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Anticancer activity of Co doped ZnS nanoparticles synthesized by chemical method

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Abstract - Co doped ZnS nanoparticles were prepared by a simple chemical precipitation method. Zinc blende structure is confirmed by X-ray diffraction (XRD) analysis. The surface morphology and chemical compositions of material were recorded by SEM with EDAX.. High Resolution Transmission Electron (HRTEM) micrographs showed the confirmation of particle size around 2nm. The results from selected area electron diffraction (SAED) consistent the XRD results . In FTIR spectra the peaks formed at 616-1103 cm^{-1} are attributed to the bond between cobalt and sulphur. The magnetization (M - H curve) measured at room temperature exhibited saturated hysteresis loop for higher Co concentration exhibit at room temperature indicating ferromagnetism where as samples having higher Co concentrations showed such saturation confirming the strong ferromagnetism of the sample. Anticancer properties of the Co doped ZnS nanoparticles showed a direct dose response relationship and activity increased at higher concentrations level. Invirto anticancer study of Co doped ZnS nanoparticles against HT 29 colon cancer cell lines revealed significant anticancer effect and concentration dependent inhibition.

Key Words: Anti-cancer activity , Nanoparticles, direct dose, ferromagnetism.

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I. Introduction

Cancer is one of the main meticulous health problem of people. The last World Cancer testimony of the WHO states that the occurrence of cancer increased from 18.1 million in 2015 to 25.1 million in 2020 [1]. The heal of cancer involves various treatment based on alkylating agents, antimetabolites and biological agents but one of the main trouble is the side effects due to difficulties in differentiating between cancerous and normal cells, which produces systemic toxicity [2]. When exploring new strategies for the heal of cancer, one alternative is the use of nanotechnology. For more than 40 years, nanomaterials have been used as pharmaceutical carriers to develop the *in vivo* antitumor effectiveness of drugs. The first studies in the 1970s used nanoscale drug carriers, such as liposomes entrapping antitumor pharmaceuticals [3]. Growth of nanostructured devices for drug release and controlled delivery constituted new antitumor chemotherapies. Presently, growing field of research into the applications of nanoparticles against tumor formation, development and evolution, due to their intrinsic antitumor effects. Recently, studies reported that the remedial effects of nanoparticles loaded with chemotherapeutic agents in digestive cancer tumors [4]. Recently, Pesic and coworker study

recommended anticancer activity of nanoparticles on cancer and compared with normal human cells and concluded that the toxicity of these nanoparticles is specific to the cancer cells. The low inhibitory possible of cerium oxide in normal human cell lines indicates that they may be safer for human usage in industry and medicine [5].

Semiconductor nanoparticles (NPs) have suit much important because of their greater prospective in opto-electronic devices and biological applications[6]. Nanoparticles are fast becoming shows potential agents for drug delivery in the healing of diseases. At present, the nanoparticle delivery systems that have been developed for cancer therapy, including liposomes, polymer–drug conjugates, and micellar formulations[7]. The small size particle can accumulate naturally taking place proteins and different types of bio-molecules in the cell, and is especially smaller than the typical diameter (~7 μm) of many human cells.. It able them to interact in distinctive ways with cell bio-molecules and facilities their physical transport into the internal structure of cells [8]. Serious experiments and studies have exposed that the nanoparticles of magnesium oxide (MgO), calcium oxide (CaO) and zinc oxide (ZnO) hold strong antimicrobial property when verified against various Gram positive and Gram negative organisms. Zinc sulphide (ZnS) is a inorganic compound identified for its convenient applications in photoconductors, solar cells, field effect transistors, sensors transducers, optical coatings and light emitting materials. It may be noted that simple inorganic substances as antimicrobial agents may prove to be beneficial as they contain mineral substances necessary for human utilization and may reveal significant action even when administered in small amounts[9]. In vision of the information on presence of anticancer action in nanoparticles of MgO, CaO and ZnO, nanoparticles of doped ZnS NPs were prepared in our laboratory and were evaluated for the anticancer activity. Hence, the present work deals with biomedical applications such as anticancer properties of Co doped ZnS nanoparticles.

II. Materials and Methods

Human and animal rights and informed consent under the section "Compliance with ethical standards".

