Eco-friendly synthesis of nanocrystals and determination of their antimicrobial effects on pathogenic strains.

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Abstract

The main advantage of the synthesized 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). The antimicrobial effects of the zinc sulphide (ZnS) nanocrystals 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*.

Keywords: eco-friendly, cost effective, zinc sulfide, nanoparticles, aqueous chemical synthesis, antimicrobial activity.

Introduction:

Extensive and often indiscriminate application of antimicrobial 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[1-4].

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[5]. 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 action.

Materials & Methods:

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) \

Method of preparation of nanoparticles:

Synthesis of ZnS nanoparticles was carried out by aqueous chemical method using $ZnCl_2$ and Na_2S 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 28ºC temperature and ambient conditions. In a typical preparation solution of 1M Na_2S was added drop by drop to 1M $ZnCl_2$ solution which was kept on stirring using a magnetic stirrer at 70º 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 120º C for 2 hours.

Characterization of 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 CuKα radiation at 1.5409Å ($2\Theta = 10^0$-70^0, 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 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[10].

Preparation of 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.

In vitro tests for determination of Minimum Inhibitory Concentration (MIC) of 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 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 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.10^5 colony forming units (CFU), on the plates following the guidelines of CLSI [11]. The plates were incubated at 37 ºC. 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.

Results:

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 2θ values of 28.978^0, 47.62^0, 56.65^0 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 λ is the wavelength (Cu Kα), 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 29 nm which was also supported through FESEM[10-13].

FESEM analysis and EDAX study:

Figs 2 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+ ion and about 26.45% S ion by mass present in the sample.

Antibacterial activity of ZnS 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. baumanii* 462 and *P. aeruginosa* 27853 were inhibited at 100µg/ml of ZnS; *E. coli* K12 Row, *S. enteric* 11 and *V. cholerae* 14033 were inhibited at 200µg/ml of ZnS; *Sh. sonnei* 9774 remained totally resistant to ZnS.

Discussion:

Our 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. 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. baumanii* 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 14033and *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.

Since these results reveal that the synthesized nanoparticles possesses potent antibacterial action, further studies are in progress to explore the possibility of their application in routine therapy against infections of human cell lines.

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Tables:

Bacteria	Source	ZnS
Bacillus subtilis UC 564	Upjohn Lab, USA	100
S. aureus NCTC8531	S.P.Lapage, London	100
S. aureus NCTC8532	S.P.Lapage, London	100
E. coli K12 row	J.D.Abott, U.K.	200
E. coli C600	J.D.Abott, U.K.	100
Shigella sonnei NCTC9774	J.Taylor,London	>400
Shigella flexneri 6NCTC 396/3	J.Taylor,London	100
Salmonella enteritidis NCTC11	J.Taylor,London	200
Klebsiella pneumoneae ATCC10031	Central Drugs Lab, Calcutta	100
Acinitobacter boumanii 462	Dr.S Das, Calcutta	100
Vibrio Cholerae ATCC 14033	S. Mukherjee, Calcutta	200
Pseudomonas aeruginosa ATCC27853	Central Drugs Lab, Calcutta	100

Table 1: Determination of minimum inhibitory concentration of ZnS nanoparticles against pathogenic strain.

Figures:

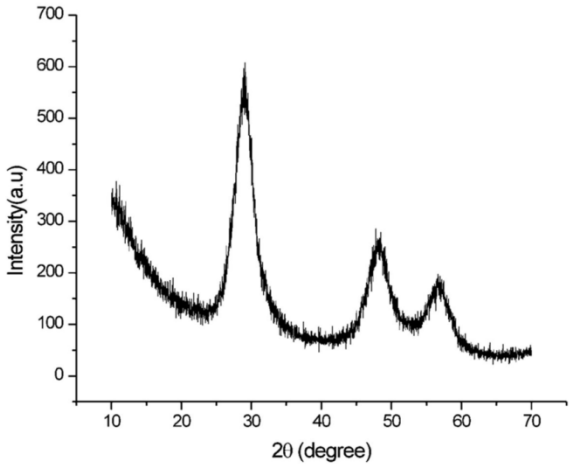

Fig 1. XRD pattern of ZnS nanoparticles synthesized by aqueous chemical method.

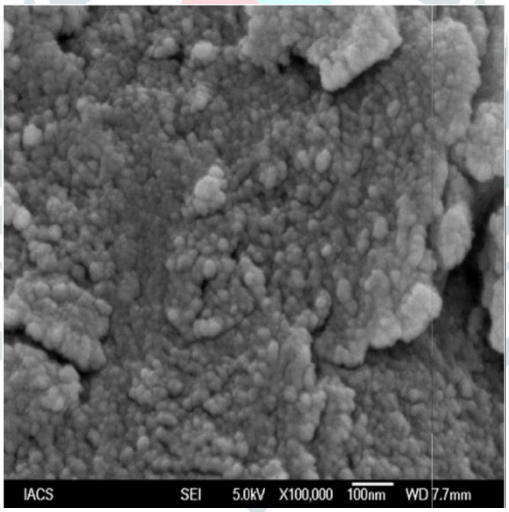

Fig 2. FESEM micrographs of the synthesized ZnS nanoparticles.