

**Research Article**

# Evaluation of antimicrobial effects of synthesized Zinc sulphide nanoparticles and their potentiation by the anticancer drug imatinib

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**ABSTRACT**

Zinc sulphide (ZnS) nanoparticles were synthesized by simple aqueous chemical reaction of zinc chloride and sodium sulphide in an aqueous solution. The main advantage of ZnS nanoparticles of diameter 29 nm was that the sample could be prepared by using of cheap precursors in a cost effective and eco-friendly manner. Structural, morphological and chemical composition of the prepared nanoparticles were investigated by X-Ray Diffraction(XRD), Scanning Electron Microscopy(SEM) with energy dispersion X-ray dispersive fluorescence spectroscopy (EDAX) and Fourier transform infrared (FTIR) spectroscopy. The antimicrobial effects of the zinc sulphide (ZnS) nanocrystals and the anticarcinogenic agent imatinib were studied by spot inoculation technique and also by well diffusion technique against twelve pathogenic bacterial strains. Nanoparticles of ZnS showed antimicrobial activity against both Gram positive and Gram negative strains except *Shigella sonnei*. The anticancer drug imatinib was inhibitory for only Gram positive organisms, *Bacillus subtilis* and *Staphylococcus aureus*. Synergism between imatinib and ZnS nanoparticles was distinctly observed in all the Gram positive strains.

**Keywords:** zinc sulfide; nanoparticles; aqueous chemical synthesis; imatinib; antimicrobial activity

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## 1. Introduction

Antibiotics and antimicrobial chemotherapeutic agents have acted much like magic bullets against almost all bacterial pathogens when they were first discovered. However, extensive and often indiscriminate application of these agents for many years has resulted in an explosion of multiple drug resistant pathogens throughout the world. Thus, there is an urgent need to identify and develop new antimicrobial compounds, either natural or synthetic, to offer appropriate and efficient therapy for various types of infections. In the search for novel antimicrobial drugs, attention has been given to detect such an activity among known anticancer agents. Experimental studies [1] showed that methotrexate possesses moderate to powerful antimicrobial action against *Staphylococcus aureus* strains. Recently Bruns et al reported on the bactericidal action of imatinib on *Mycobacterium tuberculosis* [2]. Based on such observations the anticarcinogenic compound imatinib was investigated in this study for determination of its antimicrobial action against several different bacteria.

Imatinib is a drug used to treat certain types of cancer like chronic myelogenous leukemia (CML), gastrointestinal stromal tumours (GISTS) and some other diseases [3]. Imatinib is the first member of a new class of compounds that act specifically by inhibiting a certain enzyme that is characteristic of a particular cancer cell, rather than non-specifically inhibiting and killing all rapidly dividing cells, this has served as a model for other targeted therapy modalities through tyrosine kinase inhibition [4].

Although most of the chemotherapeutic antimicrobial agents are complex chemical compounds, several nanosized simple inorganic compounds have also exhibited presence of such an action [5].

Interests on nanoparticles have been generated in recent years due to their simple structure, characteristic physical, chemical and biological properties that are usually distinctly different

from those of the bulk materials. Intensive experiments and studies have revealed that the nanoparticles of magnesium oxide (MgO), calcium oxide (CaO) and zinc oxide (ZnO) [6] possess potent antimicrobial property when tested against various Gram positive and Gram negative organisms. Zinc sulphide (ZnS) is a simple inorganic compound known for its practical applications in photoconductors, solar cells, field effect transistors, sensors transducers, optical coatings and light emitting materials [7]. It may be pointed out here that simple inorganic substances as antimicrobial agents may prove to be advantageous as they contain mineral substances essential for human consumption and may exhibit powerful action even when administered in small amounts.

In view of the information on presence of antibacterial action in nanoparticles of MgO, CaO and ZnO, nanoparticles of ZnS were prepared in our laboratory and were evaluated for the antimicrobial potentiality along with that of imatinib singly and by combination of the two agents.