Synthesis Co doped ZnS NPs by chemical -precipitation method

Zinc nitrate [$Zn(NO_3)_2.6H_2O$] (from Sigma-Aldrich, India), Sodium sulphide (Na_2S) and Cobalt nitrate[$Co(NO_3)_2$] were used to prepare Co:ZnS NPs. The Co:ZnS NPs were prepared by the chemical -precipitation method as follows. Initially, desired mole concentrations of $Zn(NO_3)_2.6H_2O$ and (cobalt nitrate) were dissolved in de-ionized water and then stirred for 30 min at room temperature to achieve complete dissolution, thus 0.1 mole concentration solutions were obtained. Sodium sulphide also dissolved in de-ionized water separately as per mole concentration. Further, sodium sulphide solution was added drop by drop to the prepared solution and maintained at constant stirring for 5 hours at room temperature using magnetic stirrer. The precipitated solution were centrifuged at 5000 rpm for 30 min to separate undesired agglomerates, and washed with de-ionized water and ethanol. The samples were dried for one hour at 150 ^{0}C to eliminate residual gas, water impurities and to obtain fine powders of Co doped ZnS NPs. All the reactions were carried out at room temperature under ambient condition using de-ionized water as a solvent for its inherent advantages of being simple and eco-friendly.

Invirto anticancer activity -Cell line

The human colon cancer cell line (HT 29) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

MTT assay

Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. MTT assay – to know the value of the specific concentration

Characterizations

XRD analysis have been carried out using PANalytical X-ray diffractometer, Surface morphology of the samples have been studied using SEM (JEOL JSMS 800-V). Compositional analysis of the samples has been studied using energy dispersive analysis of X-rays (JEOL Model JED-2300). HRTEM images of the prepared nanopowders have been recorded using a Philips TECNAI-F20 microscope. The functional groups present were identified from Fourier transform-infrared (FTIR) spectroscopy using a Thermo Nicolet Avatar 370 with a spectral range of 4000–500cm^{-1} and resolution of 4 cm^{-1} . M-H curve measured by Vibrating Sample Magnetometer (VSM) Lakeshore VSM 7410 .

III. Results

X-Ray diffraction (XRD)-Structural Analysis

The observed diffraction peak are found to be very broad, confirming that the particles are in very small size. From Fig.1 the three broad peaks observed in the XRD patterns at 2θ equal to 28.66^0, 47.99^0 and 56.66^0 are respectively corresponding to the orientation planes (111), (220) and (311) indicates that the materials are crystallized in zinc blende structure in a cubic lattice [10]. This is in good agreement with the standard JCPDS data (JCPDS# 05-0566). From X-ray diffraction pattern, crystallite size has been measured by using Scherrer formula [11].

$$D = \frac{K\lambda}{\beta \cos\theta}$$

θ is the Bragg angle, λ is the X-ray wavelength, β is the Full width half maximum (FWHM) and K is the shape factor (for spherical shape K= 0.9, evident from HRTEM). The mean crystallite size is found to be 2 nm .The observed diffraction peak are found to be broad when compared with bulk ZnS confirming that the sample is nanocrystalline in nature. The increase in diffracted angle indicates particle size contraction due to quantum confinement and higher surface to volume ratio [12].

