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Structural Properties and Antibacterial Activity of Zinc Sulfide Nanoparticles

Ftema Waniss Aldbea, Zenab Abdoorhman[2], Ruqayah Abdulsalam Almahdi[1], Mabrok Kraini[3], Nada Abusalah Imrigha[1], Pramod Kumar Singh[4] and Carlos Vázquez Vázquez[5]

[1]*Physics Department, Faculty of Science, Sebha University, Libya*

[2]*Zoology Department, Faculty of Science, Sebha University, Libya*

[3]*Laboratory of Physics of Materials and Nanomaterials Applied at Environment, Gabes University, Tunisia*

[4]*COE on Solar Cell and Renewable Energy, Department of Physics, Sharda University, Greater Noida, India*

[5]*Laboratory of Magnetism and Nanotechnology, Institute of Materials (iMATUS) and Department of Physical Chemistry, Faculty of Chemistry, Universidade de Santiago de Compostela, Santiago de Compostela, Spain*

***Correspondence to:**
Ftema Waniss Aldbea
Physics Department,
Faculty of Science,
Sebha University, Libya.
E-mail: fte.aldbea@sebhau.edu.ly

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Abstract

Zinc sulfide (ZnS) nanoparticles powders were prepared using the sol-gel method at pH values of 5, 6, and 7. The samples were treated at 200 °C for 1 h. The X-ray diffraction (XRD) results showed that the samples have a cubic crystal structure. The crystalline size decreased to 2.22 ± 0.6 nm at pH 7 due to an increase in the micro-strain. The higher lattice parameter value of ZnS is observed at pH 7 and reached a value of 5.38 ± 0.006 Å, close to bulk ZnS's value of 5.4 Å. The synergistic effect of as-prepared ZnS with chloramphenicol (C), Gentamycin (CN), and nalidixic acid (NA) antibiotics showed significantly decreased gram-negative *Salmonella typh*i and gram-positive *Bacillus cereus* bacteria activity at pH 5 and 7. The calcinated ZnS has a good synergistic effect with NA and C antibiotics against *S. typhi* at pH 5 and 7. The *B. cereus* bacteria was sensitive to the ZnS with CN antibiotic only at pH 5 and pH 7, with zone of inhibition (ZOI) diameters of 21 and 24 mm, respectively. ZnS nanoparticles at pH values of 5 and 7 promoted the efficiency of C, NA, and CN antibiotics and they had a good impact against *S. typhi* and *B. cereus* bacteria.

Keywords

Sol-gel, X-ray diffraction, Antibacterial, pH, *Salmonella typhi*, *Bacillus cereus*