## **2. Experimental**

### ***2.1. Materials and method***

**Drugs:** The anticancer drug imatinib was obtained as a pure dry powder from Novartis, India.

**Bacteria:** A total of 12 pathogenic bacteria belonging to 8 genera comprising 9 Gram negative and 3 Gram positive strains were tested. These were of human origin, identified as described by Barrow and Feltham [8] and preserved in freeze dried state.

**Chemical compounds:** Analar zinc chloride ( $ZnCl_2$ ) and sodium sulphide ( $Na_2S$ ) were purchased from Merck, Germany; these were allowed to react to produce ZnS nanoparticles.

*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) and Mueller Hinton agar (MHA, Oxoid)

## **2.2. Method of preparation of ZnS nanoparticles**

Synthesis of ZnS nanoparticles was carried out by aqueous chemical method using  $\text{ZnCl}_2$  and  $\text{Na}_2\text{S}$  as source materials. All the reagents were of analytical grade and used without further purification. 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  $28^\circ\text{C}$  temperature and ambient conditions. In a typical preparation solution of 1M  $\text{Na}_2\text{S}$  was added drop by drop to 1M  $\text{ZnCl}_2$  solution which was kept on stirring using a magnetic stirrer at  $70^\circ\text{C}$  for 2 hours; this resulted in formation of ZnS nanocolloid. The nanoparticles were collected by centrifugation at 2000 rpm for 15 minutes and further purification was made in ultrasonic bath. The resultant product was finally dried at  $120^\circ\text{C}$  for 2 hours.

## **2.3. Characterization of ZnS nanoparticles**

The prepared sample was subjected to characterization by XRD (Model D8, Bruker AXS) to determine the phase purity and average particle size of the sample, using  $\text{CuK}\alpha$  radiation at  $1.5409\text{\AA}$  ( $2\theta = 10^\circ$ - $70^\circ$ , scan speed = 0.2 s/step, increment = 0.02, operating voltage = 40 kV and operating current = 40 mA). The nanophase was identified by comparing peak positions and intensities (finger print method) [9].

To determine the structural features of all the samples, Fourier transform infrared (FTIR) spectroscopy was carried out using an FTIR spectrometer (FTIR-8400s, Shimadzu), with 150 scans for wave numbers ranging from  $400$ - $4000\text{ cm}^{-1}$  and resolution  $4\text{ cm}^{-1}$ . The potassium bromide (KBr) pellet method was used to prepare the samples [10].

To investigate the morphological structure of sample surfaces, surface textures were examined by field emission scanning electron micrography (FESEM) and energy dispersion X-ray fluorescence spectroscopy (EDAX) (JSM6700F JEOL LTD, Tokyo, Japan), was also carried out to ascertain the composition.

#### ***2.4. Preparation of zinc sulphide nanoparticle solution***

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 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 alone and in combination with Imatinib.

#### ***2.5. In vitro tests for determination of Minimum Inhibitory Concentration (MIC) of imatinib and ZnS nanoparticles***

The Gram negative bacteria were grown in MHB and the Gram positive ones in NB for 18h to obtain optimum growth.

An aqueous 10mg/ml stock solution of imatinib was prepared in sterile distilled water. This was added to molten nutrient agar at 50°C 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 inocula consisted of suitably diluted 18h broth culture of a bacterium. The MIC of imatinib was determined by spot inoculating one 2mm (internal diameter) loopful of a culture containing ca.10<sup>5</sup> colony forming units (CFU), on the plates following the guidelines of CLSI [11]. The plates were incubated at 37 °C. Growth was recorded at 18h as well as after 72 h. Experiments were carried out with the same varying amounts of ZnS nanoparticle solutions also by following the same technique.

