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Distinct anti-microbial effects of synthesized ZnS, Ag-N co-doped ZnS and ZnS-Fe_2O_3 composite nanoparticles against some pathogenic bacterial strains

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ABSTRACT

*ZnS, Ag-N co-doped and ZnS/Fe_2O_3 composite nanoparticles were synthesized by chemical and sol-gel methods. As-synthesized nanomaterial have been characterized by XRD and TEM techniques and their antimicrobial effects were studied by paper disc diffusion technique against gram positive (*Staphylococcus aureus and *Streptococcus) as well as gram negative (*Pseudomonas aeruginosa *and* Escherichia coli*) bacteria. Doping of Ag and N into ZnS showed enhanced antimicrobial activity of ZnS. Synthesized antibiotic material exhibited higher activity against Gram positive bacteria than Gram negative bacteria. Antimicrobial activity of Ag-N co-doped ZnS was also compared with the activity of two commercially available antibiotics- Gentamycin and Azithromycin. Compositing of Fe_2O_3 with ZnS showed antagonistic effect towards the antimicrobial activity of the later.*

Keywords: Antimicrobial, *Escherichia coli*, nanoparticles, TEM, *Streptococcus*, XRD

INTRODUCTION

In the recent years, due to the development of new strains, resistance of some bacteria to commonly used bactericide antibiotics has increased. Some of the antimicrobial agents, being used today, are irritant and even toxic; therefore, there is much interest in finding ways to formulate new types of safe and cost-effective biocidal materials, either natural or synthetic. Nanosize inorganic material such as metals and semiconductors during their antibiotic action can cause denaturation of proteins present in bacterial cell walls [1]. According to some previous reports antimicrobial formulations in the form of semiconductor nanoparticles could be used as effective bactericidal materials [2-4]. It has, earlier, been reported that nanoparticles of Zinc oxide, calcium oxide and magnesium oxide possess antibacterial property when tested against some Gram positive and Gram negative bacteria [5, 6].Also, the simple inorganic substances possessing antimicrobial activity are beneficial as they contain minerals which are essential for human consumption and may exhibit potent antibacterial action even when administered in small amounts as nanocrystals. In the present work, nano-size ZnS, Ag-N co-doped ZnS, Fe_2O_3 and ZnS/Fe_2O_3 composite have been prepared by chemical and sol-gel methods and characterized using XRD and TEM techniques. As-synthesized nanomaterial has been tested for their antimicrobial activity against gram positive (*Staphylococcus aureus* and *Streptococcus*) and gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria and also compared with the activity of some commonly used antibiotics.

MATERIALS AND METHODS

2.1. Chemicals

Zinc nitrate ($Zn(NO_3)_2.6H_2O$, MW=297.47g/mol MERCK); Sodium sulfide ($Na_2S.9H_2O$, MW=250.04g/mol, MERCK); Glucose ($C_6H_{12}O_6$, MW=180.16 g/mol SRL), Silver nitrate ($AgNO_3$, MW=169.87g/mol, MERCK); Urea (CH_4N_2O, MW=60.06 g/mol, MERCK); Ferric chloride ($FeCl_3$, MW=162.21 g/mol, MERCK), Citric Acid

Monohydrate ($C_6H_8O_7.H_2O$, MW=210.14 g/mol, SRL); Oxalic acid ($C_2O_4.2H_2O$, MW= 126, SRL), Nutrient Agar and Muller-Hinton Agar; Nutrient Broth and Muller-Hinton Broth.

2.2. Methods

2.2.1 Synthesis of ZnS Nanoparticles

1.0 M sodium sulfide was added, drop-wise, at 70^0C to equal volume of 1.0 M Zinc nitrate aqueous solution with simultaneous magnetic stirring for 15 hours. To the precipitates thus obtained, 1.0 M glucose solution was added drop-wise. The resultant mixture was incubated at 70^0C for 6 hours and centrifuged at 2000 rpm for 15 minutes and the product was dried at 50 ^{0}C for 4 hours and then crushed to a fine powder [7].

2.2.2 Synthesis of N-doped ZnS

10 gm. of as-synthesized ZnS photo-catalyst and 30 g of urea was mixed well in an agate mortar. The mixture was transferred into a ceramic crucible and calcined at 400ºC for four hrs and then cooled.