Introduction

Since they discovered, nanomaterials have attracted more attention because they have unique feathers such as small particles size and large surface area [1, 2]. Furthermore, nanomaterials have many types of crystal structures and shapes that give them specific physical, chemical, and biological properties such as chemical stability, sensors, optical devices, solar cell, gyrators, thermal energy, electrical conductivity, catalytic, drug delivery, antimicrobial properties, etc. [2-4]. Nanomaterials were synthesized by chemical and physical methods in order to enhance their properties and create new shapes of nanoparticles [5, 6]. There are many scientific works focused on the development of the biological properties of nanomaterials in order to find a suitable treatment for serious diseases caused by microbial diffusion [7]. Metal oxide nanoparticles such as CuO, FeO, ZnO, TiO_2, and MgO have played a good role as antibacterial activity [8-14]. On the other hand, ZnS materials have attractive more attention due to their excellent chemical and physical properties. It is considered as one of the important II-VI semiconductor groups, because of it has features such as a wide and direct bandgap between 3.6 and 3.8 eV, nontoxic, available in nature [15-19]. The crystal structure of ZnS can be in a cubic or hexagonal structure [20] and it has various applications such as solar cells, storage devices, electronic devices, sensors, optical devices, biomedical devices, light-emitting diodes, degradation and biological applications, etc. [21-24]. Many methods have been used to prepare ZnS powder, including co-precipitation [25], thermal decomposition [26], spray pyrolysis [27], ultrasonic [28], hydrothermal process [29], sonochemical method [30] and sol-gel method [31]. The sol-gel method is favorable because it produces particles in the nano-range, low-cost apparatuses [32]. Many works have been done to produce ZnS using the sol-gel method and they studied its properties at different parameters such as annealing temperature, aging time, doping concentration, and pH value of the solution [33-36]. The changes of pH values in the solution have noticeable effects on the structural properties and biological applications of ZnS materials. ZnS nanoparticles in the cubic structure exhibited good antibacterial activity at pH values in the range of 5.5 to 8.0 [37]. The antibacterial activity of ZnS nanoparticles at pH 9.5 showed a good effect on the growth of *Staphylococcus aureus* bacteria but it is still less effect compared to the ZnO nanoparticles [38]. Furthermore, sol-gel ZnS nanoparticles at pH 4 were examined on *Escherichia coli* and *S. aureus*, the reduction on the particle size of the ZnS have a notable effect on *E. coli* bacteria and the larger inhabitation zone was in the range between 12 and 22 mm [39]. Another method was created to produce a high quality of ZnS nanoparticles by modified a used Zn=C battery with pH 7. The samples restrain the growth of bacteria when the ZnS concentration values were about 0.312 and 0.156 mg/ml for *S. typhi* and 1.25 and 0.625 mg/ml for *B. subtilis*, respectively [40]. To date, several studies have been carried out to prepare ZnS nanoparticles at different chemical and physical scales. Changes in the pH value of the solution have a significant impact on the properties of ZnS nanoparticles. In addition, this parameter (pH) has an effective effect on the crystal structures of ZnS nanoparticles, which makes them play an important role as an antibacterial agent since bacterial contamination is dangerous to the environment and humans. Even though, there are vast reports on the structural properties and biological applications of ZnS, few works were published on the antibacterial investigation of ZnS nanoparticles performed on the gram-negative *S. typhi* and gram-positive *B. cereus* under variation of pH values less than 8. Therefore, this work reports the preparation of ZnS powders using the sol-gel method and the study of their structural properties at pH values of 5, 6, and 7, in order to find a good sample to be suitable for antibacterial activity application.

Materials and Method

Preparation of ZnS powders

Zinc acetate dehydrate ($ZnC_4H_6O_4$, 99% purity; Sigma-Aldrich) and thiourea ($CS(NH_2)_2$, 99% purity; Sigma-Aldrich) powders were dissolved separately in methanol (99% purity) and distilled water. Then, the zinc acetate/thiourea solution was mixed using a magnetic stirrer at 50 °C. The zinc acetate/thiourea solution was maintained at pH of 5, 6, and 7 by adding the amount of ammonia solution. A clear and transparent solution was obtained. Then, the solution stirred for 2 h at 50 °C to obtain a homogeneous gel. The obtained gel was stirred for 4 h at 50 °C until it turned into a fine powder. The obtained powder was dried at room temperature for 24 h. Finally, it was calculated at 200 °C for 1 h. The steps of preparing ZnS powders are shown in figure 1.

Characterization

The crystal structure of the samples was measured by an X-ray diffractometer (XRD Bruker D8 Advance diffractometer using monochromatic Cu-Kα radiation (λ = 1.5406 Å)) in the 2θ range of 20° - 80°. The gram-negative *S. typhi* and gram-positive *B. cereus* were provided from the Zoology laboratories at Sebha University, Libya. The statistical analysis was performed by calculating the mean and standard deviation (mean ± standard deviation).

Results and Discussion

XRD measurements

Figure 2 shows the XRD patterns of as prepared ZnS powders with pH values of 5, 6, and 7. The obtained peaks were indexed according to the standard JCPDS card no. 5-566 for the cubic structure. At pH 5, the sample showed an amorphous structure. Three peaks related to (111), (220), and (311) for the ZnS structure with some peaks of impurity appeared at pH 6 and 7. The low intensities are due to the samples not being well calcinated.

