*Sutapa Ganguly et al. / International Journal of Pharmacy & Therapeutics, 5*(3), 2014, 230-234.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

# STUDY OF ANTIMICROBIAL EFFECTS OF THE ANTICANCER DRUG OXALIPLATIN AND ITS INTERACTION WITH SYNTHESIZED ZNS NANOPARTICLES

**Sutapa Ganguly[1],Sukhen Das[1]*,Sujata Ghosh Dastidar[2]**

[1] Department of Physics , Jadavpur University, Kolkata- 700 032, India.
[2] Department of microbiology, Herbicure Healthcare Bio-Herbal Research Foundation,Kolkata- 700 104 , India.

**ABSTRACT**

The global problem of increasing drug resistance has extended the search of antimicrobial agents to a new class of compounds keeping aside the known antibiotics. The antimicrobial effects of the zinc sulphide (ZnS) nanocrystals and the anticarcinogenic agent oxaliplatin were studied by spot inoculation technique and also by well diffusion technique agains t twelve pathogenic bacterial strains. Nanoparticles of ZnS showed antimicrobial activity against both Gram positive and Gram negative strains except *Shigella sonnei*. The anticancer drug oxaliplatin was inhibitory for only Gram negative organism *Pseudomonas aeruginosa* ATCC 27853. Synergism between oxaliplatin and ZnS nanoparticles was distinctly observed case of Gram negative *Pseudomonas aeruginosa* ATCC 27853 strain.

**Key Words:-** zinc sulfide, nanoparticles, oxaliplatin, antimicrobial activity.

## INTRODUCTION

Antibiotic resistance is a form of drug resistance whereby some (or, less commonly, all) sub-populations of a microorganism, usually a bacterial species, are able to survive after exposure to one or more antibiotics. Pathogens resistant to multiple antibiotics are considered *multidrug resistant* (MDR) organisms (CDC Guideline, 2013).

Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century, particularly as it pertains to pathogenic organisms (the term is especially relevant to organisms which cause disease in humans). Some pathogens, such as *Pseudomonas aeruginosa*, also possess a high level of intrinsic resistance (CDC Guideline, 2006)

Corresponding Author

**Sukhen Das**
Email:- sukhendasjuphy@gmail.com

Over the past few decades, inorganic nanoparticles, whose structures exhibit significantly novel and distinct physical, chemical, and biological properties, and functionality due to their nanoscale size, have elicited much interest. Nanostructure materials are attracting a great deal of attention because of their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical applications (Nair S *et al*., 2009). In medicine, nanotechnology has been explored for early detection, diagnosis and treatment of diseases (Rema Devi BS *et al*., 2007).

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 (Sutapa G *et al*., 2013)

To combat the problem of drug resistance 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 (Sutapa G *et al*., 2013).

The search for antimicrobial agents has been extended to a new class of compounds known as non antibiotics.

Oxaliplatin is a platinum-based antineoplastic agent that is used in cancer chemotherapy and more commonly used as an advanced colorectal cancer treatment (Ehrsson H *et al*., 2002; Nail JW *et al*., 2010).
The compound features a square planar platinum (II) center. In contrast to cisplatin and carboplatin, oxaliplatin features the bidentate ligand 1,2-diaminocyclohexane in place of the two monodentate ammine ligands. It also features a bidentate oxalate group.

The cytotoxicity of platinum compounds is thought to result from inhibition of DNA synthesis in cancer cells. In vivo studies showed that Oxaliplatin has anti-tumor activity against colon carcinoma through its (non-targeted) cytotoxic effects (Graham J *et al*., 2004)
Oxaliplatin functions by forming both inter- and intra-strand cross links in DNA(Becouarn Y *et al*., 1998) Cross links in DNA prevent DNA replication and transcription, resulting in cell death.

In view of the information on presence of antibacterial action in nanoparticles of MgO, CaO and ZnO (Padmavati N & Vijayaraghavan R, 2008), nanoparticles of ZnS was prepared in our laboratory and was evaluated for the antimicrobial potentiality along with that of oxaliplatin singly and by combination of the two agents.

