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Synthesis and Spectroscopic Characterization of Zinc Sulphide nanoparticles using Microwave irradiation of Zinc complex of Thiosemicarbazone ligand as a Single Molecular precursor : Pharmacological activities

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Single molecular precursors are appropriate starting materials for synthesis of semiconductor nanoparticles (NPs), which allow for the control of atomic ratio, monodispersity, composition and particle size of nanoscaled metallic sulfide nanoparticles. In the present study, we have reported the synthesis of nanostructured chalcogenides pharmacologically active active zinc sulfide nanoparticles (ZnS NPs) using Zn (II) ion inserted thiosemicarbazone ligand as a single molecular precursor .The precursors were thermally pyrolysized using high energy microwave radiations to obtain very fine ZnS nanoparticles. In this synthesis, we use DMSO as a nonpolar solvent for the synthesis of all compounds. The heating of Zinc complex in the non- aqueous environment of DMSO plays a very crucial role in decreasing reaction time, reducing the chances of side reactions and proper conversion of Zn complex into ZnS nanoparticles. In this reaction Zn complex of thiosemicarbazone ligand provides both Zn^{2+} and S^{2-} ions for synthesis of ZnS nanoparticles. The microwave synthesis of ZnS NPs from Zn complex is a very simple, fast, highly effective, efficient and low cost method. All synthesized compounds were characterized by various structural, electronic, vibrational, optical, morphological and pharmacological characterizations. The prepared ZnS NPs were found to crystallize in cubic phase, which generally forms at low temperatures, with the dimensions dependent upon the molar ratio of molecular precursors used. Synthesized ZnS nanomaterials had surface sulfur vacancies that extend their absorption spectra towards the visible region and decreased the bond gap. This allowed ZnS nanoparticles to demonstrate various pharmacological activities like antibacterial, antioxidant and anti-inflammatory activities under normal conditions. Powered X-ray diffraction studies confirms the formation of well -defined equispaced crystalline ZnS NPS. TEM and FE SEM microscopic studies confirmed the elongated tubules structure of ZnS NPs with an average particle size of 60 nm. Sharpe electronic absorption band at 390 nm indicates the synthesis of good quality ZnS NPs. The FT-IR spectral studies confirmed the presence of Zn-S stretching, N-H bending and C=N stretching, vibrations in molecular precursor as Zn(II) complex. The thermal analysis of molecular precursor was performed to investigate the thermal stability of zinc complex. The Zn complex was stable up-to 380^0c. All synthesized compounds demonstrated excellent pharmacological activities like antibacterial, antioxidant and anti-inflammatory activities as compared to standards used in analysis of compounds. The microwave synthesis of ZnS nanoparticles via single molecular precursor in proper stoichiometric ratios is an excellent and an efficient method for synthesizing highly effective bioactive agents which can be considered as good drug candidate for the treatment of various diseases in future.

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Keywords: Schiff base ligand, Zinc complex, ZnS nanoparticles, Microwave radiations, Structural, Morphological studies, Antibacterial, Antioxidant, Anti-inflammably activity

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1. Introduction

During the last decades, microwave utilised semiconductor nanoparticles have been widely explored mainly for their unique structural, morphological, optical, industrial and pharmacological properties. The tiny dimensions and high surface area to volume ratio of nanoparticles make their physicochemical properties unique from those of the bulk materials. These unique properties are attractive for applications in various fields, such as optics, electronics, biomedicine, sensing, photo-catalysis, and so on. The different unique properties of semiconductor nanomaterials are well known and result from their physical, chemical and phase composition, nanoparticle size and shape, surface chemistry, and crystal defects. ZnS, as a type II-VI semiconductor, is among the widely investigated materials, and many methods have been proposed for the synthesis of its nanostructures with tailored or modified properties and potential applications in the biomedical and optoelectronic field, such as to prepare biosensors[1], bio composites[2], light emitting diode (LED), screens, sensors and laser[3] or nanocomposites[4].For example, the hydrothermal method is generally used for the synthesis of nanostructured ZnS from starting reagents such as thiourea, thiosemicarbazides, semicarbazides, sulfur powder, sodium sulfide and hydrogen sulfide [6-10] . The nano particle size and chemical composition can be controlled by altering the pH of solution / or adding a stabilizing agents[10-14]. A thousands of methods have been used to prepare very stable unique and defect- rich nanomaterials with enhanced properties, such as the microwave irradiation technique [15-16], aerosol micro precipitation [17], low temperature micro-emulsion[18,19], capping ZnS NPs with polymers[20] or even biological methods like stevia-assisted precipitation [21] and sulfate reduction by leaf extract [22] .

