EVALUATION OF ANTIBACTERIAL EFFICACY OF ZINC PHENOTHIAZINE OLEATE

Neha Mathur[1] & Biplab Manna[1]* & Preeti Sharma[2] & Aksha Sharma[2]
[1] Department of Chemistry, PNKS Govt. Science and Commerce College, Dausa, Rajasthan, India.
[2] Department of Zoology, Stani Memorial PG College, Jaipur, Rajasthan, India.

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ABSTRACT *Metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry. Tricyclic and bicyclic aromatic compounds like phenothiazine and its derivatives are found to be useful as drugs, industrial antioxidants, thermal stabilizer, pesticides, as dyes and pigments. In the present study, a new complex of Zn oleate with phenothiazine has been prepared and complexation has been proved by spectral studies.The antibacterial activities of this eco-friendly complex have been studied against E. coli, Bacillus, Staphylococcus and Streptomycesby using agar well diffusion-method. It is found to be an active antibacterial complex.*

Keywords: *Phenothiazine; Pestisides; Zinc oleate; antibacterial activity.*

INTRODUCTION

Heterocyclic nucleus such as indole [1], carbazole, thiazole [2], phenothiazine[3], imidazole etc. is a part of most of the medically important compounds.Among these, phenothiazine nucleus has been found to be applicable in drug development for the treatmentof cancer[4], various microbial infections[5]and inhibits intracellular replication of HIV[6,7].

Zinc is a vital element for normal functioning of most creatures[8,9]. It functions as an antioxidant and is involved in many serious biochemical reactions[10]. The Zn (II) ions have a high affinity towards oxygen, nitrogen and sulphur donor ligands[11,12].It is the second most abundant transition metal ion in human body after iron and has mostlybeen used as a medicine for growth disorders due toundernourishment[13,14]. Some complexes have attracted special attention as classicalcompounds for the active sites of compounds containing zinc as enzymes[15].

In this study, the antibacterial character of zinc phenothiazine complex by agar well diffusion method was premediated. It is most commonly used method to study*in vitro* antibacterial activity of metal complexes [16,17]. In this method,the agar plate surface is injected by spreading a volume of the microbial inoculum over the total agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume(20–100 mL) of the zinc phenothiazine complexsolution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism [18]. The zinc phenothiazine complex solution diffuses in the agar medium and inhibits the growth of the microbial strain tested. Results of antibacterial testing is subjected to statistical measurement by using ANOVA technique.

MATERIAL AND METHODS:

2-bromo-7-nitrophenothiazine has been prepared as ligand species by using Smiles Rearrangement procedure [19,20] in whichnitro substituted 2-aminobenzenethiol was prepared and then reactedwith meta-dibromo nitrobenzene in alkaline medium.

For preparation of Zn metal complex, zinc oleate soap is reacted with 2-bromo-7-nitrophenothiaze ligand.

Preparation of Substituted phenothiazine: To prepare bromo substituted phenothiazine at first, nitro substituted benzenethiol was synthesized and reacted with meta dibromo nitrobenzene in alkaline medium. In present study, 3.32 g (0.01 mole) of benzenethiol, 0.4 g (0.01 mole) sodium hydroxide and 2.02 g (0.01 mole) bromonitrobenzene in absolute alcohol (25 mL) was used and this mixture was refluxed for 1-2 hours. Then the mixture was cooled and filtered. Residue was washed with hot water and 80% ethanol and then crystallized from acetone.

Preparation of Zinc surfactant: Zinc oleate was prepared by mixing one gram of oleic acid into 25 mL ethyl alcohol, the mixture was shaked in hot water-bath at 50°C and then one drop of phenolphthalein was added. Saturated solution of KOH was prepared in another beaker and added into the oleic acid solution drop by

drop until the appearance of light pink colour. Then a saturated solution of $NiCl_2$ (about 3-4 g in 5 mL of water) was prepared in another beaker and was mixed into the above solution with constant stirring till a coloured soap (whitecolour for Zincoleate soap) is formed which is then filtered and washed with warm water and 10% ethyl alcohol, dried and recrystallized with hot benzene.