## MATERIALS & METHODS

***Drugs:*** The anticancer drug oxaliplatin was obtained as a pure dry powder from Dabur, 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 (1993) and preserved in freeze dried state.

***Chemical compounds:*** Analar zinc chloride (ZnCl2) and sodium sulphide (Na2S) 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 Zinc Sulphide nanoparticles

Synthesis of ZnS nanoparticles was carried out by aqueous chemical method using ZnCl2 and Na2S 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 low temperature and ambient conditions. In a typical preparation solution of 1M Na2S was added drop by drop to 1M $ZnCl_2$ solution which was kept on stirring using a magnetic stirrer at 70$^oC$ 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 ºC for 2 h (John R & Sasiflorence S, 2010).

### Preparation of zinc sulphide nanoparticle and oxaliplatin solution

To prepare ZnS nanoparticle solution 0.01g of the synthesized ZnS nanoparticles were dissolved in 10ml of sterile distilled water. The final concentration of ZnS nanoparticles in the solution was 1µg/ml. Aquous solution of oxaliplatin was prepared in a similar way.

### Characterization of ZnS nanoparticles

To investigate the morphological structure of sample surfaces, surface textures were examined by field emission scanning electron micrography (FESEM).

### Preparation of zinc sulphide nanoparticle solution

To prepare ZnS nanoparticle solution 0.01g of the synthesized ZnS nanoparticles were dissolved in 10ml of sterile distilled water. 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 oxaliplatin.

### *In vitro* tests for determination of Minimum Inhibitory Concentration (MIC) of oxaliplatin 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 oxaliplatin 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 oxaliplatin 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. The plates were incubated at 37$^{o}$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.

***Determination of antimicrobial action of ZnS and oxaliplatin 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 (1996). 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 (Dasgupta A *et al*., 2008).

**RESULTS**

**FESEM analysis and EDAX study**

Fig 1 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 and the anticancer drug oxaliplatin 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 100-150µg/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.

The anticancer drug oxaliplatin was found to possess antibacterial activity against 1 Gram negative bacterial strain out of 12 pathogenic bacteria. It was found that *P. aeruginosa* 27853 was completely inhibited by the drug at a concentration of 100µg/ml. Other strains were found to be resistant to the drug even at a concentration as high as 500µg/ml.

**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 for Gram positive organisms when the amount of ZnS was 100 ug per well .The diameters of inihibitory circles for Gram negative bacteria increased in size as the amount of ZnS was increased. The greater sensitivity of Gram positive organisms by ZnS was further confirmed by this test.

**Action of ZnS nanoparticles singly and combinedly with oxaliplatin by well diffusion**

oxaliplatin being inhibitory for only Gram negative bacteria was tested singly and combinedly with ZnS nanoparticles by well diffusion assay in the same plate. Singly 150 ug ZnS produced 9 mm wide zone of inihibition against *Pseudomonas aeruginosa* ATCC 27853 while the same due to 100 ug oxaliplatin was 8 mm. In combination the diameter became 11 mm wide.

**DISCUSSION**

The present study clearly indicates that ZnS nanostructures have been 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.

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

| Bacteria | Source | Minimum Inhibitory Concentration (MIC) | (µg/ml) |
|---|---|---|---|
| | | ZnS | oxaliplatin |
| *Bacillus subtilis* UC 564 | Upjohn Lab, USA | 100 | >500 |
| *S. aureus* NCTC 8531 | S.P.Lapage, London | 100 | >500 |
| *S. aureus* NCTC 8532 | S.P.Lapage, London | 100 | >500 |
| *E .coli* K12 row | J.D.Abott, U.K. | 200 | >500 |
| *E. coli* C600 | J.D.Abott, U.K. | 100 | >500 |
| *Shigella sonnei* NCTC 9774 | J.Taylor,London | >500 | >500 |
| *Shigella flexneri* 6 NCTC 396/3 | J.Taylor,London | 100 | >500 |
| *Salmonella enteritidis* NCTC 11 | J.Taylor,London | 200 | >500 |
| *Klebsiella pneumoneae* ATCC 10031 | Central Drugs Lab,Calcutta | 100 | >500 |
| *Acinetobacter baumani*i 462 | Dr.S.Das,Calcutta | 100 | >500 |
| *Vibrio cholerae* ATCC 14033 | S.Mukherjee,Calcutta | 200 | >500 |
| *Pseudomonas aeruginosa* ATCC 27853 | Central Drugs Lab,Calcutta | 100 | 100 |

