Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(2):21-26

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Polarographic determination and antifungal activity of Cu (II) complex with 3-hydroxy-3-m-tolyl-1-p-sulphonato(sodium salt) phenyltriazene

Pooja Joshi, Neelam Pareek, Rehana Khanam, Dipen Upadhyay, Amit Bhandari, R. S. Chauhan and A. K Goswami*

Department of Chemistry, M.L Sukhadia University.Udaipur(Raj.)

ABSTRACT

The electrochemical behavior of complex of Cu (II) with 3- hydroxy-3-m-tolyl-1-p-sulphonato (sodium salt) phenyltriazene (HTST) was studied . It was observed that HTST forms 1:3 complex with Cu(II) in between pH 6.5 to 7.1 . It was found that the reduction process of Cu(II)- HTST complex is two electron reversible reduction process.The logarithm value of stability constant of 1:3 Cu(II)-3-hydroxy-3-m-tolyl-1-p-sulphonato(sodium salt) phenyltriazene complex is 13.02. The present paper also reports antifungal activity of hydroxy-3-m-tolyl-1-p-sulphonato(sodium salt) phenyltriazene, and its Cu (II) complex against Rhizoctonia solani, the fungi causing disease in fennel. The result has been expressed as percentage inhibition (PI) of mycelial growth as well as sclerotium formation at 500 ppm for both ligand as well as complex. The result shows that PI value in complex is 88.03 whereas for ligand 78.63. Thus it is clear that complex is better in activity against the fungi compared to its respective ligand. The result has been interpreted in terms of theory of drug action proposed by Paton.

Keywords: Hyroxytriazene, Polarography, HTST-Cu (II) complex, Stability constant, Rhizoctonia solani.

INTRODUCTION

Hydroxytriazenes are well established chelating agents as revealed by reviews appearing on them during last few years[1-4].These compounds have been used as spectrophotometric and complexometric reagents for determination of transition and non-transition elements[5-7] . In the

present work complex formation of Cu (II) with HTST at D.M.E in aqueous and alcoholic medium has been studied polarographically. Overall stability constant of Cu (II)-HTST has been determined. Hydroxytriazenes and their transition metal complexes have shown excellent biological activities in recent years[8-11] . The present work has been centered upon study of linear antifungal activity against *Rhizoctonia solani* (fennel) which is an important crop in India. The study has been extended to compare action of ligand v/s there Cu (II) complex and thus an overall mechanism of drug action has been attempted.

EXPERIMENTAL SECTION

Synthesis of hydroxytriazene 3-hydroxy-3-m-tolyl-1-p-sulphonato (sodium salt) phenyltriazene (HTST)

(i) Synthesis of m-tolyl-hydroxylamine: In a one litre beaker (0.1mol) of m-nitrotoluene, 5 gm of NH_4Cl 50 ml water and 50 ml C_2H_5OH were mixed, stirred mechanically and cooled to 0° C by surrounding the beaker with ice salt mixture, 20 gm Zn dust was added in small lots such that the temperature of reaction mixture remained between 0 to 5° C. Addition of Zn dust was completed in 40 min. The reaction mixture was stirred mechanically for another 15 min. The solution was filtered under suction and washed with ice cold water. The filtrate was taken in a beaker and kept in freezer and used as such for coupling with diazotized product.

(ii) Diazonium salt of sulphanilic acid: In a 500 ml beaker 0.1 mol of sulphanilic acid was dissolved in warm mixture of 25 mL of concentrated HCl and 25 mL of water. After constant stirring the mixture was kept in a freezer to cool.In another beaker 6.9g of $NaNO_2$ was dissolved in 20 mL of distilled water and kept it in the freezer. The beaker which contained sulphanilic acid solution was put in an ice bath to maintain temperature between 0 to 5º C. To this The $NaNO_2$ solution was added drop by drop with continuous stirring. The diazotized product so obtained was directly used for coupling.

