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Biological Studies of Some Transition Metal Complexes

Abstract

In recent decades the co-ordination chemists have explored numerous approaches for finding out and developing organic liquids and metal chelates. It is a fact that a number of biologically important compounds are chelates and they have opened many approaches to chemotherapy. The ligand (HQ, HQS, en, pn, TRA, CAT, and CTA) and their complexes were screened for their antifungal and antibacterial activities in the present study. The antifungal activity of the ligand and complexes has been conducted on the fungi and bacteria by agar diffusion method. Metal complexes and ligand displayed acceptable microbial activity against both types of bacteria.

Keywords: Biological Activity, Transition Metal Complexes, MIC.

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Introduction

A number of naturally occurring chelates are present in biological systems mainly of trace metals such as copper, cobalt, zinc, nickel etc. with amino acids, proteins and other related compounds[01]. It is used as an antioxidant for stabilization of drugs which rapidly detoriate in the presence of trace metals and as an antidote for heavy metals (Pb, Cu,Cr,Fe and Ni). The chelation may be used for diversified purposes including- Sequestration of metals to control the concentration of metal ion, Stabilization of drugs, Elimination of toxic metals from intact organism, Improvement of metal adsorption, Increasing the toxic effects of a metal. Albert at al [02,03] described the structural activity relationship of chelates as biocidal agents. When 8-hydroxyquinoline[04-06] is coordinated with suitable metal ion the mixed ligand complexes acts as antimicrobial agent. S, N and O- donor ligands also find application as antifungal[07], antihelmintics[08], antiemetics[09], antipyretic[10], analgesic[11]and diuretic[12] activity. Pharmacological significance of some transition metal complexes has been reported in the literature[13-15]. 8- hydroxyl quinoline was observed to precipitate a number of heavy metals under physiological conditions of temperature and pH therefore, it was suggested that the antifungal and antibacterial properties of oxime may be due to the removal of trace metals essential for the metabolism of the organism, it was advised that the site of action of oxime and its analogous is inside the bacterial cell or may be on cell surface[01,03]. It was observed that all biological active compounds may from metal chelates but not all the chelated biologically active[01].

Metal complexes of oxine, substituted oxines, 1,10-phenenthroline, 2,2’-bipyridyl, anthroquinone, phenols, substituted phenolic compounds and other ligands have been found highly biologically active. It indicates that nitrogen containing heterocyclic and hydroxyl compounds possess an increased activity on being coordinated with metal ion. It was therefore considered worthwhile to isolate some mixed ligand complexes of transition metals with 8-hydroxyquinoline and other bifunctional ligands like ethylene diamine, 1,2-diaminopropane, catechol, tiron, chromatropic acid. Biocidal activity of these isolated mixed ligand complexes in comparison to the involved ligands and the metal ion has been studied against some available pathogenic micro organism. The previous studies pointed out that some complexes can be used in the treatment of diseases, such as copper (II) complexes which are used in the treatment of rheumatoid arthritis and as antiulcer agents[16]. Copper plays a very important role in the treatment and prevention of gastrointestinal damage of acidic anti- inflammatory agents[17]. It is obvious that not only copper has medical applications, but also many complexes have medical applications, some of them being used as model molecules for biological oxygen carrier systems[18] and some having applications in the fields of analyticalchemistry[19]. The coordination of Schiff base ligands with many transition metal ions gives stable and coloured complexes with interesting properties, chemical and physical, as

well as beneficial biological activities [20]. The coordination of ligands with metal ions often enhances the activities[21] as has been reported for pathogenic fungi[22].

In the present paper the biological studies of the complexes were conducted using Gram- positive and Gram- negative organisms. The antibacterial behaviour was calculated by evaluating the inhibition region (mm) and minimum inhibitory concentration (MIC50).

Materials and Methods

Among the available methods [23-28] for determining antimicrobial sensitivity of compounds the methods are generally used involving the same principle namely the preparation of a concentration gradient of the test compounds in a nutrient medium which is seeded with the indicated micro organism, incubated and the observation on the growth taken place in terms of concentration of the compound. Out of them Broth serial dilution method is considered best suitable and accurate on providing constant conditions and proper controls and therefore during the investigation[25,26,28] has been used for determining the minimum inhibitory concentration of some isolated ternary complexes in comparison to the involved ligands and the metal ion.

Broth Serial Dilution Method (Tube Method)

In this method following steps[25, 26, 28] have been taken using the asceptic technique[29].

Culture Media Preparation

This is essential to obtain a culture by growing the micro organism in a suitable artificial culture media having necessary nutrients growth promoting factors and free from other containing micro organism at the suitable temperature, period and proper pH in asceptic conditions.

During the course of present investigation following culture mediums (medium-1 and medium-2)[25] have been used for cultivating the bacteria and fungi as a broth or agar slant and for conduct of the test.