**Fig 1. SEM image of ZnS Nanoparticles**

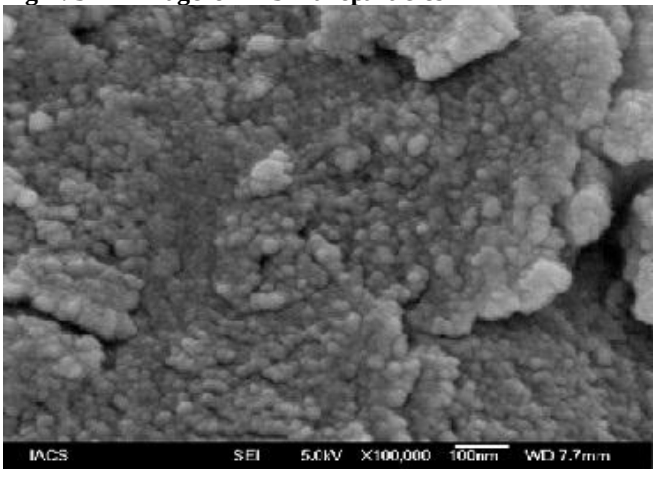
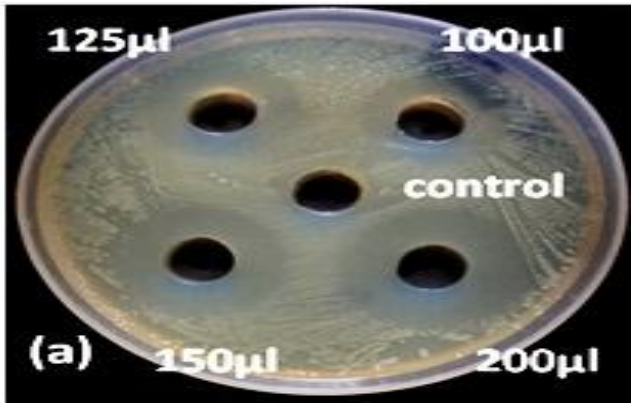



**Fig 2. Petriplate showing antimicrobial activity of ZnS NPs by well diffusion method against *P. aeruginosa* ATCC 27853 after 24 hrs of incubation**




Oxaliplatin was discovered in 1976 at Nagoya City University by Professor Yoshinori Kidani, who was granted U.S. Patent 4,169,846 in 1979. Oxaliplatin was subsequently in-licensed by Debiopharm and developed as an advanced colorectal cancer treatment. Debio licensed the drug to Sanofi-Aventis in 1994. Eloxatin gained European approval in 1996 (firstly in France) and approval by the U.S. Food and Drug Administration (FDA) in 2002.

In clinical studies, Oxaliplatin by itself has modest activity against advanced colorectal cancer (De Gramont A *et al*., 2000) It has been extensively studied in combination with Fluorouracil and Folinic Acid (a combination known as FOLFOX). When compared with fluorouracil and folinic acid administered according to the "De Gramont regimen" there was no significant increase in overall survival with the FOLFOX regimen (specifically, FOLFOX4), but progression-free survival, the primary end-point of the phase III randomized trial, was improved with FOLFOX (Pasetto LM *et al*., 2006).

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 JE, 1992; Dasgupta A *et al*., 2008; Jeyaseeli L *et al*., 2012). Our present study indicates the potential of oxaliplatin as a noteworthy antimicrobial agent since it has shown significant inhibitory effect against Gram negative pathogenic bacteria. Furthermore, the antimicrobial efficiency of oxaliplatin 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 oxaliplatin possesses potent antibacterial action, further studies are in progress to explore the possibility of their application in routine therapy against infections of animals.

**ACKNOWLEDGEMENTS**

The authors are grateful to UGC (University Grants Commission) Government of India, for financial support to carry out this study.

