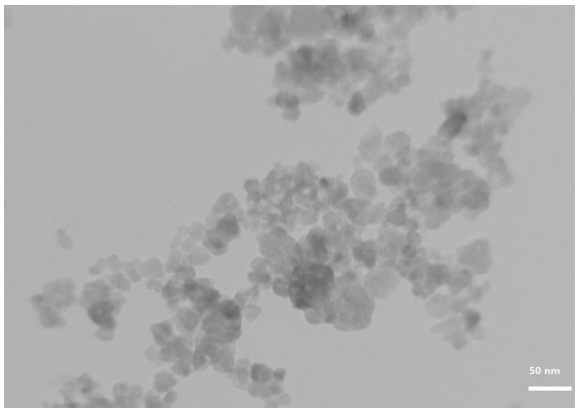


Fig. 2. Transmission Electron Microscope of ZnS:Ag QDs

The absorption spectra of ZnS:Ag QDs are presented in Fig. 3. The UV-Vis absorption spectrum at 37 °C showed that the absorption peak of the obtained ZnS:Ag QDs, is 272 nm (4.56eV) whereas it is 340 nm (3.68eV) for bulk cubic ZnS:Ag [5]. The size can also be estimated by Brus equation, as being (2- 3 nm) [12].

$$\Delta E_g = E_g^{QD} - E_g^{Bulk} = \frac{\pi^2 \hbar^2}{2MR^2}$$

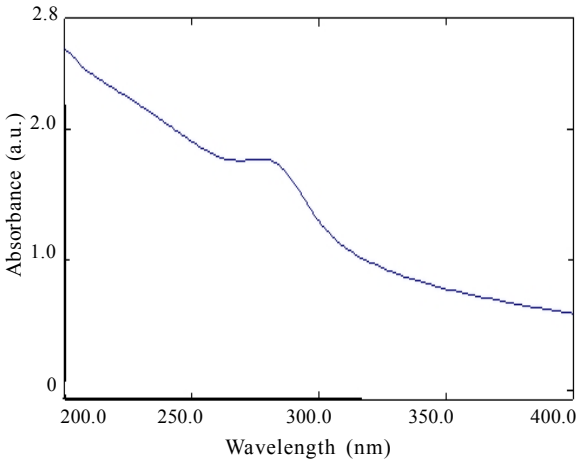
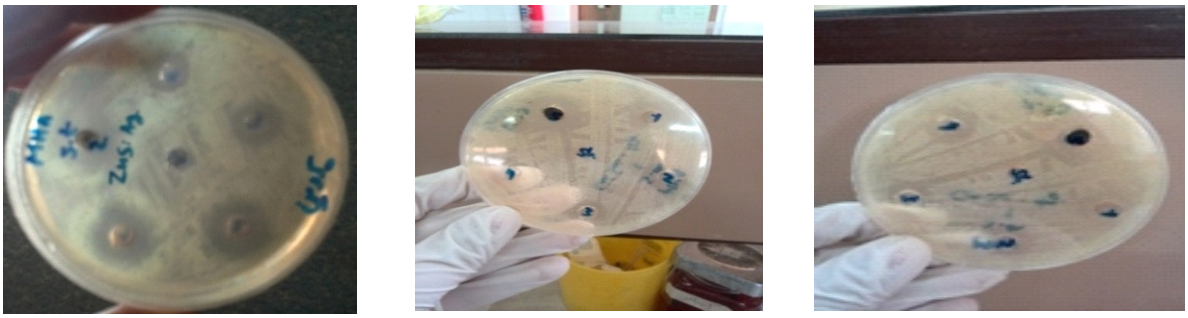


Fig. 3. UV-Vis absorption spectrum of ZnS:Ag QD



S. Typhi **P. aeroginosa** **S. aureus**

Fig. 4. Antibacterial activity of ZnS:Ag nanoparticles against S. aureus, P. aeroginosa and S. typhi

Table 2. Zone of inhibition

ZnS:Ag concentration (mg/ml)	S. aureus (mm)	P. aeroginosa (mm)	S. typhi (mm)
12.2	23	22	19
6.1	21	19	15
3.05	20	16	14
1.52	18	13	11

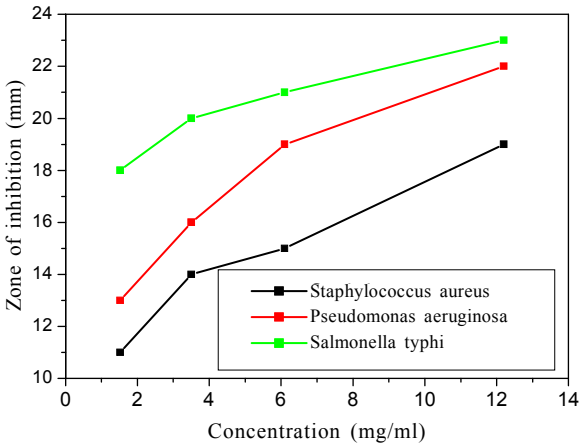


Fig. 5. Comparison of the zone of inhibition for different ncentration of ZnS:Ag quantum dots

Where E_g^{QD} and E_g^{Bulk} are the energy gap of nanoparticle and bulk respectively, h is the Plank constant, R is the nanoparticle redius and M is the redused mass. m_e is the electron mass and m_h is the hole mass.

Redused mass = $\left(\frac{1}{m_e}+\frac{1}{m_h}\right)$

Therefore, ZnS:Ag band gap changes depending on the nanoparticle size. Our findings are completely compatible with previous studies [5].

The antibacterial activity of ZnS:Ag QDs was estimated by the disc and well diffusion agar methods and the results are shown in Table 2, Figs. 4 and 5. The size of the inhibition zone indicated the antibacterial effect of ZnS:Ag QDs [1]. As can be seen, by increasing the ZnS concentration in wells and discs, the growth inhibition has also been increased. The size of inhibition zone was different according to the type of bacteria and the concentrations of ZnS:Ag QDs.Based on the results obtained from Fig. 4 (the diameter of inhibition zone for different baterias) it can be concluded that the maximum inhibition activity is happened for *Staphylococci aureus* in comparison with *P. aeroginosa* and *S. typhi.* Fig. 5 demonstrated the similar extended results for different concentarations of ZnS nanoparticles antibacterial activity and it can be seen that the same results obtained.The results of MICs obtained fromBroth Dilution for *Pseudomonas aeruginosa* , *Staphylococcus aureus* and *Salmonella typhi*, are 3.05 , 3.05 and 6.1 mg/ml whereas the amounts of obtained MBCs are 12.2 , 6.1 and 12.2 mg/ml respectively.

CONCLUSION

In this study, ZnS:Ag QDs were synthesized by chemical method. The antibacterial activity of ZnS:Ag nanoparticles was assessed by the disc and well diffusion agar methods and the results showed the antibacterial effect of ZnS:Ag nanoparticles. In fact, by increasing the nanoparticle concentration in wells and discs, the growth inhibition and diameter of inhibition zone has also been increased. The size of inhibition zone was different according to the type of bacteria and the concentrations of ZnS:Ag QDs. The maximum diameter was happened for *S. aureus*. We understood that these reasons are not enough to understanding how ZnS:Ag nano-particles affect on bacterial cell. It is proposed to work on the resistance mechanism of resistant strains which were encountered in contact with ZnS:Ag QDs. It could be performed by studying the plasmid profile and identification of the resistant gene.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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