Among various chemical methods for the synthesis of semiconductor nanoparticles, very high temperature pyrolysis of a molecular precursor is a functional approach to achieve mono-dispersed nanoparticles with well- tunable size [23-28]. In this method, atomic rearrangement takes place throughout the nanoparticles formulation process from a single molecular source compound, resulting in a uniform distribution of metal ions and sulfide ions and retention of the initial molar stoichiometry in the finished product. In collateral, an incremental degradation of the molecular precursor permits for the control of nucleation and crystal growth of nanoparticles. Furthermore, ligands tightly coordinated to the metal-ion centre of transition metal complexes may influence the nanoparticle formation processes and change the crystal phase of the formed nanostructures, as previously explained by Cheng et al.[29] .Synchronized or coordinated organic molecules of the precursor are able to act as in-situ/ in-vivo capping agents for the synthesis of nanoparticles [30]. Thermal degradation of coordinated compounds with sulfur- containing schiff base ligands such as thiosemicarbazone, thiobiuret dithiocabamate , ditho carbonate, and trithiocarbonate, which serve as sulfide ion sources, has already been applied to produce metal sulfide nanoparticles with good crystalline nature, nanosize distribution and adjustable dimensions[31-34]. Generally all metal sulfide nanoparticles synthesis was carried out at high temperatures and proceed in an inert medium. Pradham et al. reported the production of metal sulfide nano-crystalline particles with adjustable size and shape via metal complexes by using alkylamines as solvent, which were found to play a very important role in the synthesis, significantly lowering the decomposition temperature of metal-complex molecular precursors[35], This finding promoted us to explore the effect of primary amines on the formation of metal sulfide nanoparticles from thiosemicarbazone Zn (II) complex as a single molecular precursor. Therefore, our work aimed to synthesize metal sulfide nanoparticles, from a thiosemicarbzone source in ambient conditions and with ethanol as suitable medium. To the best of our knowledge, least information on microwave synthesis of ZnS nanoparticles from thiosemicarbazone Zn (II) complex is available to till date [36-37] which is why it is worth exploring how such nanostructure can be controlled under different reaction condition. Now-a-days microwave synthesis of metallic nanoparticles have been reported as a unique way to synthesize highly effective, very fine good quality nanomaterials. In the present research work, we have studied the synthesis of ZnS nanoparticles from thiosemicarbazone zinc complex as a single molecular precursor which provides both zinc ions as well sulfide ions in the reaction medium which acts as both nucleophile and a capping agent at room temperature. In this paper we have reported a unique method for the synthesis of ZnS nanoparticles complex from zinc complex of thiosemicarbazone ligand with adujustable size used as a sulfide ion source. Considering the

extraordinary interest in applying ZnS nanostructures in diverse pharmacological applications under normal temperature and pressure [38-39] upon characterizing them properly, we then evaluated the performance of synthesised ZnS nanoparticles against various biological activities like antibacterial, antioxidant and anti-inflammatory activities ,which was used as a good drug candidate for various biological applications in future for treating microbial infections.

2. Experimental

2.1. Materials and methods

Zinc sulfide nanoparticles were synthesised by using high energy microwave irradiation approach with the help of synthesised Zn complex by the liquid phase process. Analytical grade chemicals and reagents Zinc chloride, aromatic amine, diethylmalonate, hydrazine hydrate, 4-chloroaceto phenone, hydrazine hydrate ,thiosemicarbazide hydrochloride, Ethanol, DMSO are obtained from Merck, India and used as received without further purification. Triple distilled water was used for all the experiments, chemical analysis, spectroscopic analysis and pharmacological analysis.

UV- Vis absorption spectra were recorded on a Perkin -Elmer UV/Vis Lambda 25 UV/Visible spectrophotometer using a 1cm path length cuvette with dichloromethane as a solvent for dissolution of all compounds. The Fourier-transform infrared spectra were recorded using a Perkin Elmer FT-IR spectrophotometer (K Br pellets) with the wave length range from 400 to 4000 cm^{-1}. Melting points of compounds were obtained by an electro thermal melting point apparatus via open capillary method and were not corrected. The elemental analysis was conducted using CHN Analyser ,Thermo- Flash EA-1112 series at the temperature up to 900°-1000° c and vandium penta oxide was used as an oxidezer to prevent oxidation caused by the presence of sulfur element in Zn complex. TLC was performed using a normal hexane and ethyl acetate n-hexane/ EtOAc in 1:3 ratio as an eluent. X-ray measurements of compounds were performed at room temperature using a Bruker axis D8 using Cu K$\propto -$ radiation.

2.2. Synthesis of ZnS nanoparticles

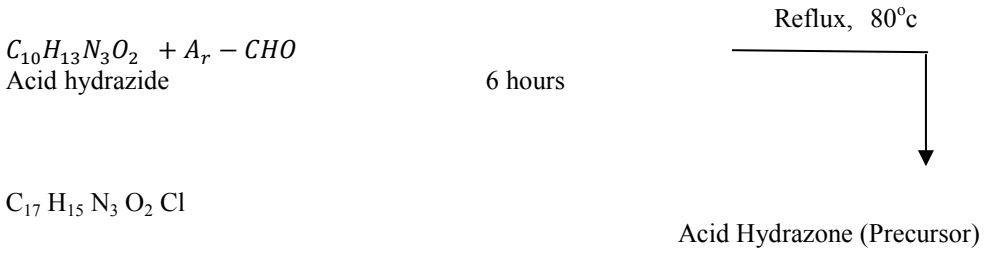
The whole synthetic process was divided into four steps-

(1) Synthesis of Schiff base ligand from primary amine (precursor)
(2) Synthesis of Zn complex from precursor
(3) Synthesis of ZnS nanoparticles

2.2.1. Step -1 Synthesis of precursor

Precursor was prepared by the condensation reaction between acid hydrazide and aromatic aldehydes in absolute ethanol in 1:1 molar ratio . The process of synthesis of precursor is -

For the synthesis of precursor, (0.01 M 2.96 g) of acid hydrazide was dissolved in absolute ethanol at a temperature of 60°c then add equimolar quantity of 4- chloroacetophenon (0.01M, 1.26g) in ethanol in the same round bottom flask and mixed both the solutions with continuous mechanical stirring at 70°c for half an hour. After complete mixing of both components, the mixture was refluxed for 6 hrs at 80°c. After 6 hours of refluxing, the colourless crystalline material was obtained. After 24 hrs of aging process, the crystalline material was filtered with whatman filter paper no. 41, washed with warm absolute ethanol and recrystallized from petroleum ether and dried the crystals over silica gel.