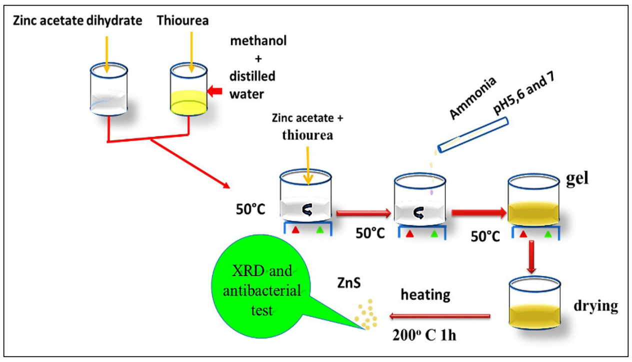

Figure 1: Preparation steps of ZnS nanoparticles.

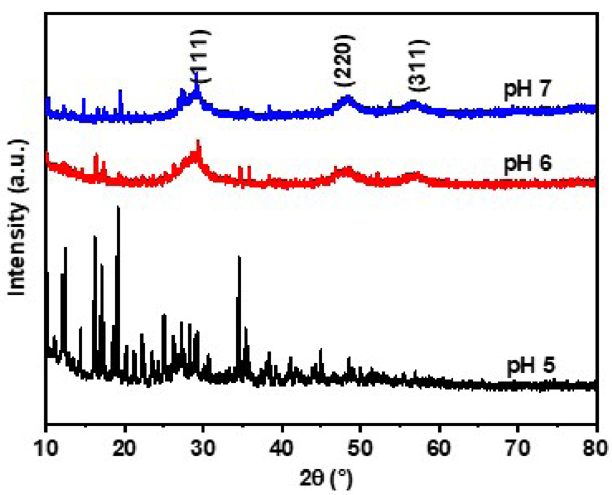

Figure 2: XRD patterns of as prepared ZnS at pH 5, 6, and 7.

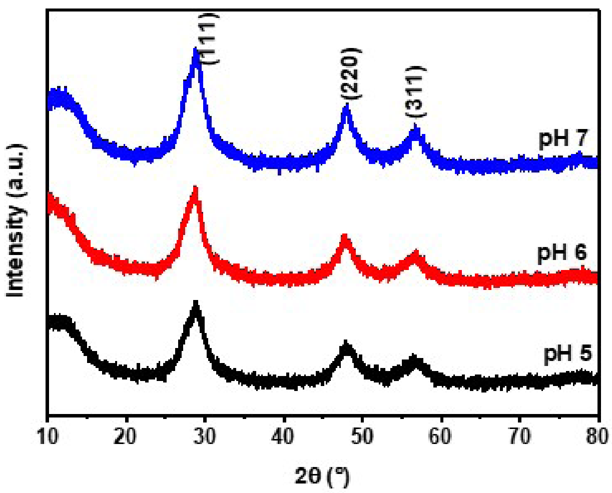

Figure 3: XRD patterns of calcinated ZnS nanoparticles with different pH values.

Figure 3 shows XRD patterns of ZnS powders calcinated at 200 °C for 1 h with pH values of 5, 6, and 7. From figure 3, the samples at different pH values showed the three peaks related to the (111), (220), and (311) plans of a cubic ZnS structure. With increasing pH values from 5 to 7, the diffraction peaks become broad with a slight shift towards the higher 2θ. The intensities of the peaks are also increased with an increase in pH values. This indicated that an increase in pH up to 7 can affect the ZnS structure. The wide peaks are a sign for ZnS nanoparticles. The crystalline size (D) of samples was calculated using Sherrer`s equation (Equation 1) [41, 42].

$$D = \frac{0.9\lambda}{\beta \cos\theta} \quad (1)$$

Where D is the crystallite size, θ is the angle of diffraction, λ is the wavelength of X-ray (1.54056 Å for CuKα) and β is the full width at half maximum of each diffraction peak.