## ***2.6. Determination of antimicrobial action of ZnS and imatinib singly and in combination by well diffusion assay***

The *in vitro* effect of the agents was determined by well diffusion technique as described by Miles and Amyes [12]. Each well having 5mm diameter was cut with the help of sterile cork 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. Thereafter the wells were made for two agents whose relationship was to be determined in such a manner that the inhibitory circles would touch each other tangentially, leaving only a very thin ridge of growth in case of complete indifference. In the instances of antagonism the inhibitory circles would recede away from each other at their facing surface assuming a somewhat kidney shape. However, in case of synergism the ridge of growth would disappear and the two circles would merge to form a single asymmetric ellipse [12].

## **3. Results and discussion**

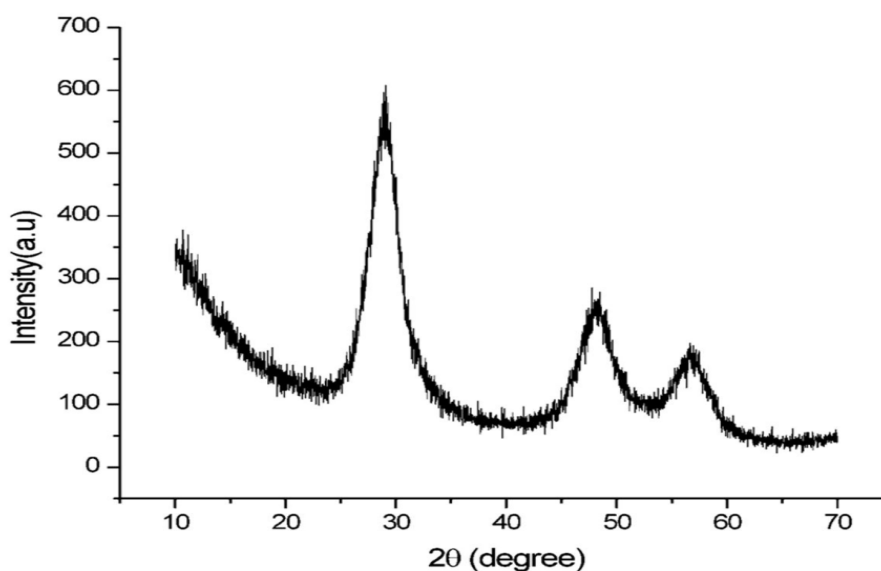
### ***3.1. X-Ray Diffraction (XRD) analysis***

From the XRD results as shown in Fig 1, it is clear that pure ZnS nanoparticles were obtained in powder form. The broadened peaks in the XRD pattern indicated the formation of ZnS nanocrystals with small crystallites. The three diffraction peaks at  $2\theta$  values of  $28.978^\circ$ ,  $47.62^\circ$ ,  $56.65^\circ$  corresponding to the (111), (220) and (311) diffraction planes, respectively of the spherical nanocrystalline structure of ZnS were observed. These values were very close to those reported by Jia Xiang Yang et.al (Yang J X et al. 2008) [9].

The average crystallite size ( $D$ ) was calculated from the full-width at half-maximum (FWHM) of the most intense peak of the (111) plane of ZnS nanoparticles using the Debye-Scherrer formula for spherical particles [Eq. (1)].

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

Where  $\lambda$  is the wavelength (Cu  $K\alpha$ ),  $\beta$  is the full width at the half-maximum of the ZnS nanoparticles and  $\theta$  is the diffraction angle. From this equation the average particle size was estimated to be 29 nm which was also supported through FESEM.



**Fig. 1.** XRD pattern of ZnS nanoparticles synthesized by aqueous chemical method

### 3.2. Fourier transforms infrared (FTIR) studies

The FTIR spectrum of the ZnS nanoparticles is demonstrated in Fig 2. The spectra exhibited strong bands appearing in the 1114, 1259, 1384 & 3200–2900  $\text{cm}^{-1}$  corresponding to ZnS nanoparticles [10]. The peaks at 612  $\text{cm}^{-1}$  can be assigned to the ZnS band (i.e., corresponding to sulphides) [9]. The O–H bending region due to absorbed water appears at

1620  $\text{cm}^{-1}$ . The stretch vibration adsorption of ZnO at 420–460  $\text{cm}^{-1}$  is not detected which indicates that ZnS was not oxidized to ZnO during the preparation as reported by She Yuan-yuan et.al [13].