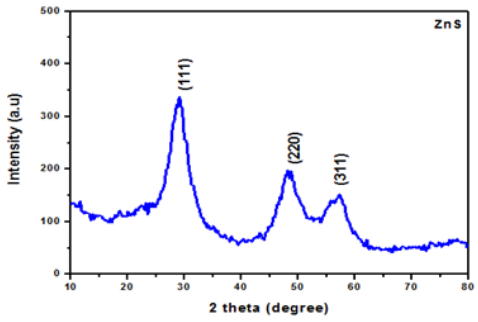

Fig.1: XRD pattern of Co-doped ZnS

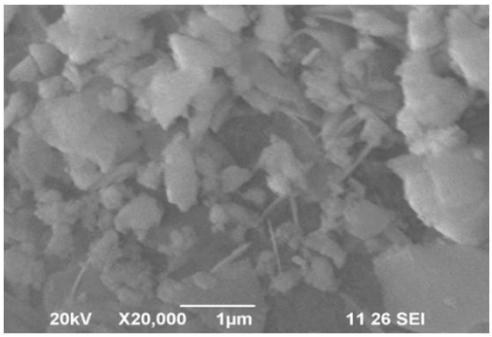

Fig. 2*:* SEM image of Co doped ZnS

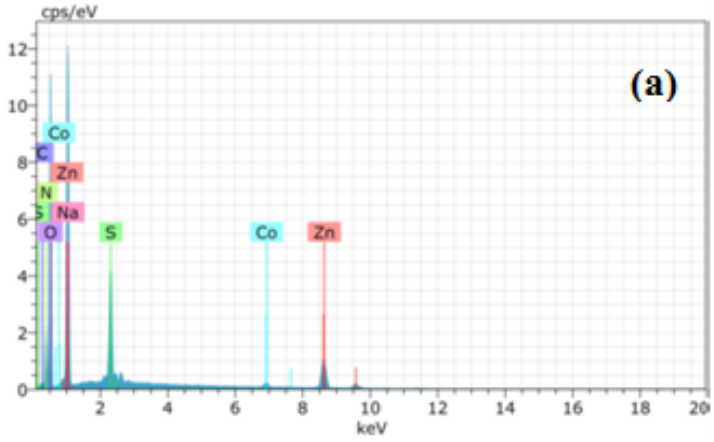

Fig. 3: **EDAX spectra of Co doped ZnS nanoparticles**

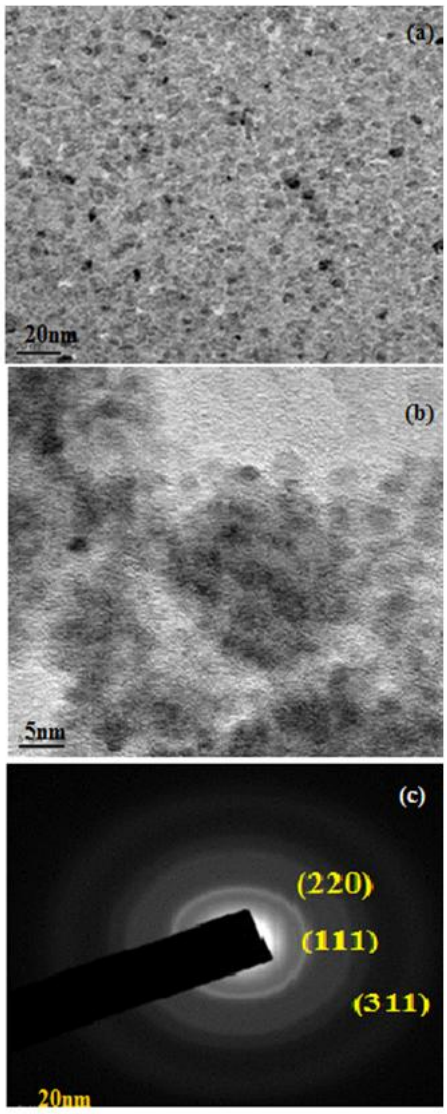

Fig. 4: (a-b) HRTEM images and (c) SAED pattern of Co-ZnS

Scanning Electron Microscopy (SEM) with EDAX

The SEM image of the Co doped ZnS samples shows in Fig.2. The SEM images clearly show the occurrence of nano and micro level petals throughout the sample. Nanosized grains were agglomerated over the petals. Fig. 3 shows the EDAX spectra of the Co doped ZnS. EDAX analysis reveals that Zn, Co and S are present in the sample[13].

High Resolution Transmission Electron Microscope (HRTEM) and Selected Area Diffraction Pattern (SAED)

Fig.4(a-c) shows the HRTEM image and SAED pattern of the prepared Co doped ZnS sample. The image shows lattice fringes and the observed plane matches well with the reported interplanar spacing of the cubic ZnS. Using the particle number (frequency %) and the average particle diameter of the particles in the HRTEM image, the average particle size has been calculated and was found to be 2 nm. The three rings seen in the selected area electron diffraction (Fig. 4.11c) can be indexed as (111), (220) and (311) lattice planes of cubic ZnS [14]. The results obtained from SAED pattern are in line with the XRD results. The obtainedparticle size from HRTEM image are in consistent with that of XRD results.