2.2.3 Synthesis of Ag-doped ZnS

10 g as-synthesized ZnS and 10 ml 0.2 M $AgNO_3$ aqueous solution was mixed in a ceramic crucible. The mixture was well agitated with glass rod and the solvent (water) was evaporated in an oven at 110ºC. The dried powder was calcined in a furnace at 400 ºC for four hrs and then cooled to room temperature.

2.2.4 Synthesis of Ag and N co-doped ZnS

In a ceramic crucible, 10 g of as- synthesized N-doped ZnS was mixed with 10 ml of 0.2 M $AgNO_3$ aqueous solution. The liquid was evaporated at 110º C and the dried powder was calcined in a furnace at 400 ^{0}C for 4 hours and then cooled to room temperature.

2.2.5 Synthesis of Fe_2O_3 Nanoparticles

Using ferric chloride as precursor, nanosize Fe_2O_3 was prepared by modified sol-gel method [8]. Ferric chloride was dissolved in minimum quantity of distilled water. The solution was acidified with nitric acid followed by stirring at 65-70 ºC. A mixture of Citric acid and oxalic acid, in 1:1 ratio, was added to the reaction mixture with simultaneous stirring for one hour. The gel thus obtained was dried at 110 ^{0}C and then calcined at 500 ^{0}C for 4 hrs, cooled to room temperature and grinded to fine powder.

2.2.6 Synthesis of ZnS/Fe_2O_3 Nanocomposite

In a crucible, 10 gm each of as-synthesized ZnS and Fe_2O_3 was thoroughly mixed and then calcined at 400^0C for 2hrs and then cooled to room temperature.

2.3 Characterization of Synthesized Nanomaterial

2.3.1 X-ray diffraction (XRD) analysis:

For determining the crystallite structure and size of as-synthesized photocatalysts powders, their X-ray diffraction (XRD) pattern were obtained using a diffractometer equipped with a Cu target K_α radiation (λ=1.5405 A^0) source. The measurement was made at room temperature using accelerating voltage and the applied current as 40 kV and 30 mA, respectively, over 2θ range 40 –64º.

2.3.2 Transmission Electron Microscopy (TEM) Analysis:

Transmission electron microscopic (TEM) images of synthesized material were obtained at SAIF Punjab University, Chandigarh, using accelerating voltage 80 KV and magnification $2x10^5$. The photo-catalyst powder was dispersed in acetone by stirring in a tank for 15 minutes. A drop of the suspension thus obtained was mounted on a carbon-coated copper grid; the solvent was allowed to evaporate before TEM images were obtained.

2.4 Antimicrobial activity of Nanomaterial

Antimicrobial activity of synthesized nanomaterial was tested against both Gram positive (*Staphylococcus aureus and Streptococcus)* as well as Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria using paper disc diffusion technique [9]. The test bacterial strains were streaked on nutrient agar in sterile Petri dish. Using a micropipette, 10, 20 and 30 µl 5mg/ml of the nanomaterial, dispersed in water, were impregnated, separately, on paper discs, each of 5 mm diameters. The antimicrobial activity of the synthesized nanomaterial was evaluated in terms of magnitude of the measured inhibition zone after incubating at 37 ^{0}C for 24 hrs.

RESULTS AND DISCUSSION

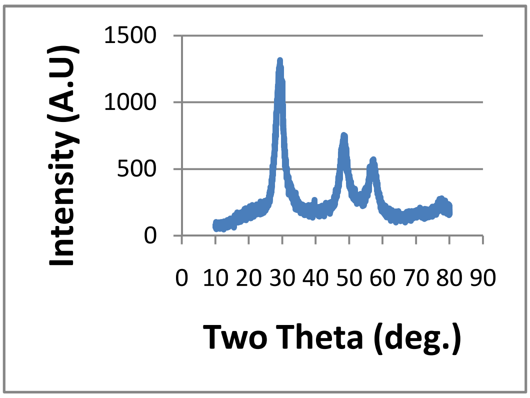
3.1. XRD Analysis

XRD patterns of as synthesized ZnS, Ag doped ZnS, N-doped ZnS , Ag-N co-doped ZnS Fe_2O_3 and ZnS-Fe_2O_3 composite are exhibited in Figures 1.Diffraction peaks at 2θ= 29.2, 48.3 and 57.3^o correspond to (101), (110) and (111) planes of hexagonal wurtzite phase of pure ZnS [10,11] . In the XRD pattern of Ag-N-co-doped ZnS two additional peaks appeared at 33^o, 38^o, 63^0 and 67^0 indicates the creation of new crystal phases on doping Ag and N in ZnS. However, it is difficult to assign the exact position (interstitial or substitutional) of Ag and N in the doped ZnS. In case of XRD pattern of synthesized Fe_2O_3, diffraction peaks at 2 θ = 24.1, 33.1, 35.6, 40.8, 51.0 54.0, 57.5, 63.9 and 65.0^0, correspond to rhombohedral *α*-Fe2O3 phase [8]. In the XRD pattern all the diffraction peaks of ZnS and Fe2O3 are observed indicating the successful formation ZnS-Fe_2O_3 composite.