1. Culture media(medium no-1) for the cultivation of bacteria: Peptone 0.60%; Pancreatic digest 0.40%; Yeast extract 0.30%; Beat extract 0.15%; Dextrose 0.10%; Agar-Agar (Only for agar slant preparation) 1.50%; Water to make the total volume 100 ml; pH adjusted to 6.5-6.6
2. Culture media (medium no 2) for the cultivation of fungi: Peptone 1.00%; Dextrose 2.00%; Agar-Agar (Only for agar slant preparation) 2.50%; Water to make the total volume 100 ml; pH adjusted to 5.40

The basic ingredients of each of the above mentioned medium were weighed and dissolved in a given volume of distilled and sterilized water in a sterilized large flask. The mixture was heated over a water bath to hasten the solution of the ingredients in water after the solution was effected the flask was removed from water bath and the solution was filtered through cotton gauge and its pH was adjusted. After this the media was poured into the test tubes or flasks as per requirements and same were closed with cotton plugs and sterilized by steam pressure moist heat sterilization method[30] at 121^{0}C temperature and 15 lbs pressure for 15 minutes in an autoclave. After the sterilization the tubes in the case of agar slant were laid on flat surface with their mouth raised so that, when the medium cooled and solidified. It was in slant position. The broth and slant prepared were stored at 4^{0}C temperature.

Cultivation of Indicated Micro Organism Culture

The principle cultures of the following available pathogenic bacteria and fungi were taken as test microorganism.

Bacteria

1. Bacillus substilis (NCTC-8236)
2. Stabhylococcus aureus (ATCC-6538P)
3. Salmonella typhinurium (NCTC-786)
4. Escherichia coli

Fungi

5. Candida albicans
6. Crypto coccus neoformans
7. Tychophyton mantagrophitis
8. Aspergillus niger

Sub cultures of above micro organisms were prepared from the principle cultures monthly and broth cultures from sun cultures weekly. All cultures were stored at 4^{0}C. The inoculation process[30] was carried under Laminar flow clean bench. The incubation temperature and period for indicated micro organism were kept as

Bacteria

1. Bacillus substilis at 37^{0}C for 24 hours
2. Stabhylococcus aureus at 37^{0}C for 24 hours
3. Salmonella typhinurium at 37^{0}C for 24 hours
4. Escherichia coli at 37^{0}C for 24 hours

Fungi

5. Candida albicans at 28^{0}C for 48 hours
6. Crypto coccus neoformans at 28^{0}C for 48 hours
7. Tychophyton mantagrophitis at 28^{0}C for 96 hours
8. Aspergillus niger at 28^{0}C for 96 hours

Seeded broth for the test conductance was prepared by diluting 1:100 of the overnight kept broth culture of the respective organism incubated at optimum temperature.

Preparation of Stock Solution of Mixed Ligand Complexes, Involving Ligand and the Metal Ions

The stock solutions of mixed ligand complexes 1:1:1 M $^{(+2)}$-HQ-CAT/TRA/CTA, 1:1:1 M $^{(+2)}$-HQS-en/pn ; M $^{(+2)}$- Cu$^{(II)}$, Ni$^{(II)}$, Co$^{(II)}$ and Zn$^{(II)}$. Ligands 8- hydroxyquinoline (HQ), 8-hydroxyquinoline-5- Sulphonic Acid (HQS), Catechol (CAT), Tiron (TRA), Chromotropic acid (CTA) were prepared by dissolving 1 ml/1 mg of substance in 95% alcohol and the solution of metal nitrates by weighing and dissolving the metal nitrate having equivalent amount of respective metal ion as 1mg/1ml in sterilized distilled water. The solution was further sterilized by passing through a G-5 sintered glass filter and dispensed in small amounts (1 or 2 ml) into well stoppered test tubes and these solutions were stored in deep freezer at 20^{0}C until needed.

Conduct of the Test

The sets of "two fold serial dilution" of the test complexes ligands or the metal ions were prepared as follows:

1 ml of the seeded broth (obtain by 1:100 dilution of the indicated micro organism broth culture

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in broth) was taken in 10 well sterilized stoppered tubes (3x100 mm size) keeping the first tube empty and 2 ml of the seeded broth having 100μg/ml of the test complex/ligand/metal ion (prepared by dissolving 0.2 ml of the respective stock solution in 1.8 ml of seeded broth) was placed in first empty tube. Using fresh sterilized pipette 1 ml content from the first tube were withdrawn and added into the second tube and mixed well. 1 ml content from the second tube were pipette out with another sterilized pipette and added into third tube and shaken. This gradient dilution process is continued till the last tenth tube using fresh pipette in each case. 1 ml content taken out from the both tubes was rejected. All these tubes were labeled as 100μg/ml, 50μg/ml, 25μg/ml, 12.5μg/ml, 6.25μg/ml, 3.125μg/ml, 1.56μg/ml, 0.78μg/ml and 0.20μg/ml respectively starting from first tube to the last 10th tube. On the indicated micro organism the sets of two fold dilution of the solvent were carried out under similar experimental conditions of the method as given above for the test complexes, ligands or metal ions. 1 ml each of the seeded broth and the broth was placed in two separate tubes for the control of culture and control of broth media respectively in every set of above experiment simultaneously.