Optical properties-Fourier Transform Infrared Spectroscopy

Fig.5 shows the FTIR spectra of Co doped ZnS and it has been compared with reported values of different stretching and bending modes in standard references [15]. The observed measurements are in line with the reported values. The changes observed are due to the formation of nanophase. Bands around 3468 cm^{-1} corresponds to valence vibrations of the occluded water. Bands of 1112-1375 cm^{-1} may be due to the oxygen stretching and bending. The additional weak band at 1613 cm^{-1} may be due to the micro structural creation of the sample. Band around 1100 cm^{-1} is due to the characteristic frequency of inorganic ions. These modes indicate the occurrence of resonance interaction between Co vibrational modes of sulphide ions in the crystal. The peak around at 616 cm^{-1} is corresponding to symmetric bending of ZnS band (i.e., corresponding to sulphide). This peak confirms the formation of ZnS.

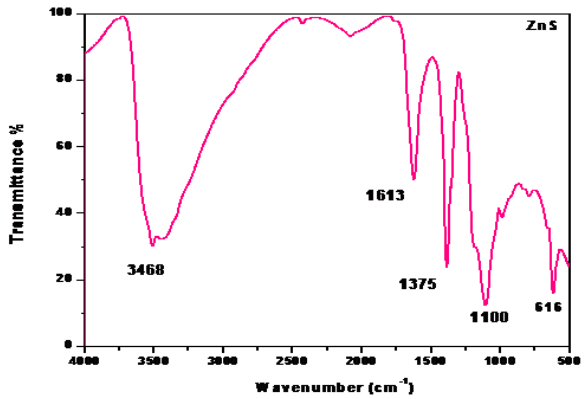

Fig .5: FT-IR spectra of Co doped ZnS nanoparticles

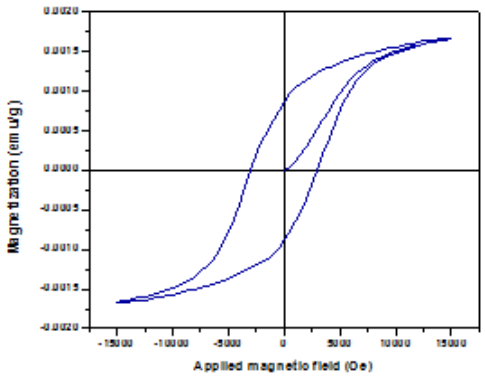

Fig. 6: M-H loop of Co-doped ZnS nanoparticles (a) 0.025 mol, (b) 0.05 mol and

Magnetic Properties

M-H curve for the Co doped ZnS at room temperature are shown in Fig.6 the magnetic parameters of Co doped ZnS such as saturation magnetization (Ms-0.16 emu/g) and coercivity (H_c-270 Oe) have been calculated from the hysteresis curve. Ferromagnetic behavior with notable magnetic moment was observed for all the samples of Co doped ZnS nanoparticles, which reflect the contribution of high magnetic moment for obtaining the ferromagnetic hysteresis [16]. Due to remarkable magnetic properties Co doped ZnS nanoparticles samples are further used anticancer applications.

IV. Discussion and Conclusion

Invitro cytotoxicity of on HT 29 cell line of Co doped ZnS nanoparticles

The *invitro* cytotoxicity of the chemical precipitatly synthesized Co doped ZnS nanoparticles was evaluated against the human colon cancer cell line (HT 29) at different concentrations. Fig.7. depict 73.64 % of cell death at maximum dosage level at the concentration of 200 µg/ml of Co doped ZnS nanoparticles. More than 55.12 % of cell death was observed at dosage level of 100 µg/ml concentration of Co doped ZnS nanoparticles[17] .

The morphological observation of control and different concentration of Co doped ZnS nanoparticles treated HT human colon cell lines are shown in Fig.8. The chemical precipitate synthesized Co doped ZnS nanoparticles *invitro* cytotoxicity analysis showed direct dose-response relationship with cytotoxicity increased at higher concentration[18]. Co doped ZnS NPs shows good activity against cancer cell line. AT the concentration of 100 ug of Co-ZnS NPs inhibit the 55.12 % of cell line..