Yield: 2.26g (75.7%),m.p. :215 – 220°c,Analytical calculation for [$C_{17}H_{15}N_3O_2Cl$] : C , 67.05 ; H , 5.45 ; N, 8.78 ;Cl, 17.1 ; Found (%) : C , 67.46, H , 5.65 , N, 8.90 ; Cl 16.9 Characteristic FTIR absorption Peaks are (KBr disc , cm^{-1}) :3436 (S), 3276(S),1615(S),960 (m).'H N M R [DMSO – d_6,S] :11.60 [S, $1H_z$, N-H],11.24 [S, $1H_z$, Ar – OH], 7.688 [d, $1H_z$, J= 8.$1H_z$,Ar-H],6.98[m, $4H_z$, Ar-H],3.42 [S, $3H_2$, CH_3],UV-Vis [DCM, ⋋max] : 340nm.

2.2.2. Step -2 Synthesis of Zn complex from precursor

For the Synthesis of single molecular precursor of Zn complex from precursor, dissolved 1mM of precursor in 50 ml absolute ethanol and 1mM of thiosemicarbazide hydrochloride in 50 ml absolute ethanol (as a source of S^{2-}ion) in separate beakers. Mix both the solutions and stirred the mixture continuously for 1 hour at room temperature and add slowly 1 mM of Zinc chloride di hydrate salt as a source of Zn^{2+} ions and again shake the mixture continuously for 1 hour at room temperature. After 1 hour of stirring, we get colourless precipitate of Zn Complex. Then precipitate was sonicated for 2 hours at 80°c temperature .Cool the Solution and left for overnight for aging process . After 24 hours of aging, a colourless crystal of Zn Complex was obtained which was filtered and re -crystallised from hot ethanol and dried the crystals over silica gel.

$$2C_{17}H_{15}N_3O_2Cl + 2H_2N - \underset{C}{\overset{S}{II}} - NH - NH_2 + Zncl_2.2H2O\text{-----Reflux, Ethanol } 80^oC\text{--}$$

$[Zn(C_{38}H_{37}N_{14}S_2Cl_2]+4\ H_2O$

Acid hydrazide Thiosemicarbazide Hydrochloride Zn salt ---- Zn (II) Complex

2: 2 : 1 molar ratio

Yield : 1.90 g (93%), m.pt. : 290-300°c,Analytical Calculation for [$Zn(C_{38}H_{37}N_{14}S_2Cl_2)$]:C, 57.81 ; H, 5.16 ; N, 7. 80 ; S, 10.14, Cl, 17.38 ; Found (%) : C, 57.78 ; H, 5.26 ; N, 8.14 ; S, 11.12, Cl, 17.40.Characteristic FT-IR absorption peals [KBr, disc, cm^{-1}): 3448 (S), 1624 (S),964(m), 996(w), 662(m). H^1 NMR [CD_2Cl_2 S] : 8.12 [Cl, 1H,J= 6.2 H_Z, Ar – H], 6.90 [d s t, 2H J= 7.1 H_z, Ar – H], 6.74 [d, 2H, J = 9.1 H_z Ar – H], 6.92 [d &t, 2H, J = 7.2 H_z,Ar-H], 6.76 [d, 2H, J = 9.3 H_z, Ar-H], 3.68 [S, CH_3, 3H] . UV-Vis [DCM, ⋋max] : 355 nm

2.2.3. Step -3 Synthesis of ZnS nanoparticles

For synthesis of nanoparticles, 2 g of zinc complex was dissolved in 50 ml of non - polar solvent DMSO in a RB flask .A ultrasonic treatment was given to the solution mixture for half an hour at 70^0 C to proper mixing of solution mixture and then left for one hour for initiation of nucleation process of nanoparticles. After one hour ageing process of solution, the final product was pyrolyzed in microwave oven for 5 minutes with 800 W power microwave irradiations resulted immediate formation of ZnS nanoparticles. The solution containing ZnS nanoparticles was then cooled at room temperature, centrifuged and washed with absolute ethanol any times and stored for further spectroscopic characterization and pharmacological analysis.

2.3. Characterization of compounds

All the required chemicals were purchased from sigma- Aldrich and Merck. All the chemicals were used without purification. The crystalline structure of the ZnS nanoparticles was measured by X-ray diffraction (XRD) technique at room temperature using a D_8 Advance BRUKER diffractometer equipped with Cu K ∝ (1.54 A°) as the incident X- ray radiations. The Scherrer equation was used for the calculation of crystal size of nanoparticles. The Scherrer equation was

$$D = \frac{.9\,\lambda}{\beta.CoS\theta}$$

where D = Crystal size / Particle size (A°)

λ = Wavelength of the Cu - K$\propto$ radiations (1.54 A^o)
β = Corrected half – width of the diffraction Peak.

The FT-IR Spectra (KBr) pellets were reached on Perkin - Elmer Spectrophotometer with the wavelength range from 40^o – 4000 cm^{-1}. The fine structures of synthesised nanoparticles was analysed by SFM (Scanning Electronic microscopy. EDX (Energy – dispersion X-ray spectroscopy of the nanoparticles was carried out on a Sigma ZEISS, Oxford Instruments Field Emission Scanning Election Microscope. The optical, antioxidant and anti-inflammatory activities and antimicrobial properties of ZnS nanoparticles were investigated using a Perkin Elmer UV/Vis Lambda 25 spectrophotometer using a 1 cm path length with dichloromethane solvent.

2.4. Antibacterial study of compounds

The antibacterial activities of compounds was carried out for precursor, Zn complex and ZnS nanoparticles using disc diffusion method ; one colony of each bacterial strain *E. coli* (MTCC-1687), *E. faecalis* (MTCC- 439), and *S. aureus* from a streak plate was inoculated in 30 ml of LB broth after 16 hour of incubation period . The optical density of the microbial inoculums were measured and further diluted to achieve Mc Farland standard of 0.5 using sterile cotton swab, Again sterile plates were uniformly swabbed with diluted inoculums of bacterial strains include both gram positive and gram negative strains. After inoculation of bacterial strains sterile filter paper discs (6 mm) were impregnated with different molar concentrations of precursor, Zn complex and ZnS nanoparticles as 50 , 25 , 12.5 , 6.25 and 3.125 μg / ml using dichloromethane as the solvent) . Load the sample on discs filter paper for about 3-4 times with equal quantities so that concentration of compounds become same throughout the experiment. The zone of inhibition in mm were measured after 24 hour of incubation process. In this experiment vancomycin antibiotic used as a standard drug for comparison .For accuracy, all the experiments were performing in triplicates mode.