**REFERENCES**

Barrow GI & Feltham RK. A Cowan and Steels manual for the identification of medical Bacteria, Cambridge university press, Cambridge, UK, 1993, 329.

Becouarn Y, Ychou M, Ducreux M *et al*., Phase II trial of oxaliplatin as first-line chemotherapy in metastatic colorectal cancer patients. Digestive Group of French Federation of Cancer Centers. *J Clin Oncol*, 16(8), 1998, 2739–2744.

CDC Guideline. Antibiotic Resistance Questions & Answers. Get Smart: Know When Antibiotics Work.Centers for Disease Control and Prevention, USA. 30, 2013.

CDC Guideline. Management of Multidrug-Resistant Organisms in Healthcare Settings. 2006.

Dasgupta A, Dastidar SG, Shiratki Y, Motohashi N. Antibacterial activity of artificial phenothiazines and isoflavones from plants. *Bioactive Heterocycles VI Topics in Heterocyclic Chemistry*, 15, 2008, 67-132.

Dasgupta A, Dastidar SG, Shiratki Y, Motohashi N., Antibacterial activity of artificial phenothiazines and isoflavones from plants. *In: Bioactive Heterocycles VI*, 15, 2008, 67-132.

De Gramont A, Figer A, Seymour M *et al*., Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer". *J Clin Oncol*, 18(16), 2000, 2938–2947.

Ehrsson H, Wallin I, Yachnin J. Pharmacokinetics of oxaliplatin in humans. *MedicalOncology*, 19(4), 2002, 261-265.

Graham J, Mushin M, Kirkpatrick P. Oxaliplatin. *Nature Reviews Drug Discovery*, 3(1), 2004, 11–12.

Jeyaseeli L, Dasgupta A, Dastidar SG, Molnar J *et al*., Evidence of significant synergism between antibiotics and the antipsychotic, antimicrobial drug flupenthixol. *Eur. J. Clin. Microbiol. Infect. Dis*, 3, 2012, 1243-1250

John R & Sasiflorence S. Optical, structural and morphological studies of bean like ZnS nanostructures by aqueous chemical method. *Chalcogenide Let*, 7, 2010, 269.

Kristiansen JE. The antimicrobial activity of non antibiotics, Report from a congress on the antimicrobial effect of drugs other than antibiotics on bacteria on bacteria, viruses, protozoa, and other organisms. *APMIS*, 30, 1992, 7-14.

Miles & Amyes. Laboratory control of antimicrobial therapy, 1996, 225.

Nair S, Sashidharan A, Divyarani VV, Menon D, Nair S, Manzoor K, Raina S, Role of size scale of ZnO nanoparticles and microparticles on toxicity towards bacteria and osteoblast cancer cells. *J.Mat.Sc:Mater. Med*, 20, 2009, 235.

Nial JW, Shonagh W, Gemma EC and Rabbab O. The status of platinum anticancer drugs in the clinic and in clinical trials. *Dalton Transactions*, 39, 2010, 8113-8127

Padmavati N & Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles-an antimicrobial study. *Sci.Technol.Adv.Mater*, 9, 2008, 1468.

Pasetto LM, D'Andrea MR, Rossi E, Monfardini S. Oxaliplatin-related neurotoxicity: how and why?" *Crit. Rev. Oncol. Hematol*, 59 (2), 2006, 159–168.

Rema Devi BS, Raveendran R, Vaidyan AV. Synthesis and characterization of Mn+2 doped ZnS nanoparticles. *Pramana-J.Phy*, 68, 2007, 679.

Sutapa G, Parveen S, Sukhen D, Sujata GD. Characterization of newly synthesized ZnS NPs by a simple aqueous chemical method and determination of its antimicrobial activity against various pathogenic bacterial strains. *International Journal of Biological & Pharmaceutical Research*, 4(5), 2013, 377-381.

Sutapa G, Subhajit G, Sukhen D, Sujata GD. Evaluation of antimicrobial effects of the synthesized ZnS NPs and their potentiation by the anticancer drug imatinib. *International Journal of Phytopharmacology*, 4(4), 2013, 266-271.