Preparation of Zinc complex with 2-bromo-7-nitrophenothiazine: The complex of zinc oleate and phenothiazine was prepared by adding 0.621 g (0.001 mole) zinc oleate with 0.646 g (0.002 mole) substituted phenothiazine in 25-30 mL ethyl alcohol and the mixture was refluxed for about two hours with constant stirring. After cooling, the solid separated out was filtered, dried and recrystallizedwith hot benzene.

Antimicrobial Studies: Chemically derived complex of zinc oleate with substituted phenothiazine was being subjected to antibacterial studies. Four bacterial strains were selected for this purpose.

Micro-organisms used: Pure cultures of *Staphylococus*, *Streptomyces*, *E. coli*, *Bacillus* for antibacterial activity obtained from S.M.S. Medical College, Jaipur, India, were used as indicator organisms. Each culture was further maintained on the same medium after every 48 hours of transferring. A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

Determination of Antibacterial Assay:*In vitro* antibacterial activity of the zinc complex wasstudied against gram positive and gram-negative bacterial strains by the agar well diffusion method [21]. Mueller Hinton agar no.2 (Hi Media, India) was used as the bacteriological medium. Theextracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentration of 5 mg/ml. TheMueller Hinton agar was melted and cooled to 48-50ºC and a standardized inoculum (1.5×108 CFUmL-1, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petridishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100µL) was introduced in the well (6 mm). The plates were incubated overnight at 37^0C. Theantimicrobial spectrum of the extract was determined for the bacterial species in terms of zone of inhibitionaround each well. The diameters of zone of inhibition produced by the agent were compared withthose produced by the commercial control antibiotics i.e. streptomycin. For each bacterial strain controlswere maintained where pure solvents were used instead of the extract. The control zones weresubtracted from the test zones and diameter of the resulting zone was measured with antibiotic zonereader to nearest mm. The experiment was performed three times to minimize the error and the meanvalues were calculated.

RESULTS AND DISCUSSION

The synthesised complex was dark coloured and solid in nature whichwas very much stable at room temperature.It was insoluble in water, moderately soluble in organic solvents like ethanol, benzene etc. while highly soluble in binary solvent mixture.

IR Spectra: The IR spectra of complex provide valuable information about complex and ligand [22,23].Various frequenciesranging in 2931-2850 cm^{-1} were observed which showed the presence of $-CH_2$ and $-CH_3$ groups of soap segment present in complex.Due to the presence of $-CH_2$ and $-CH_3$ groupsνfrequency were also observed at 1108 cm^{-1} and 725 cm^{-1} which were due to C-H rocking vibrations. Frequencies in range of 1460-1470 cm^{-1} shows the presence of fatty acid group of metal soap in complex.
Skeletal bands between 1600 to 1430 cm^{-1} represent heteroaromatic ring system present in the complex.The ν(Ar-N) frequency for the complexes are observed around 1300-1340 cm^{-1}which is lower than that of free ligand and this evidence supports the coordination of phenothiazine nitrogen in the complexation. From IR spectral data, it is evident that ligand act as a monodented, bonded to metal ion (Zn) with secondary nitrogen atom of NH. The band near to 1560 cm^{-1}shows characteristic signal of N-H bending vibration. Compare to the N-H group in the free ligand, this signal is shifted to lower frequency i.e. 1543-1539 $cm^{-1}$for the complex, indicate that the secondary nitrogen is the coordinating site in the complex.

NMR Spectra: By using NMR spectra, complex and free ligand have been compared to determine bonding [24]. Signal 7.2δto 6.8δ represents the presence of aromatic group in complex. Peak at 8.18δindicates the presence of an -I group (here $-NO_2$) in the complex. A peak at 3.6δshows the presence of secondary -N-H group in phenothiazine complex. It indicates the coordination take place threw this secondary amine withzinc ionof soap.The signal was shifted significantly downfield due to deshielding effect employed by zinc metal ion.