### **3.3. FESEM analysis and EDAX study**

Figs 3 shows the FESEM results of as prepared ZnS nanoparticles. It is seen that the ZnS nanoparticles are homogenously dispersed and almost spherically shaped with an average diameter of about 29 nm. From the EDAX result the composition of the prepared sample could be obtained which was about 73.55% of Zn<sup>+</sup> ion and about 26.45% S ion by mass present in the sample.

### **3.4. Antibacterial activity of ZnS and the anticancer drug imatinib as determined by spot inoculation technique**

The MIC of ZnS nanoparticles against different bacteria as observed by spot inoculation method is presentated in Table 1. This shows that *B. subtilis* UC 564, *S. aureus* 8531, 8532, *E. coli* C600, *Sh. Flexneri* 6, *K. pneumonia* 10031, *A. baumannii* 462 and *P. aeruginosa* 27853 were inhibited at 100 $\mu\text{g/ml}$  of ZnS; *E. coli* K12 Row, *S. enteric* 11 and *V. cholerae* 14033 were inhibited at 200 $\mu\text{g/ml}$  of ZnS; *Sh. sonnei* 9774 remained totally resistant to ZnS.

The anticancer drug imatinib was found to possess antibacterial activity against 3 bacterial strains out of 12 pathogenic bacteria. It can be seen from Table 1 that *B. subtilis* UC 564, *S. aureus* 8531 and 8532 were inhibited by the drug at a concentration of 300 $\mu\text{g/ml}$ . Other strains were found to be resistant to the drug even at a concentration as high as 500 $\mu\text{g/ml}$ .



**Table 1.** Determination of minimum inhibitory concentration of ZnS nanoparticles and imatinib against pathogenic strains

Bacteria	Source	Minimum Inhibitory Concentration(MIC)( $\mu\text{g/ml}$ )	
		ZnS	Imatinib
<i>Bacillus subtilis</i> UC 564	Upjohn Lab, USA	100	300
<i>S. aureus</i> NCTC8531	S.P.Lapage, London	100	300
<i>S. aureus</i> NCTC8532	S.P.Lapage, London	100	300
<i>E. coli</i> K12 row	J.D.Abott, U.K.	200	>400
<i>E. coli</i> C600	J.D.Abott, U.K.	100	>400
<i>Shigella sonnei</i> NCTC9774	J.Taylor,London	>400	400
<i>Shigella flexneri</i> 6NCTC 396/3	J.Taylor,London	100	>400
<i>Salmonella enteritidis</i> NCTC11	J.Taylor,London	200	400
<i>Klebsiella pneumoneae</i> ATCC10031	Central Drugs Lab, Calcutta	100	>400
<i>Acinitobacter boumanii</i> 462	Dr.S Das, Calcutta	100	>400
<i>Vibrio Cholerae</i> ATCC 14033	S. Mukherjee, Calcutta	200	400
<i>Pseudomonas aeruginosa</i> ATCC27853	Central Drugs Lab, Calcutta	100	>400

### 3.5. Effects of ZnS nanoparticles by well diffusion

The nanoparticles of ZnS produced inhibition zones around the wells that varied from 7 mm to 18 mm when the amount of ZnS was 100  $\mu\text{g}$  per well (Table 2).The diameters of inhibitory circles increased in size as the amount of ZnS was increased (Table 2).The greater sensitivity of Gram positive organisms by ZnS was further confirmed by this test.