The variation of crystalline size with increasing pH values is listed in table 1. The crystalline size is increased initially from 2.22 ± 0.6 to 2.23 ± 0.6 nm as the pH value is increased from 5 to 6, respectively which less than that reported by Barman for ZnS nanocomposite [43]. With further increase in pH value to 7, the crystalline size is decreased to 2.22 ± 0.6 due to higher surface to volume ratio and the increase in strain (Table 1) [44]. The decreasing crystalline size with increasing pH values confirmed the peak broadening as seen in figure 3.

The lattice parameter (*a*) of ZnS samples at different pH values was calculated using equation 2 for the cubic structure [45].

$$a = \sqrt{\frac{\lambda^2}{4\sin^2\theta}\left(h^2 + k^2 + l^2\right)} \quad (2)$$

Where λ is the wavelength of the CuKa radiation (λ = 1.5406 Å), θ is the diffraction angle and (*h*, *k*, and *l*) are Miller indices.

The lattice parameter values at different pH values are summarized in table 1 and observed to be about 5.37 Å, which is close to the actual lattice parameter value for ZnS (5.4 Å)

Table 1: Changing the crystalline size, lattice parameter, lattice volume, dislocation, and micro-strain of ZnS at pH 5, 6, and 7.

Parameters	pH 5	pH 6	pH 7
D (nm)	2.22 ± 0.6	2.23 ± 0.6	2.22 ± 0.6
a (Å)	5.37 ± 0.009	5.37 ± 0.009	5.38 ± 0.006
V ($Å^3$)	155.13	155.22	155.60
δ (nm^{-2})	0.203	0.201	0.203
ε	0.1560	0.1553	0.1559

[21]. With the increasing pH value, the lattice parameter is increased, which leads to an increase in the lattice volume (V = a^3). The dislocation (δ = $1/D^2$) is observed to increase with increasing pH value, the higher value is recorded to be 0.203 nm^{-2} at pH 7. The micro-strain (ε) values of ZnS samples at pH 5, 6, and 7 are calculated using equation 3 [46].

$$\varepsilon = \frac{\beta \cos\theta}{4} \quad (3)$$

Where β is the full width at half maximum.

The micro-strain values at pH 5, 6, and 7 are listed in table 1. It was observed that the ε value dropped first from 0.1560 to 0.1553 as pH increased from 5 to 6. Further increasing of pH value to 7, ε value is increased to 0.1559, where micro-strain has inverse relation between micro-strain and crystalline size as observed in table 1. This supports the decrease in crystalline size that occurred at pH 7.

Antibacterial activity of ZnS powders

Two types of bacteria were grown on a solid medium (nutrient agar) and placed in the incubator for 18 - 24 h at 37 °C. After that, a swab was taken, and cultured in nutrient broth liquid medium, and grown for another 18 - 24 h at 37 °C. Chloramphenicol (C, 30 mg), (Gentamycin (CN, 30 mg) and Nalidixic acid (NA, 30 mg) are three types of antibiotics that are used to perform the synergistic effect with ZnS powders. The ZnS solution (control) and the synergistic test between the antibiotic and the ZnS solution were tested by the saturated tablets method [47], where filter paper and antibaiotics were saturated separately with 100 μl of ZnS solution (concentration 1 mM) distributed to the bacteria in a petri dish and placed in the incubator at 37 °C for 24 h. The sensitivity of bacteria to ZnS and ZnS with antibiotic solutions was examined by measuring the ZOI diameter.