(iii) Coupling of m-tolyl-hydroxylamine with diazonium salt of sulphanilic acid: The m-tolylhydroxylamine prepared in step a was coupled with the diazotized product of b step at 0 to 5°C under mechanical stirring with occasional addition of sodium acetate solution for maintaining the pH close to 5 during coupling process. The compound was obtained as yellowish fluffy powder after crystallization from ethanol.

Melting points of all synthesized compounds were taken in open capillaries and are uncorrected. C H N analysis corroborated the purity of compound. Further the compound was subjected to IR spectral analysis and following bands were observed :

IR (KBr) cm^{-1}: 3249 (O-H str.), 3078 (C-H str. Ar), 2981 (C-H str., CH_3), 1632 (N=N str.), 1419 (N-N str.).The spectra showed the compound to be in pure state. IR spectra (KBr) were recorded on FT IR RX1 Perkin Elmer Spectrometer. A Systronics Polarograph 1632 was used for obtaining current voltage curves. Physical and analytical data are given in **Table I.**

Table-I Elemental analysis of 3-hydroxy-3-m-tolyl-1-p-sulphonato (sodium salt) Phenyltriazene

Molecular formula	Melting point		%C	%N	%H
(C12H10N3O4.S.Na) H2O	180° C (d)	Th.	43.2	12.6	3.6
		Exp.	42.4	12.4	3.6

Polarographic study of Cu(II)-HTST complex :

A systronics polarograph 1632 was used for obtaining current voltage curves. Metal solution (1mM) was prepared using $CuSO_4$.$5H_2O$ and ligand solution was prepared by dissolving requisite quantity of HTST(.01 M) in double distilled water. Citric acid and Na_2HPO4 solution were used as buffer to maintain pH.Ionic strength was kept constant by using KCl as supporting electrolyte, gelatin (.002%) was use as maximum suppressor. The capillary had following characteristics t=1 drop/sec .IR drop correction were applied.

The polarographic study of Cu(II)-HTST has been done at D.M.E in aqueous medium. Solution was deareated by purging of oxygen free nitrogen through the polarographic cell.

Determination of half wave potential of Cu(II) with HTST :

A 1×10^{-3} M Cu(II) solution in N/10 KCl has been used to obtain polarograms of Cu(II).This showed an $E_{1/2}$ at 0.25 vs SCE. polarographic study was done on Cu(II) with various concentration of HTST. The polarogram showed the half wave potentials shifted towards more negative value with increasing concentration of ligand indicating complex formation and the diffusion current was found to decrease regularly with increase of HTST concentration.

Bioassay

Following method was used to determine the antifungal activity. For fungal bio-assay Potato Dextrose Agar (PDA) medium was used.

(a) Cleaning and Sterilization: Corning and Borosil glass wares were used for the experiment. Cleaning was done by dilute chromic acid followed by Teepol. Glasswares were thoroughly washed with distilled water and dried before being autoclaved in hot oven at 180º C for 2 hrs. Throughout the experiment the media (PDA) was sterilized by autoclaving at pressure of 1.045 kg/cm^2 for 20 minutes. Further, polyethylene bags were sterilized using 5% formalin.

(b) Isolation of the Fungi: The fungi were isolated from fennel (*Foneculum vulagare* mills) seeds using Standard Blotter and Agar Plate Method. Fungal purifications were done by using single spore and hyphal tip method.

(c) Pathogenicity test: Pathogenicity was tested using seed inoculation and seed rolled method. In the first method fennel seeds were inoculated with 7 days old culture of the test fungi. These seeds were placed on three layered moist blotter paper. However, in seed rolled method seeds were rolled with 7 days old culture of test fungi and then they were sown in 30 cm earthen pots filled with sterilized soil, observation in both the cases were recorded after 21 days of sowing, it was observed that symptoms in case of *R. Solani* were observed on the roots of the plant. Resolution was made and culture was compared with original culture. These pathogenic fungi were used for further experimentation.

RESULTS AND DISCUSSION

A single well defined wave was obtained for Cu(II)-HTST system between pH 6.5-7.1. Diffusion controlled nature of each wave was verified from id vs C and id vs √h plots where id =diffusion current in µA; C=conc. In m mole lit^{1-}, h=height of mercury column.