**Table 2.** Antimicrobial activity of ZnS nanoparticles determined by well diffusion technique

Bacteria	Amount of ZnS applied in each well		
	100 $\mu\text{g}$	150 $\mu\text{g}$	200 $\mu\text{g}$
<i>Bacillus subtilis</i> UC 564	15	21	24
<i>S. aureus</i> NCTC 8532	16	21	26
<i>S.aures</i> NCTC 8531	18	28	30
<i>E .coli</i> K12 Row	0	7	9
<i>E. coli</i> C600	16	21	23
<i>Shigella sonnei</i> NCTC 9774	0	0	0
<i>Shigella flexneri</i> 6 NCTC 396/3	7	13	15
<i>Salmonella enteritidis</i> NCTC 11	0	7	11
<i>Klebsiella pneumonia</i> ATCC 10031	7	13	15
<i>Acinetobacter baumannii</i> 462	16	23	25
<i>Vibrio cholerae</i> ATCC 14033	0	0	7
<i>Pseudomonas aeruginosa</i> ATCC 27853	0	9	11

### 3.6. Action of ZnS nanoparticles singly and combinedly with imatinib by well diffusion

Imatinib being inhibitory for only Gram positive bacteria was tested singly and combinedly with ZnS nanoparticles by well diffusion assay in the same plate. Singly 100 ug ZnS produced 15 mm wide zone of inhibition against *B subtilis UC564* while the same due to 300 ug imatinib was 14 mm. In combination the diameter became 21mm wide. Similar increase in inhibition zones was recorded in *S aureus* strains as well (Table 3).

**Table 3.** In vitro antibacterial activity of zns nanoparticles and imatinib alone and in combination with each other

Bacteria	Diameter of inhibition zone in mm		
	ZnS (100 µg)	Imatinib (300µg)	ZnS(100µg)+Imatinib(300µg)
<i>B. subtilis UC 564</i>	15	14	21
<i>S. aureus NCTC 8531</i>	16	12	25
<i>S. aureus NCTC 8532</i>	18	12	23

### 3.7. Discussion

The present study clearly indicates that ZnS nanostructures could be synthesized by a simple aqueous chemical method using pure aqueous route resulting in primary particle sizes of 29 nm. This particle size was calculated from Debye –Scherrer formula. FESEM image was used to study the morphology of the synthesized nanoparticles. FTIR spectra showed the possible stretching and bending modes of the ZnS nanoparticles. These ZnS nanoparticles synthesized by us showed significant antimicrobial activity when tested against pathogenic bacterial strains. While sensitive bacterial strains included *B. subtilis UC 564*, *A. baumannii* 462, *E. coli C600*, *K. pneumoniae ATCC 10031*, *S. aureus 8531* and *8532* and *P. aeruginosa ATCC 27853*. It was found to be less active against *Sh. sonnei 9774*, *V. cholerae ATCC 14033* and *E. coli K12 Row*. It may be pointed out here that ZnS nanoparticles demonstrated a pronounced inhibitory action against *S.aureus 8531*, an organism which is known to be

multidrug sensitive. ZnS nanoparticles were found to be bacteriostatic *in vitro* against both Gram positive and Gram negative bacteria.

Imatinib which is also known as ST1-571 is used as a drug for targeted anticancer therapy [14,15]. Targeted therapy is the result of about 100 years of research dedicated to understanding the differences between the normal cells and cancer cells. Imatinib belongs to the signal transduction inhibitor category of targeted therapies [16,17].

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 which demonstrate significant antimicrobial activity against some of the most pathogenic infectious agents (Kristiansen J E. et al. 1992; Dasgupta A et al. 2008; Jeyaseeli L. et al. 2012) [18-20]. Our present study indicates the potential of imatinib as a noteworthy antimicrobial agent since it has shown significant inhibitory effect against Gram positive pathogenic bacteria. Furthermore, the antimicrobial efficiency of imatinib was much enhanced when tested in combination with ZnS nanoparticles as revealed by the study for determination of synergism.

Since these results reveal that the combination of ZnS nanoparticles and imatinib possesses potent antibacterial action, further studies are in progress to explore the possibility of their application in routine therapy against infections of animals.

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