2.5. Antioxidant study of compounds

The free radical scavenging activity (RSA) of compounds at concentration 200, 400, 600, 800, 1000 µg/ml was carried out in presence of freshly prepared solution of stable free radical DPPH (0.04% w/v) following Hataro's method using ascorbic acid as standard. All the test analysis was performed on three triplicates and results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH in the presence of test compounds and absorption of DPPH in the absence of test compounds at 517 nm by UV Visible spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using following equation-

$$\%\text{RSA} = \frac{A_C - A_S}{A_C} \times 100$$

where, A_C = Absorbance of control.
A_S = Absorbance of test sample

2.6. Anti-inflammatory study

Following are the two methods utilized for evaluation of anti-inflammatory activity of synthesised compounds.

2.6.1. Inhibition of albumin denaturation

The anti-inflammatory activity of samples are was studied by using inhibition of albumin denaturation according technique described by Mizushima et al., (1968) and Sakat et al., (2010) with suitable modifications. The reaction mixture consists of compounds and 1% aqueous solution of bovine albumin fraction, maintaining the neutral pH of the reaction mixture. This is followed by incubation at 37ºC for 20 min and then heated to50º C for 20 min, after cooling the samples, the turbidity was measured at 660nm.

2.6.2. Anti- proteinase action

The activity was performed refereeing to the methods suggested by Oyedepo& Femurewa (1995); Sakat et al.(2010) with suitable modifications. 2 ml of reaction mixture containing 0.06 mg trypsin, 1ml 20 mM Tris-HCl buffer (pH 7.4) and 1 ml compounds of different concentrations (100 -500 µg/ml). The mixture was incubated for 5min at 37oC followed by addition of 1 ml of0.8% (w/v) casein and again followed by second incubation of 20 minutes. Then the reaction was stopped by adding 2 ml of 70% perchloric acid. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm against buffer as blank. Both the experiment were performed in triplicate.

The percentage inhibition of protein denaturation in both experiments was calculated
As per the following formulae;

Percentage inhibition = Abs Control –Abs Sample/ Abs control × 100

3. Results and discussion

The present research work reports the microwave synthesis of ZnS nanoparticle from Zn complex used as a single molecular precursor by using thiosemicarbazone schiff base ligand. The zinc complex was free from any impurity and does not require continuous stirring of reaction mixture. The formation of coloured particles indicates the formation of crystalline ZnS nanoparticles indicates the formation of crystalline ZnS nanoparticles.

In the UV –Vis spectrum, ZnS NPs showed absorption peaks at 340 nm, but no peaks appeared in the Zn Complex as shown in fig.1 This is a very useful and reliable technique for the primary identification of synthesis of nanoparticles.

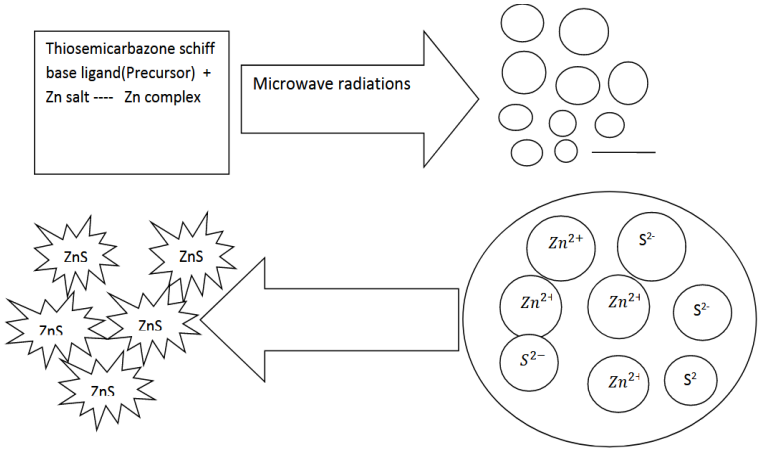


Fig. 1. Possible mechanism for the synthesis of ZnS.

FT-IR analysis is a very significant and reliable technique for the identification of presence of functional groups. The comparative FT-IR spectra of Zn complex and ZnS NPs are depicted in fig. The absorption bands of precursor at 3370,2459,1660,1146,696, and 420 cm^{1-1} are due to the presence of hydroxyl group 0-H, C-H, C = N and C –O functional groups respectively. The absorption bands of coordinated Zn complex appears at 3446, 1620, 962, 994, 666 cm^{-1}. Simultaneously, absorption bands for the ZnS nanoparticles at 2985 , 2412, 1569, 668, and 410 cm^{-1}corresponds to the presence of functional groups of O-H, C-H, C=N, C-O and C-S and might be responsible for the microwave reduction of Zn complex ions into ZnS nanoparticles.