Figure 4 shows the antibacterial activity of as prepared ZnS at pH 5, 6, and 7 with a 1 mm concentration of antibiotics (CN, NA, and C) and the control (ZnS without antibiotic) against *S. typhi* and *B. cereus* bacteria. The ZOI was measured for each sample and found to be in mm scales. The ZOI diameter values were listed in table 2. It can be seen that, the ZOI diameter of the control against *S. typhi* increases with increasing pH. The synergistic effect of as prepared ZnS with three types of antibiotics against *S. typhi* bacteria exhibited that *S. typhi* was more sensitive at pH 5 and 7, where the ZOI diameters are higher than those at pH 6 (*S. typhi* has low sensitivity). However, as prepared ZnS at pH 7 has an excellent effect on *S. typhi* bacteria when mixed with CN, NA, and C antibiotics. In the case of gram-positive *B. cereus* bacteria, the bacteria were

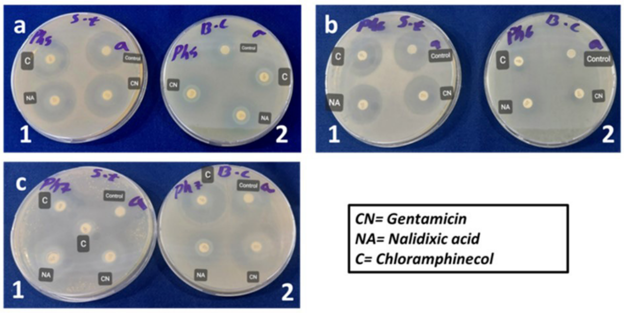

Figure 4: Synergistic effects of as prepared ZnS (1 mm) with antibiotic on *S. typhi* and *B. cereus* bacteria at pH 5, 6, and 7.

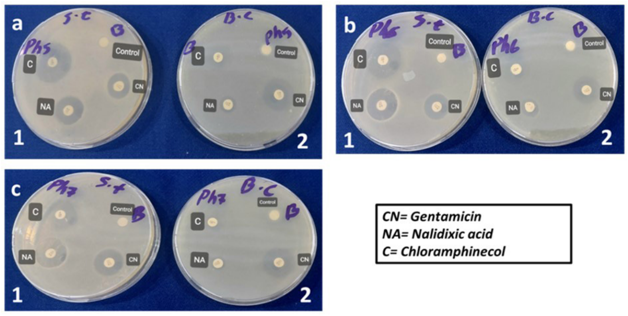

Figure 5: Synergistic effects of ZnS (1 mm) with antibiotic on *S. typhi* and *B. cereus* bacteria at pH 5, 6, and 7.

sensitive to the control at pH 5, and the ZOI diameter was 30 mm. It is noticed that there is bacterial growth within the inhibition zone, indicating the *B. cereus bacteria* has genes that enable it to resist the influence of ZnS. This case is known as the intermediate effect. As pH value increases to 6, the ZOI diameter decreases to 22 mm, and then it increases to 28 mm as the pH reaches a value of 7, meaning the sensitivity of *B. cereus* to ZnS is increased. The synergistic effect of as prepared ZnS with antibiotics against *B. cereus* showed a high effect at pH values of 5 and 7. This effect is higher than the results obtained when ZnS applied against *S. aureus* and *E. coli* [39].

Figure 5 illustrates an antibacterial test of ZnS powders (control) with pH values of 5, 6, and 7, which were calcinated at 200 °C for 1 h. The ZOI diameters in both types of bacteria were measured and listed in table 3. The ZnS (control) shows a weak effect on the *S. typhi* bacteria at pH 5 with a ZOI diameter of 7 mm. No effect for ZnS is observed at pH 6 and 7. A different effect is detected for ZnS (control) on *B. cereus* bacteria, where it is sensitive to the control at pH 6 with a ZOI diameter value of 8 mm. There was no effect of ZnS on *B. cereus* at pH 5 and 7. This means that calcinated ZnS has no effect on *S. typhi* and *B. cereus* at pH 7. In general, the as prepared ZnS sample has a good effect on *S. typhi* and *B. cereus* at pH 7, compared with calcinated ZnS. On the other hand, the synergistic effect of ZnS 1 mM concentration with antibiotics on *S. typhi* and *B. cereus* is shown in figure 3 and table 3. From the figure and table can be observed that ZnS with NA and C antibiotics showed a good effect on the *S. typhi* at pH 5 (ZOI diameter values of 14 and 19 mm) and pH 7 (ZOI diameters of 21 and 26 mm). At pH 6, *S. typhi* was sensitive to ZnS and C with an intermediate effect. *B. cereus* was sensitive to the ZnS with CN antibiotic at pH 5 and pH 7, with ZOI diameter values of 21 and 24 mm, respectively. It is obvious that the synergistic effect of ZnS with antibiotics on *S. typhi* bacteria is stronger than on *B. cereus*. This could be due to the difference between nanoparticles and cell surface which lead to an influence the penetrability of membrane, because exist of nanoparticles with higher surface are inside the cell produce oxidative stress thus leading to inhabit cell growth and cell death that bring a better antibacterial activity [48-50]. Mustafa et al. [48] have found that ZnS at pH 7 showed a strong effect on the gram-positive bacteria. Here, it can be suggested that reducing the antibiotics ratio to the ZnS concentration to obtain new synergistic effects on *S. typhi* and *B. cereus* bacteria. The role and mechanism of ZnS as an antibacterial are still in arguments.