Slope of the linear plots of log (i/id-i) vs E_{de} was found to be in the range of 30-32 mV, thereby showing the reversible nature of reduction process involving two electrons. The plot of half wave potential $E_{1/2}$ vs log Cx (where Cx = concentration of complex in m mole lit^{1-}) have been found to be a straight line showing the formation of most stable complex.

The coordination no. (j) of the metal complex is obtained from the slope of this plot, as may be expressed by:

$d(E_{1/2})/d \log Cx = -j.0591/n$

where n = no. of electrons involved (here n =2).The value of j was found to be 6. This shows that composition of the complex is 1:3 (metal: ligand). The tentative structure of complex of Cu (II)-3-hydroxy-3-m-tolyl-1-p-sulphonato(sodium salt) phenyltriazene has given in **Figure (i)**.

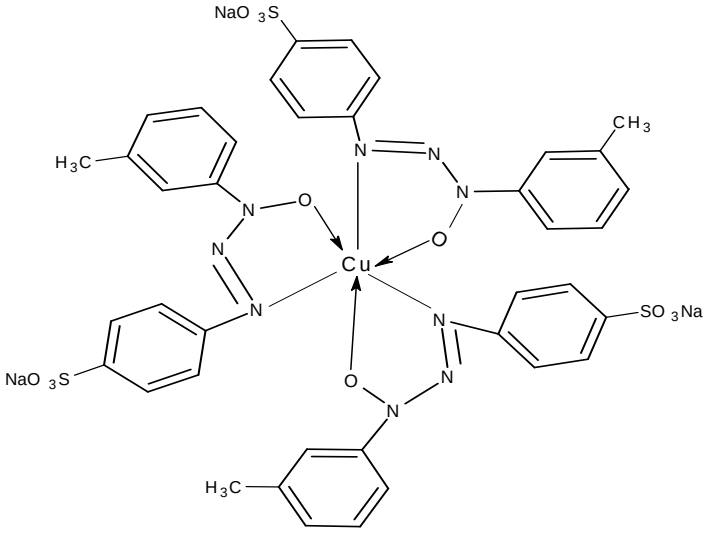

Figure (i) The tentative structure of 1:3 complex of Cu (II)-3-hydroxy-3-m-tolyl-1-p-sulphonato(sodium salt) phenyltriazene.

Determination of stability constant Cu(II)-HTST complex:

The stability constant of the Cu(II)-HTST complex has been determined by classical method of Lingane[12], as the method is applicable for maximum coordination number and for the stability constant of highest complex formed. The $E_{1/2}$ has a linear correlation with ligand concentration; which shows that there is only one complex formed. The following equation has been used to calculate the stability constant of the complex studied.

$\Delta (E_{1/2}) = 0.0591/n \log \beta + j\ 0.0591/n \log Cx$

Here, $\Delta (E_{1/2})$ =Difference of half wave potentials of simple metal ion and complexed ion, n =number of transferred electron, log β = Stability constant of complex formed, j = Coordination number, Cx = concentration of ligand.

Thus the value of log β has been found to be 13.02. Polarographic data of Cu (II)- 3-hydroxy-3-m-tolyl-1-p-sulphonato (sodium salt) phenyltriazene are given in **Table(ii).**

Table-(ii) Polarographic characteristics of Cu(II)-3-hydroxy-3-m-tolyl-1-p-sulphonato (sodium salt) phenyltriazene

S.No	Cx	Log Cx	$E_{1/2}$	Log β
1	0.00	0.00	0.250	-
2	0.01	-2	0.300	13.69
3	0.015	-1.8239	0.320	13.28
4	0.020	-1.6987	0.335	13.05
5	0.025	-1.6020	0.350	12.98
6	0.030	-1.5228	0.360	12.92
7	0.035	-1.4559	0.365	12.59
8	0.04	-1.3979	0.385	12.90
9	0.045	-1.3467	0.395	12.77

Results of antifungal activities of both ligands (Hydroxytriazenes) and their complexes have been incorporated in **table-III**.