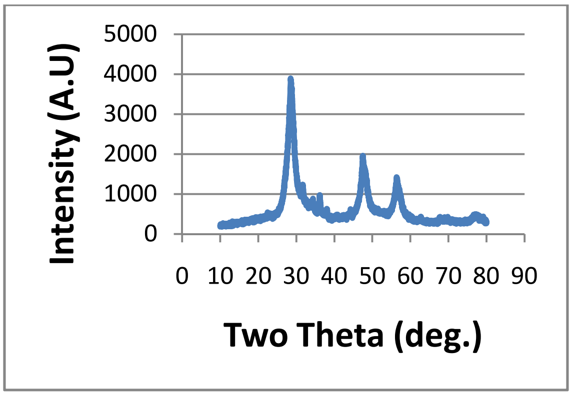
The average crystallite size of synthesized material were obtained using Debeye–Scherrer formula-

$$D = (K \lambda) / (\beta \cos \theta) \qquad (3.1)$$

Where D is the average particle size, λ is wavelength (0.15406 nm) of X-ray; β is full width at half-maxima (FWHM) of diffraction peak of highest intensity and θ is the incident angle of X-ray; K is the geometric factor equal to 0.94. The average crystallite size of as-synthesized ZnS, Ag doped ZnS, N doped ZnS , Ag-N co-doped ZnS, Fe_2O_3 and ZnS-Fe_2O_3 composite thus obtained were 2.5 nm, 3.6 nm, 2.7 nm , 2.7 nm, 13.2nm and 45.20nm, respectively.



(a)



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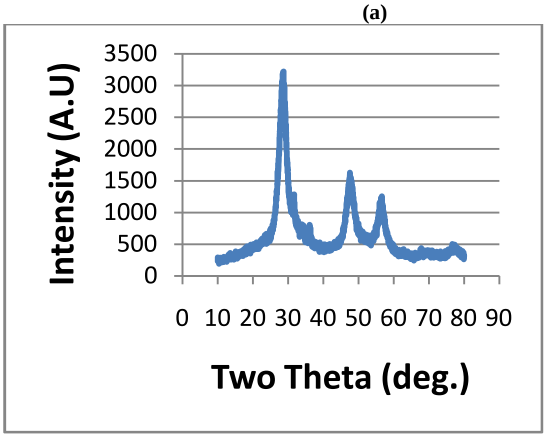

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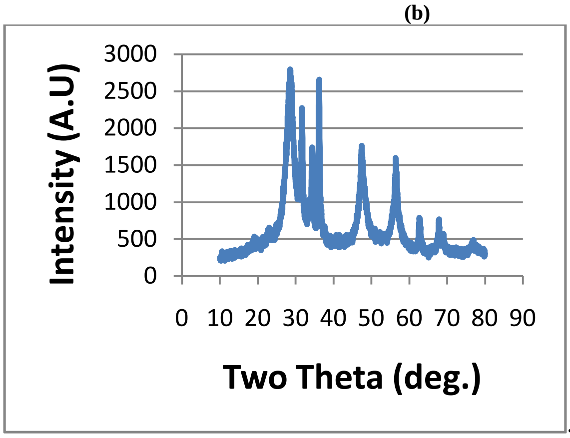

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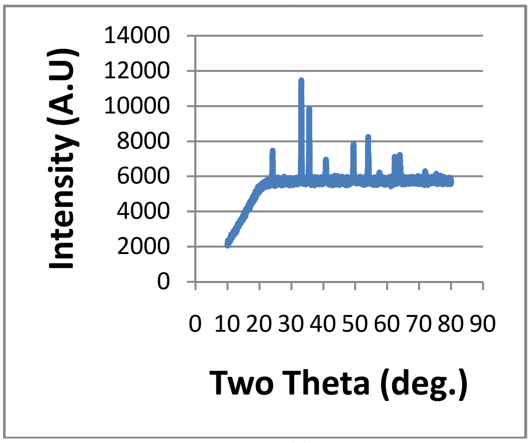
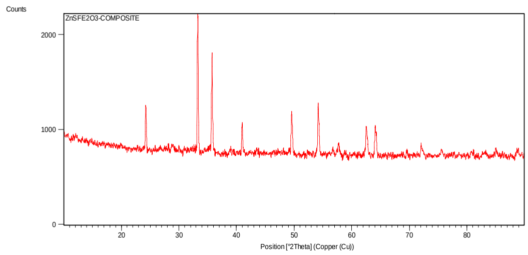