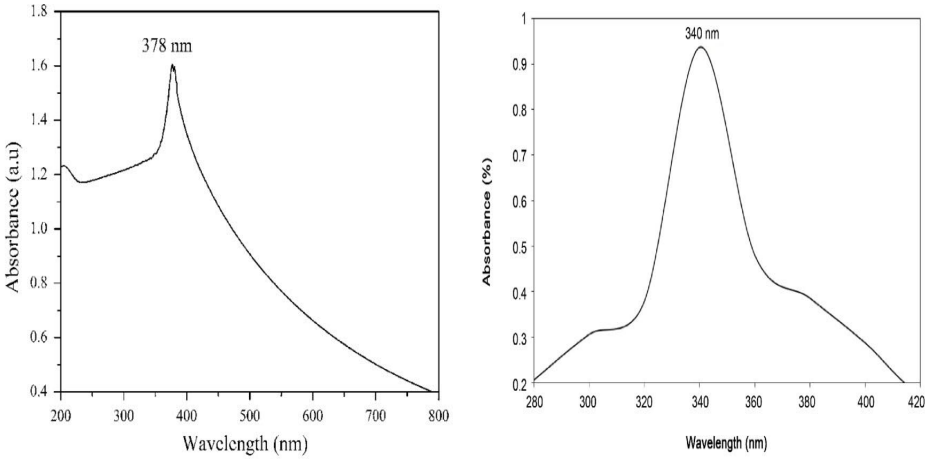


Fig. 2. UV Visible spectra of Zn complex and ZnS nanoparticles.

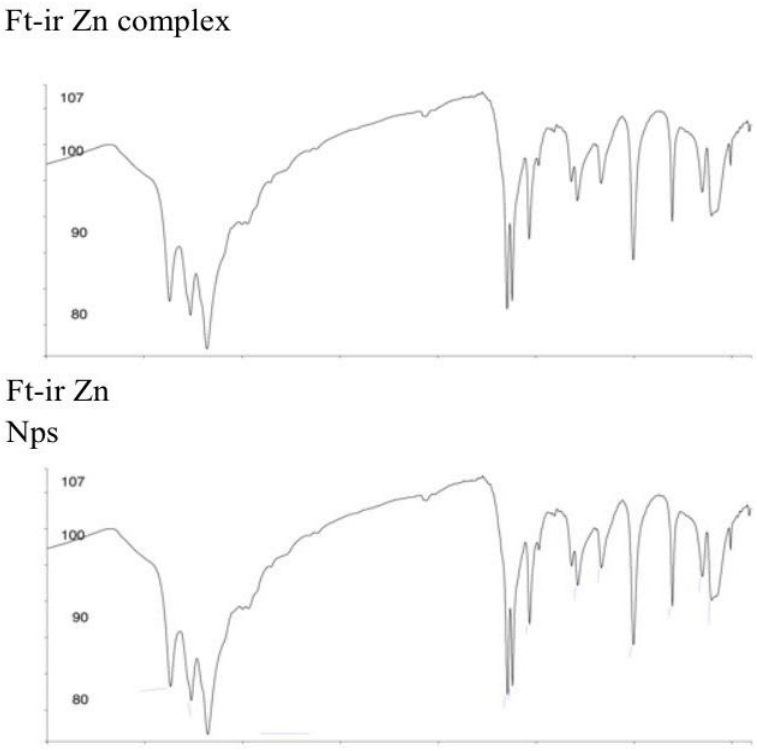


Fig. 3. FT-IR spectra of Zn complex and ZnS nanoparticles.

3.1. XRD analysis

XRD is a popular analytical technique which has been utilized for the crystalline nature of nanomaterials. The crystalline phase of the ZnS NPs was characterized by XRD analysis. The X-ray diffraction inter sites were recorded from 20^0 to 80^0 at 2ɵ angles, suggesting a monoclinic configuration. Five characteristic peaks were observed at $16.28^{o,}$ 32.36^o, 39.43^o, 49.92^o, and 54.26^o, and these corresponds to the $\hbar, \kappa, \ell$, values of the reflections from (110), (111), (220), (800) and (714) corresponding with the values of the JCPDS card (Card no. 895899). The Scherrer formula ($D=0.9\lambda/\beta Cos\theta$) was used to calculate the average crystalline size of the ZnS NPs, showing 13± 1nm on the (111) plane.

$$\text{where} \quad \lambda = X - raywavelength$$
$$\beta = fullwidthathalfmaximumatthe\theta\, angle.$$
$$\varphi = Bragg'sangle$$

The small size of ZnS nanoparticles indicated the high surface area and high surface area to volume ratio. Reflection of XRD Peaks are small in size and broad in shape and indicates the formation of ZnS nanoparticles. Due to formation of ZnS nanoparticles through Zn complex, there are no additional peaks are observed.

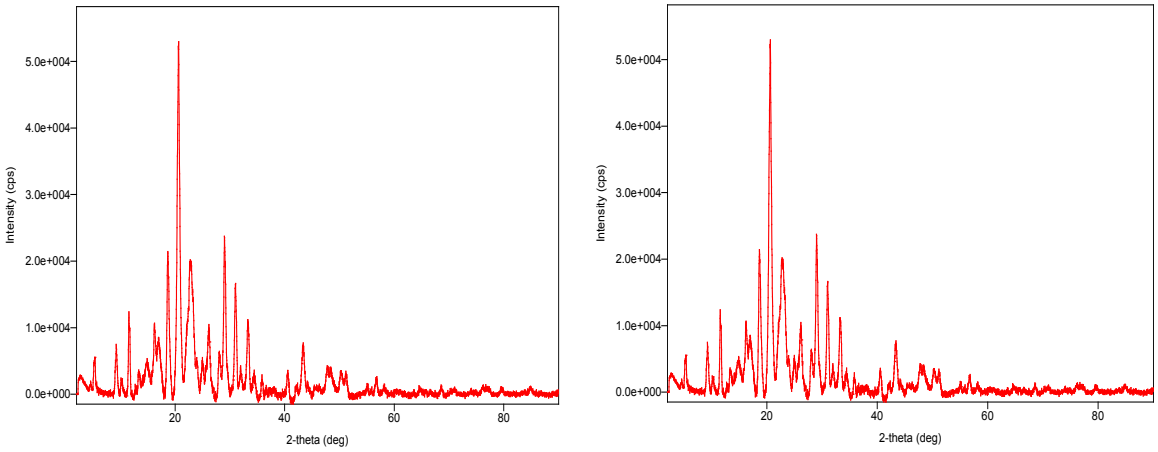


Fig. 4. XRD spectra of Zn complex and ZnS nanoparticles.