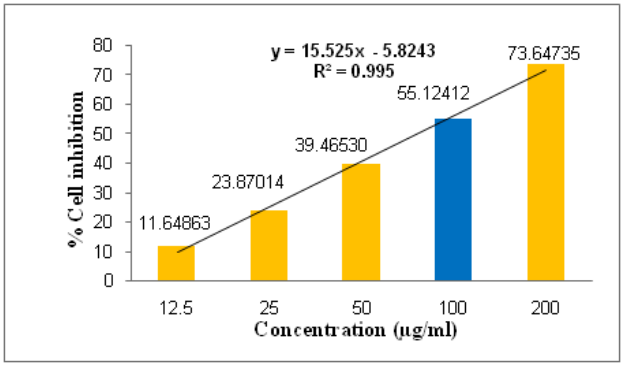

Fig .7: Effect of Co doped ZnS nanoparticles on HT 29 human colon cancer cell line

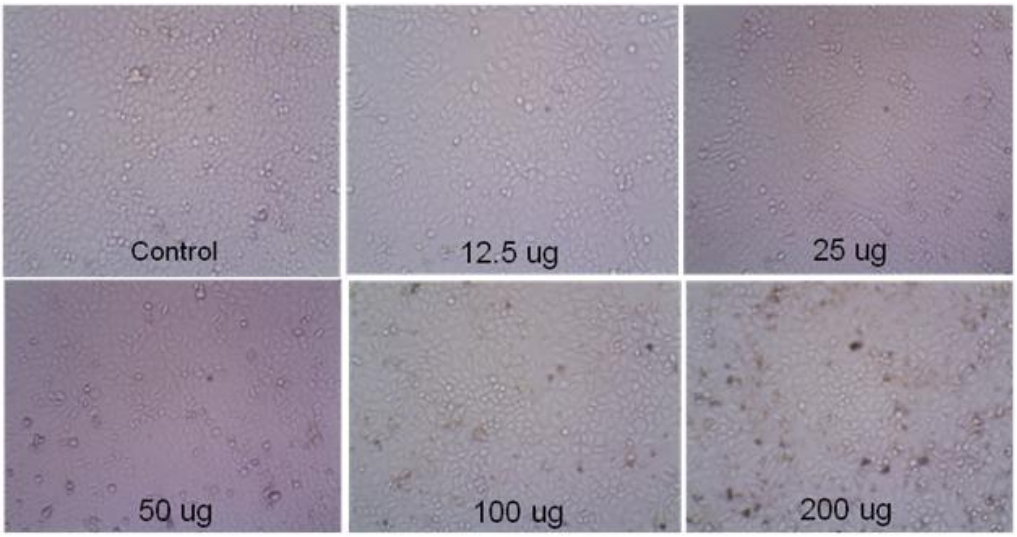

Fig. 8:Morphological observation of different concentration of Co doped ZnS nanoparticles treated HT 29 cell lines a) Control b) 12.5 µg/ml c) 25 µg/ml d) 50 µg/ml e) 100 µg/ml and f) 200 µg/ml.

XRD confirmed the zinc blende crystal structure. The average crystalline size around 2 nm. Presence of Co , Zn and S without impurity were confirmed using EDAX . The HRTEM indicated that Co doped ZnS were in narrow size distribution and confirmed that the particles were in nano regime. The SAED Pattern corresponds to reflections from three crystal planes, indicating (111), (220) and (311) and showed strong quantum confinement due to the small size of the particles. The ferromagnetism in the Co-doped ZnS samples may be a result of the exchange interaction between the Co ions on ZnS NPs. In present experiment, anticancer properties of the Co doped ZnS nanoparticles showed a direct dose response relationship and activity increased at higher concentrations level. Other reports suggest that nanoparticles are likely to interact with thiol-rich enzymes and it is possible that once penetrated into cells, nanoparticles may attack functional proteins of cells which results in partial unfolding and aggregation of proteins as it is the case in the bovine hemoglobin [19]. In this study exponentially grown of HT 29 colon cancer cell line were treated with various concentrations of Co doped ZnS nanoparticles and the percentage of inhibition were measured by the MTT assay. The significant inhibition by the nanoparticles against cell lines were observed in a dose depended manner. *Invirto* anticancer study on chemical precipitately synthesized Co doped ZnS nanoparticles against HT 29 colon cancer cell lines, it was observed that the nanoparticles possessed considerable concentration dependent inhibition and effective anticancer activity.

V. Acknowledgement

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VI. Conflict of the Interest

The author declare that they have no conflict of interest

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