(e)

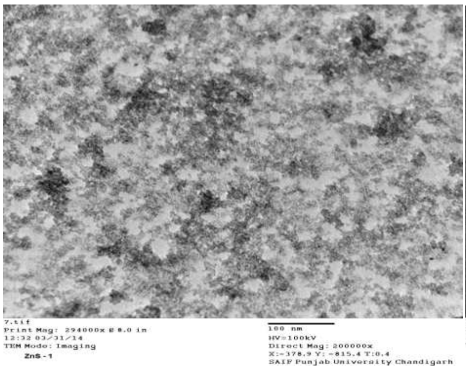


(f)

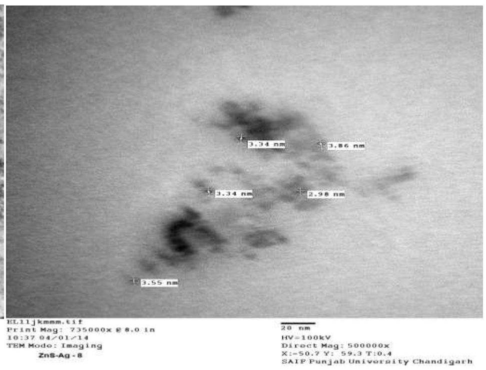
Figure- 1. XRD pattern of as-synthesized material (a) ZnS, (b) Ag-doped ZnS, (c) N-doped Zns, (d) Ag-N-co-doped ZnS, (e) Fe_2O_3 and (f) ZnS- Fe_2O_3 composite

3.2. TEM Analysis

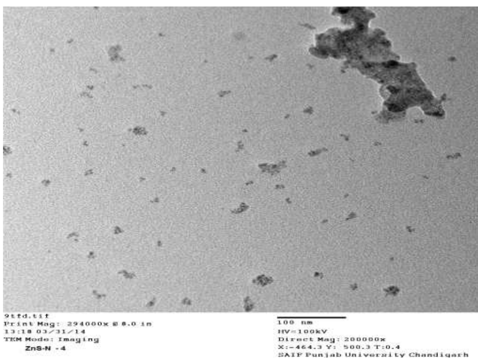
TEM images of as-synthesized ZnS, Ag-doped ZnS, N-doped ZnS, Ag-N-co-doped ZnS, Fe_2O_3 and of ZnS-Fe_3O_3 composite are presented in figures 2. Their average particle sizes were found to be 2.4 nm, 3.3 nm, 2.5 nm, 2.5 nm, 13.0nm and 45.0 nm respectively. These values are in fair agreement with those obtained using XRD technique.



(a)



(b)



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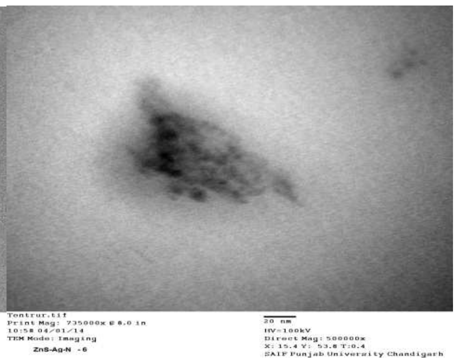
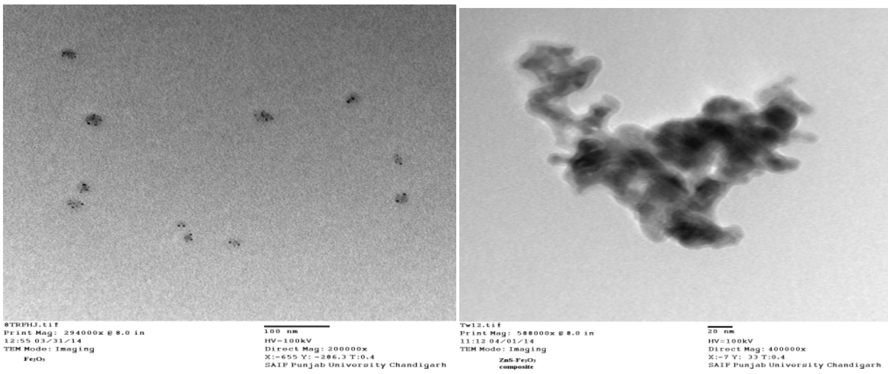