3.2. Morphology (SEM and EDX) analysis of ZnS NPs

The morphology of the ZnS NPs was examined using FESEM (Field Emission Scanning Electron Microscopy). Fig5-- represent the SEM images of the ZnS NPs at different resolutions. The elemental constituents of the synthesized ZnS nanoparticles were examined using EDX (Energy Dispersive X-ray Analysis Fig.6 shows the EDX spectra of ZnS NPs displaying the percentage of composition and major elemental peaks at 10 Kev that is specific to the Zn metal. Other small peaks also appeared due to presence of other elementals.

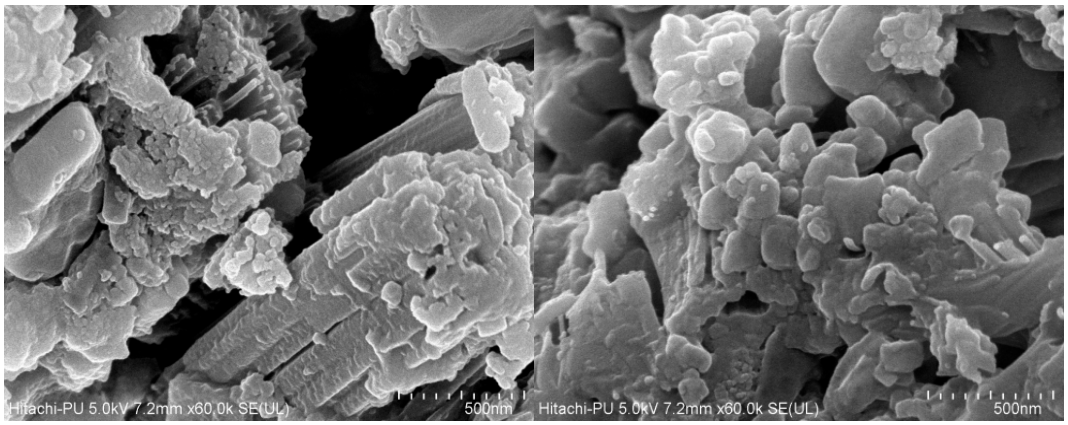


Fig. 5. SEM images of Zn complex and ZnS nanoparticles.

EDX studies of ZnS NPs confirms the presence of C, N, S and Zn elements in synthesized nanoparticles. The other impurities like Cl, Ca elements were also present due to interaction of Zn

complex with solvents during refluxing and recrystallization process in purification of Zn complex for further utilization in synthesis of ZnS NPs via microwave radiations. The presence of solvent does not change the shape and size of Zn NPs. But presence of solvent initiates the process of synthesis of ZnS NPs via nucleation process .SEM micrographs of ZnS NPs showed that the ZnS NPs were arranged in a open and quasi linear nano structures as compared to dense Packed assembly.

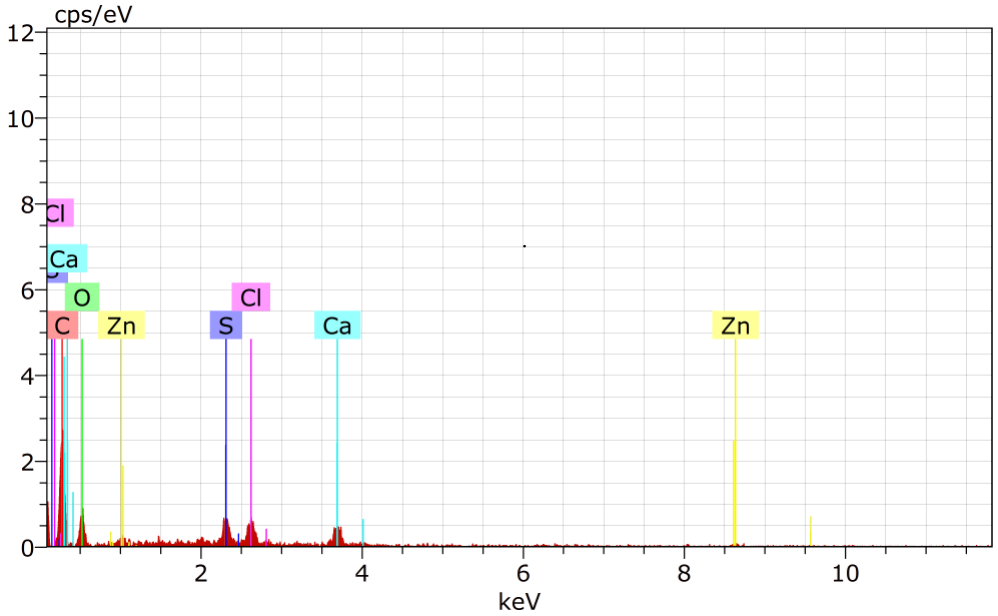


Fig. 6. EDX of ZnS nanoparticles

3.3. Transmission electron microscopic analysis (TEM) of the ZnS nanoparticle

TEM is an important characterization tool for the direct imaging of nanomaterials to obtain quantitative measurement of particle size, size distribution, shape of nanoparticles and lattice fringes. The thickness and radius of ZnS nanoparticles are estimated using Gatan Software. Reduced FFT shown in figure indicates the formation of highly crystalline nature of ZnS nanoparticles and clear occurrence of lattice fringes in ZnS nano structure. The appearance of dark and bright fringes confirms the synthesis of very fine crystalline ZnS nanoparticle via microwave radiations approach. The nanoparticles have been separated by well-defined boundaries, and are visible and uniformly distributed. TEM micrographs showed that ZnS nanoparticles were not aggregated in clusters but they were separated by equispace which was verified by microscopy visualizing under the high resolution microscope.