Conclusion

ZnS nanoparticles with various pH values have been successfully prepared by the sol-gel method. The results indicated that the pH values of 5, 6, and 7 of the precursors have an important influence on the structural properties and antibacterial activity of ZnS nanoparticles. As prepared samples showed an amorphous structure at pH 5, and wide peaks with

Table 2: ZOI diameter values at synergistic effects of as prepared ZnS (1 mM) with antibiotic on *S. typhi* and *B. cereus* bacteria.

Antibiotic + as prepared ZnS	*S. typhi*			*B. cereus*		
	pH 5	pH 6	pH 7	pH 5	pH 6	pH 7
Control	29 ± 0.57/I	25 ± 0.23/I	37 ± 0.78	30 ± 0.57/I	22 ± 0.66/I	28 ± 0.57
CN	31 ± 0.47	26 ± 0.57/I	40 ± 0.57	33 ± 0.33/I	24 ± 0.33/I	37 ± 0.78
NA	32 ± 0.57	27 ± 0.57/I	43 ± 0.52	31 ± 0.47/I	22 ± 0.66	26 ± 0.40
C	27 ± 0.57	27 ± 0.40/I	30 ± 0.57	23 ± 0.33/I	14 ± 0.23/I	33 ± 0.33

Table 3: ZOI diameter values at synergistic effects of ZnS (1 mm) with antibiotic on *S. typhi* and *B. cereus* bacteria.

Antibiotic + ZnS	*S. typhi*			*B. cereus*		
	pH 5	pH 6	pH 7	pH 5	pH 6	pH 7
Control	7 ± 0.52	R	R	R	8 ± 0.57/I	R
CN	14 ± 0.23	20 ± 0.57	23 ± 0.47	21 ± 0.33	19 ± 0.33	24 ± 0.33
NA	19 ± 0.33	18 ± 0.74	21 ± 0.33	12 ± 0.40	12 ± 0.40	9 ± 0.66
C	28 ± 0.52	28 ± 0.47/I	26 ± 0.75	15 ± 0.33	17 ± 0.78	26 ± 0.75/I

low intensities were observed at pH 6 and 7. The calcinated ZnS samples showed a cubic structure. The lattice parameter (a) increased with an increase in pH value from 5 to 7. As prepared ZnS with antibiotic showed a strong effect on two types of bacteria at pH 7. ZnS with CN antibiotic showed a high effect on B. cereus at pH 5 and 7, while S. typhi showed sensitivity to ZnS with C and NA at pH 5 and 7. However, calcinated ZnS sample at pH 7 has a good antibacterial effect on S. typhi and B. cereus than that prepared at pH 5 and 6. The obtained results at pH values 5, 6, and 7 confirmed that, as prepared ZnS samples have an antibacterial activity against S. typhi and B. cereus greater than calcinated samples of ZnS. Further analysis will be done to measure the optical and morphological properties. As well as studying the antibacterial activity of ZnS on other types of bacteria.

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Conflict of Interest

Authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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