Table-III Comparison of Test Compound (ligand against copper (II) complex) for the antifungal activities at 500 ppm for *Rhizoctonia Solani*

S. No.	Coding of Compound	Mycelial growth in diameter mm.	Mycelial growth (PI)	Sclerotium formation
1	HTST	18.33	78.63.	0
2	Control	90.00	0.00	+++
3	Cu(II)-HTST	11.67	88.03	0 (Nil)
4	Control	90.00	0.00	+++ (Good)

Comparison of Antifungal activity in ligand Vs complex:

In the screening of antifungal activity it has been envisaged that the studies must lead to an overall comparison of activities between the ligand and its metal complex so that the mechanism of the activity can be understood. To do this the present investigation was planned and solid Cu (II) complex of hydroxytriazene was screened which had shown antifungal activity. Table III includes results of comparative antifungal activity of ligand v/s Cu (II) complex. An examination of the results reveals that in the complex has shown better P.I. value than the ligand.

Mode of Action:

Although with the available information it is difficult to predict the mode of action of hydroxytriazene and their solid complex, unless the identification of active site where these compounds act is done. However, a rough guideline can be drawn on the basis of theory of drug action proposed by Paton. The theory proposes that a stable metal chelate may exert inhibitory effect on an intracellular biological process by adequately concentrating at a particular

susceptible site from which it dissociate slowly. Now if the dissociation of chelate from the biological site is at sufficiently slow rate then the inhibition induced by the metal chelates would be irreversible. If this site is by any means essential to the cell for either transport, respiration energy production or protein synthesis the resulting permanent dysfunction of any biochemical mechanism associated with the site would lead to the death of the cell. Alternatively this may also lead to the development of an alternative of biochemical mechanism for compensation of those, irreversibly impaired by metal chelate. Thus in present study it seems that the ligand although is active against the fungi it inhibit effectively as chelates via mechanism given above. This is a rough outline and definite mechanism can only be proposed when sufficient number of compounds is tested. However, this is certain that hydroxytriazene as well as their metal chelate can offer this novel application if sufficient studies are centered on their biological activities. The present work is a step further in this direction.

CONCLUSION

The present work has opened up possibility of studying Cu(II)-HTST complexes by D.C polarographic method. Stability constant (log β) was obtained with polarography .This proves the validity of polarographic techniques for studies of hydroxytriazenes metal complexes. Results of comparative antifungal activity of ligand v/s Cu (II) complex shows that the PI value of ligand is 78.63 whereas for metal chelates 88.03. Thus it is clear that complex is better in activity against the fungi compared to their respective ligand.

REFERENCES

[1] DN Purohit, *Talanta* , **1967**, 14, 207**,**
[2] D Chakrovorty: AK Majumdar: *J. Indian Chem.Soc*, **1977**, 54, 258
[3] RL Dutta : RS Sharma *J.Sci.Industr.Res.India.*, **1981,** 40, 715.
[4] DN Purohit: Nizammudin : A.M.Golwalkar, *Revs. Anal. Chem.* **1985,** 8, 76.
[5] DN Purohit: MP Tyagi; R Bhatnagar; IR Bishnoi, *Revs. Anal. Chem.***1992**, 11, 269.
[6] DK Gorgi : RS Chauhan ; AK Goswami DN Purohit, *Revs. Anal. Chem.,* **1998**, 17(4), 223,
[7] S Kumar ; AK Goswami : DN Purohit, *Revs. Anal. Chem.***2003**,22, 1,
[8] AK Goswami; DN Purohit *Analytical Science.* **2001**,17, i 789.
[9] IS Hura: N Naulakha: AK Goswami: MK Shrivastava. *Indian J. of Microbiology.* **2003**, 43 (4), 275.
[10] LS Chauhan: CP Jain: RS Chauhan: AK Goswami *Adv. Pharmacol. Toxicol,* **2006** 7(3), 73
[11] R Khanam: JC Sharma; S Khan: R Dashora , RS Chauhan : AK Goswami *.Int. J. of Pharm. Sci. and drug Research,* **2010**, 2(1),43
[12] J Lingane , *J.Chem.Rev.***1941**, 24**,** 1.