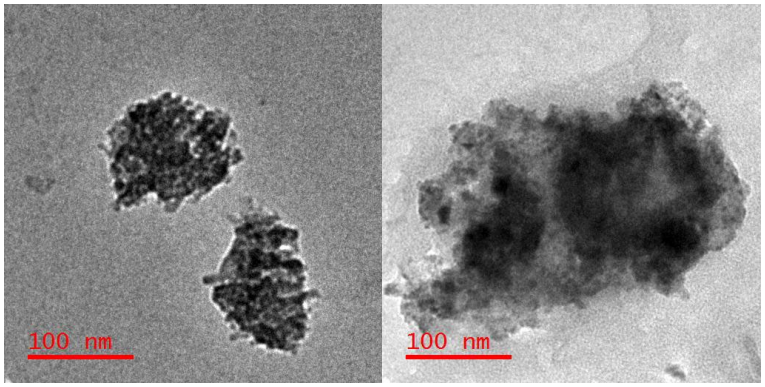


Fig. 7. TEM images of Zn complex and ZnS nanoparticles.

3.4. Evaluation of Antibacterial efficacy of ZnS NPs

In-vitro antibacterial efficacy of ZnS NPs were studies by incorporating various concentration of ZnS NPs (50 to 3.125 $\mu g/\mu l$) on agar plate was incubated with broad range of clinically important bacterial strains like *E. coli* (MTCC-1687), *E. faecalis* (MRCC-439), *S. aureus* (MTCC-737) and same concentrations of control antibiotic vancomycin was used to compare the antibacterial efficacy of ZnS NPs.The results showed that the growth of bacteria was significantly decreased in case of ZnS nanoparticles due to very small size of ZnS NPs.The higher antibacterial efficacy of ZnS NPs may be due to coordination and chelation phenomenon of Zn^{2+} ions with schiff base ligand in the formation of Zn complex which tends to make ZnS NPs act as powerful and potent bacteriostatic agents, thus inhibiting the growth of bacteria. In coordinated Zn complex the positive charges are present on Zn^{2+} ion and these positive charges are shared with the sulfide ions S^{2-} and there may be delocalization of $\pi\bar{e}$ s over the whole Zncomplex. The enhanced antibacterial activity of ZnS NPs may be explained on the basis of chelation theory of metal complexes.

Table 2. Results of antibacterial efficacy Zn (11) complex and of 1 ZnS NPS.

S. No.	Concentration ($\mu g/\mu\ell$) in Dichloromethane	*E. Coli* (MTCC-1687)		*E. faecalis* (MTCC-439)		*S. aureus* (MTCC-737)	
		Zn Complex	ZnS NPs	Zn Complex	ZnS NPs	Zn Complex	Zn NPs
1.	50 $\mu g\|\mu l$	19	22	16	25	18	20
2.	25 $\mu g\|\mu l$	17	20	14	19	15	18
3.	12.5$\mu g\|\mu l$	15	18	12	14	12	15
4.	6.25$\mu g\|\mu l$	13	15	8	12	8	12
5.	3.125C	10	12	6	10	5	10

Table 3. Inhibition of standard antibiotic Vancomycin – hydrochloride against clinically important bacterial cultures.

S. No.	Test microbe	Diameter of Zone of inhibition (in mm)at different drug concentrations				
		50$\mu g\|\mu l$	**25$\mu g\|\mu l$**	**12.5$\mu g\|\mu l$**	**6.25$\mu g\|\mu l$**	**3.125$\mu g\|\mu l$**
1.	*E.coli* (MTCC-687)	15mm	13mm	Nil	Nil	Nil
2.	*E. faecalis* (MTCC-439)	30mm	26mm	28mm	23mm	20mm
3.	*S.aureus* (MTCC-737)	26mm	24mm	20mm	17mm	12mm

Fig. 8. Antibacterial activities of Zn complex and ZnS nanoparticles.

3.5. Antioxidant activity

In this activity due to presence of paired electrons in DPPH molecule, DPPHmolecule becomes stable and immediately reacts with reducing agent. As aresults of this reaction, the pink colour of DPPH solution gradually fades due to electrons accepted by the DPPH solution. The decolouration of DPPH solution is due to the election withdrawing nature of DPPH free radical. In this experiment,ascorbic acid used as astandard substance for comparing the antioxidant activities of ZnS NPs which could be measured by UV-Vis spectrophotometer.The results of antioxidant activity of compounds showed that compounds with the low IC_{50} values are potent scavengers of free radicals than the compounds with high IC_{50} value. Compound consider in this investigation (ZnS NP's) is showed good free radical scavenging activity in DPPH method thus shows the good antioxidant potential as compared to Zn complex. Although, the antioxidant potential of ZnS nanoparticles was lower than that of standard antioxidant substance ascorbic acid with reference to the present experiment.

Table 4. Percentage DPPH free radical activity by standard antioxidant ascorbic acid at various concentration.

S. No.	Compounds	% scavenging activity				
		200(μg/ ml)	400(μg/ ml)	600(μg/ ml)	800(μg/ ml)	1000(μg/ ml)
1.	Schiff base ligand [$C_{17}H_{15}N_3O_2Cl$]	12.0	20.2	15.6	22.5	69.5
2.	Zinc Complex of schiff base ligand [Zn ($C_{38}H_{37}N_{14}S_2Cl_2$]	13.5	31.2	27.8	34.5	72.5
3.	ZnS nanoparticles	14.01	32.2	27.9	34.7	73.1
3.	Ascorbic acid(standard) (h)	50	65.4	68.2	75.3	80.6

All the compounds showed the excellent antioxidant activities at concentration of 1000 (μg / ml). As we increases the concentration of compounds antioxidant activities increases simultaneously. The tested zinc complex and ZnS nanoparticles and schiff base ligand exhibited significant antioxidant activity but less than the control standard compound ascorbic acid.