Antibacterial Analysis: To investigate antibacterial properties of zinc phenothiazine complex well diffusion method was used and *ciprofloxacin* was used as standard compound for reference.

Table 1. Antibacterial effect of Zn complex

Complex (in mg ml^{-1})	Standard (in mm)	*Staphylococcus* (in mm)	*Streptomyces* (in mm)	*E-coli* (in mm)	*Bacillus* (in mm)
20	20	--	--	10	--
40	20	12	11	15	--
60	20	07	12	13	--
80	20	11	10	15	12

Newly synthesized complex of zinc phenothiazine complex is very much active on bacterial strain. It is found to be most active for *E. coli* (Table 1).

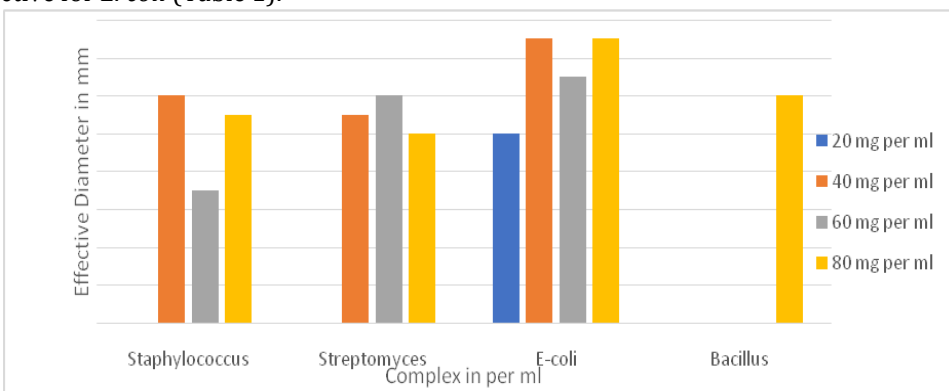

Fig. I: Antibacterial activity of Phenothiazine Zn Complex

Statistical Analysis:

In this study, effects of Zn-complex on different bacterial strains by using one-way ANOVA technique [25]. Hypothesis of activity of different bacterial strains was as follows:

H0: μM1 = μM2 = μM3 = μM4; there is no significant difference between the effects of all the four bacteria on the activity index of the test compound.

H1: at least two of the mean differ; there is significant difference between effects of different bacterial strains on the activity index of the test compound.

The above hypothesis was tested by using one-way ANOVA technique at 5% level to find out any significant difference between the activities of test complex on different bacteria. Following ANOVA table is obtained for bacterial activity on complex:

Source of variation	Ss	Df	Ms	F-Ratio
Between samples	SSB = 19.7956	k-1 = 3	MSW = 4.6786	F = MSB/MSW =1.4104
Within Samples	SSW = 32.75	N-k = 7	MSB = 6.5985	
Total	SST = 52.5456	N -1 = 10		

(SSB = Sum of square between, SSW = Sum of square within, SST = Sum of square total, MSB and MSW represents mean of square between and within)

Here α = 0.05, so critical $F_{0.05;3,7}$ = 4.35 (from F- table), while F ratio is 1.4104 which is less than critical value, thus null hypothesis cannot be reject H_0. Hence, activities of complex on different bacteria are significant morethan 0.05.

CONCLUSION

A new complex of zinc oleate with 2-bromo-7-nitrophenothiazine was prepared and complexation was studied by spectroscopic methods. It reveals that secondary amine present in phenothiazine take part in bonding for complexation. Antibacterial potential of this new complex was also evaluated by using agar well diffusion method which revealed that complex possess antibacterial properties and is most active for *E. coli*, while least active for *Bacillus.*

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