(d)



(e) (f)

Figure-2. TEM images of as-synthesized nanoaterial: (a) ZnS, (b) Ag doped ZnS, (c) N doped ZnS, (d) Ag-N co-doped ZnS, (e) Fe_2O_3 and (f) ZnS-Fe_2O_3 composite.

3.3 Antimicrobial activity

Zones of inhibition produced by some of the synthesized antibiotic material are shown in figures 3 to 6.

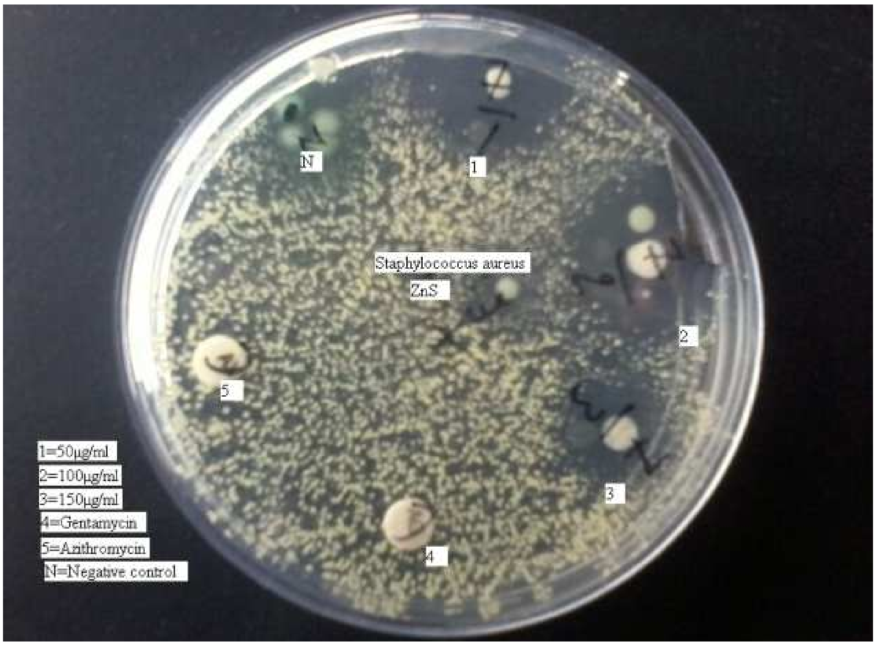


Figure-3. Zone of inhibition produced by ZnS nanoparticles against *Staphylococcus aureus*

Zone of inhibition (in mm) of as synthesized nanomaterials acting as antibiotics against different bacteria are presented in table-1. It is observed that synthesized nanomaterial act as stronger antibiotics against Gram positive (*Staphylococcus aureus* and *Streptococcus)* bacteria than against Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria. Doping of Ag and N show cumulative effect in improving the antimicrobial activity of ZnS. Order of antimicrobial activity of Ag-N co-doped ZnS against studied bacteria is:

(Streptococcus) > (Staphylococcus aureus)> (E.coli) > (Pseudomonas aeruginosa).

Among the studied salts as antibiotics Fe_2O_3 shows lowest antimicrobial activity against the studied bacteria. Further, compositing Fe_2O_3 with ZnS shows antagonistic effect in lowering the activity of the later. Minimum effective dose of each synthesized nanomaterial was found to be 150µg/ml.

Synthesized undoped ZnS as well as Ag-N co-doped ZnS exhibit much higher antimicrobial activity than the commercially obtained Gentamycin and Azithromycin antibiotics.