3.6. Anti-inflammatory activity of compounds

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation.

- The samples studied were effective in inhibiting heat induced albumin denaturation upto certain extent.
- The inhibition of zinc complex was 3.87% inhibition of albumin protein denaturation at 100 µg/ml compared to Aspirin as standard anti-inflammation drug at same concentration with maximum inhibition 70.7%. as compared to control.

Table 5. Percentage inhibition of proteinase activity of schiff base ligand against standard aspirin drugs.

S. No.	Compounds	Concentration in µg/ ml	Absorption at 210 nm	Percentage inhibition
1.	Control	100	0.733	0 %
2.	Aspirin	100	0.216	70.53 %
3.	Schiff Base Ligand	100	0.406	2.28 %
4.	Schiff Base Ligand	200	0.526	5.91 %
5.	Schiff Base Ligand	300	0.524	8.22 %
6.	Schiff Base Ligand	400	0.520	12.21%
7.	Schiff Base Ligand	500	0.497	16.20 %

Table 6. Percentage inhibition of proteinase activity of Zinc complex against standard aspirin drugs.

S. No.	Compounds	Concentration in µg/ ml	Absorption at 210 nm	Percentage inhibition
1.	Control	100	0.733	0 %
2.	Aspirin	100	0.216	70.53 %
3.	Zinc (II) Complex	100	0.566	22.78 %
4.	Zinc (II) Complex	200	0.504	31.24 %
5.	Zinc (II) Complex	300	0.477	34.92 %
6.	Zinc (II) Complex	400	0.391	46.66%
7.	Zinc (II) Complex	500	0.358	51.16%

Table 7. Percentage of proteins inhibition activity of ZnS Nano-particles against standard aspirin drug.

Sr. No.	Compunds	Concentration in on µg/ml	Absorptionat 210nm	Percentageinhibition
1.	Control	100	0.733	0%
2.	Aspirin	100	0.216	70.53%
3.	ZnSNPs	100	0.501	22.20%
4.	ZnSNPs	200	0.499	30.24%
5.	ZnSNPs	300	0.466	34.12%
6.	ZnSNPs	400	0.376	45.66%
7.	ZnSNPs	500	0.348	40.24%

3.7. Comparative protein denaturation activity of test samples

Graphical representation for percentage inhibition of protein denaturation as anti-inflammatory potential *in vitro* of samples Schiff base ligand, Zn-Complex& ZnS nanoparticles in comparison to activity of standard Aspirin at sample concentration. Proteins adsorbed on nanoparticles (NPs) are being used as biosensors and in drug delivery. However, our

understanding of the effect of NPs on the structure of proteins is still in a nascent state. Protein plays a crucial role in regulating the expression of several virulence factors in the pathogenesis of cholera. NPs increased the susceptibility of the protein to denaturation. In our study we use thiosemicarbazone schiff based ligand for the synthesis of Zinc complex as a Single Molecular precursor as well as synthesis of zinc sulphide nanoparticles. These ligands, metal complexes and their nano particles showed significant biological importance for a considerable amount of time period. When the thiosemicarbazone ligand binds with metal ions, they have shown an array of potential anticancer, antimicrobial and antioxidant activities etc. The results of anti- inflammatory activities of all synthesised compounds showed that ZnS nanoparticles showed an excellent anti - inflammatory activity as compared to thiosemicarbazone ligand and its coordinated Zn complex.

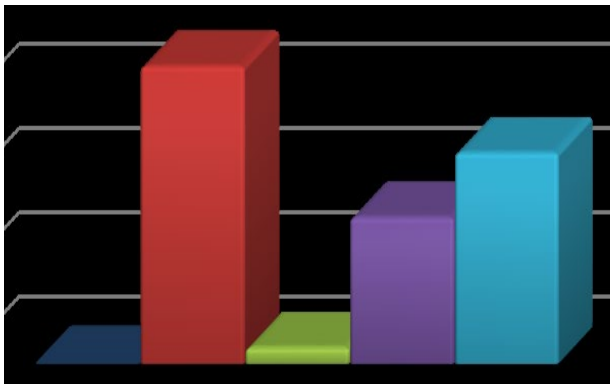


Fig-9 Comparative protein denaturation activity of ligand , coordinated Zn(II) complex and ZnS nanoparticles

4. Conclusion

In summary, novel ZnS nanoparticles were synthesised from microwave irradiation of thiosemicarbazone ligand as a single molecular processor : Pharmacological activities. During the interaction of Zn complex with microwave radiations colour of solution turned from an off white colour to a yellowish colour, indicating the formation of ZnS nanoparticles and this was also confirmed from UV –Visible spectra of ZnS nano particles. The absorption peaks at 340 and 378 nm in the UV-Visible spectroscopy results confirmed the synthesis of stable ZnS nano particles. Along with results of UV-Vis spectroscopy , FT-IR spectroscopy also confirmed the synthesis of ZnS NP's via microwave irradiation approach. The synthesised ZnS nanoparticles were found to be stable for up to 3 months without aggregation, due to the presence of various functional groups in Zn complex which acts as a chemical capping and reducing agents in the complex. The average particle size and crystalline nature of ZnS nanoparticles were found to be 60 nm, confirmed from TEM &XRD measurements. The microwave assisted synthesis of ZnS nanoparticles have effective anti- bacterial, antioxidant and anti-inflammatory efficacies as compared to standard substances. Finally, we have concluded that microwave assisted ZnS nanoparticles may be consider as a stable and efficient drug candidate for the treatment of various diseases and they are nontoxic in nature.

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