Figure-4. Zone of inhibition produced by ZnS nanoparticles against *Streptococcus*

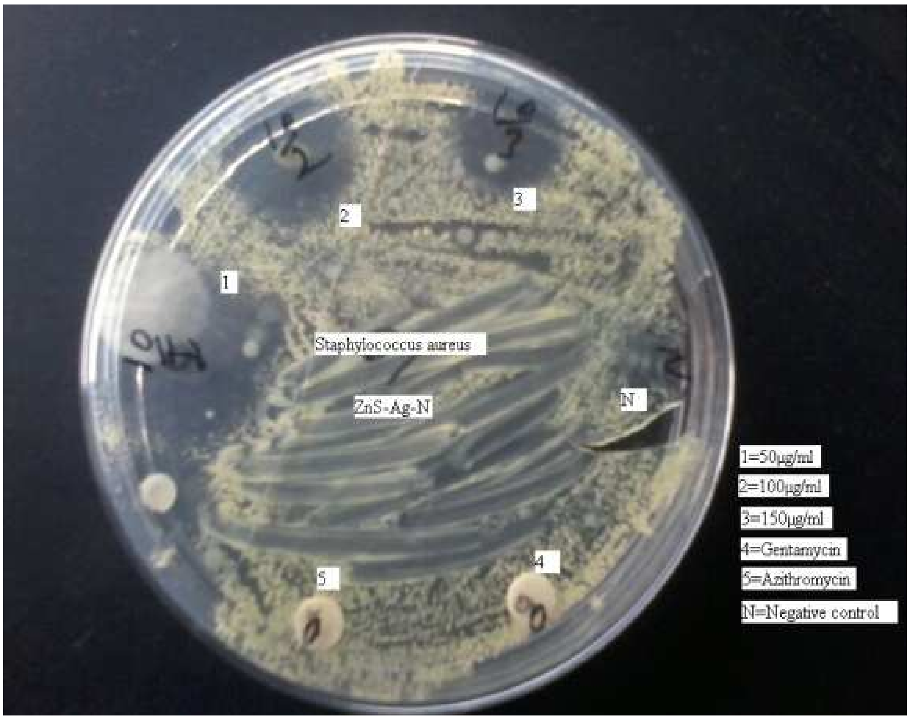


Figure-5: Zone of inhibition produced by Ag-N co-doped ZnS nanoparticles against *Staphylococcus aureus*

Table-1. Zone of inhibition (in mm) of different as-synthesized material acting as antibiotic against Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and Gram positive (*Staphylococcus aureus and Streptococcus)* bacteria

Antibiotic	*Pseudomonas aeruginosa*			*E.coli*			*Staphylococcus aureus*			*Streptococcus*		
	50 µg/ml	100 µg/ml	150 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml	50 µg/ml	100 µg/ml	150 µg/ml
ZnS	0 mm	0 mm	10 mm	8 mm	15 mm	19 mm	10 mm	17 mm	25 mm	10 mm	18 mm	24 mm
Ag-ZnS	0 mm	0 mm	11 mm	8 mm	16 mm	20 mm	11 mm	19 mm	26 mm	11 mm	19 mm	25 mm
N-ZnS	0 mm	0 mm	11 mm	9 mm	16 mm	20 mm	11 mm	20 mm	26 mm	12 mm	20 mm	25 mm
Ag-N ZnS	0 mm	5 mm	13mm	10 mm	17 mm	22 mm	11 mm	21mm	28mm	12 mm	23 mm	29 mm
Fe_2O_3	0 mm	0 mm	10 mm	5 mm	8 mm	10 mm	0 mm	8 mm	12 mm	0 mm	8 mm	11 mm
$ZnS-Fe_2O_3$	0 mm	0 mm	8 mm	5 mm	10 mm	15 mm	8 mm	15 mm	18 mm	9 mm	16 mm	19 mm

Figure 6: Zone of inhibition produced by Ag-N co-doped ZnS nanoparticles against *Streptococcus*

CONCLUSION

Nano-size ZnS, Ag-doped ZnS, N-doped ZnS, Ag-N co-doped ZnS, Fe_2O_3 and Fe_2O_3/ZnS composite have been synthesized by simple chemical methods and characterized using XRD and TEM techniques. Antimicrobial effects of synthesized nanomaterial were studied against gram positive (*Staphylococcus aureus and Streptococcus)* as well as gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria by paper disc diffusion technique. Doping of Ag and N into ZnS showed cumulative effect on enhancing the antimicrobial activity of ZnS. Undoped as well as Ag and N co-doped ZnS exhibited higher activity against Gram positive bacteria than Gram negative bacteria. Antimicrobial activity of Ag-N co-doped ZnS was also compared with the activity of two commercially available antibiotics. Gentamycin and Azithromycin. Compositing of Fe_2O_3 with ZnS showed antagonistic effect towards the antimicrobial activity of the later.

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