All the above sets of tubes were incubated in BOD incubator at the given temperature and period for the respective indicated micro organism.

Result and Discussion

The results of the "broth serial dilution" has been recorded as the minimum inhibitory concentration (MIC) of the free ligands (Table 1 & 2) and mixed ligand complexes (Table 3 & 4) in μg/ml and the growth occurred in solvent control tube, culture control tube and broth control tube were taken in account.

The minimum inhibitory concentration observed for the metal ions and free ligands reveals that most of these are in active to gram negative bacteria (Styphi, E coli). These substance has mild activity to the gram positive bacteria (B.Subtilis & S. aureus) and the fungi (C. neoformans and T. Mentagrophites). Nickel nitrate and the ligand HQ also have some activity to the other bacteria and fungi. The results obtained in the case of mixed complexes M $^{(+2)}$-HQ-CAT/TRA/CTA (Table 5 & 6), M $^{(+2)}$-HQS-en/pn indicates that the activity of the complexes have been enhanced in many folds in comparison to the constituted metal ion and the free ligands. The complexes with Zn were found highly active in comparison to the complexes of nickel and copper. The complexes M $^{(+2)}$-HQ-CAT and M $^{(+2)}$-HQS-pn were found most active in comparison to others.

The increased activity of the metal chelates in comparison to their constituted metal and ligands is probably due to the following facts.

1. By increasing the liposoluble nature of the biologically active ligand or the metal in the form of metal complexes in comparison to the metal ion or the ligand molecule alone.
2. By the replacement of the metal enzyme present in biological system with the foreign metal ion of the more lipo soluble metal complex.
3. By the displacement of protein molecule from the metal ion by the foreign ligand of the more lipo soluble metal-ligand complex rapturing the enzyme affecting a biological system.
4. Due to their combined activity effect of both ligands in the metal chelate or due to their more liposoluble nature on being co- ordinate with the metal ion forming a stable, neutral metal chelates.

Conclusion

However among all the above possible factors the more rapid diffusion of the metal complex as a whole through the cells of the micro organism may be one of the other important factors. The activity constituents may act on protein synthesis within the cell or at a site within envelope so that they must pass through part or the entire envelope to reach their target[31]. A comparison of the obtained results indicate that metal complexes in general have been found to be more biologically active in comparison to the ligand molecules alone under the same experimental and environmental conditions.

Table 1

Minimum Inhibitory Concentration (MIC, in μg/ml) Observed For Ligands against Indicated Bacterial Cultures

S.No.	Substance	Bacteria			
		B. subtilis	S.aureus	S. typhi	E.coli
1.	2- hydroxyquinoline	12.5	25	-	-
2.	2- hydroxyquinoline-5-sulphonic acid	25	25	50	50
3.	Ethylenediamine(en)	50	25	-	-
4.	1,2'-diaminopropane	50	25	-	-
5.	Catechol (CAT)	-	-	-	-
6.	Tiron (TRA)	-	-	-	-
7.	Chromotropic acid (CTA)	50	25	-	-

Table 2

Minimum Inhibitory Concentration (MIC, in μg/ml) Observed For Ligands against Indicated Fungi Cultures

S.No.	Substance	Fungi			
		C.albicans	C.neoformens	T.mentagrophites	A.nigar
1.	2- hydroxyquinoline	25	12.5	25	25
2.	2- hydroxyquinoline-5-sulphonic acid	-	25	25	50
3.	Ethylenediamine(en)	-	12.5	50	25
4.	1,2'-diaminopropane	-	50	50	-
5.	Catechol (CAT)	50	25	50	-
6.	Tiron (TRA)	-	-	50	-

7.	Chromotropic acid (CTA)	-	25	50	25

Table 3: Minimum Inhibitory Concentration (MIC, in µg/ml) Observed for the 1:1:1 M (II)-HQ-CAT/TRA/CTA Mixed Ligand Complexes against Indicated Bacterial Cultures

S.No.	Substance	Bacteria			
		B. subtilis	S.aureus	S. typhi	E.coli
1.	1:1:1, Cu (II)-HQ-CAT	3.125	12.5	-	-
2.	1:1:1, Ni (II)-HQ-CAT	3.125	12.5	25	25
3.	1:1:1, Zn (II)-HQ-CAT	3.125	6.25	-	-
4.	1:1:1, Co (II)-HQ-CAT	3.125	6.25	-	-
5.	1:1:1, Cu (II)-HQ-TRA	12.5	6.25	-	-
6.	1:1:1, Ni (II)-HQ- TRA	6.25	12.5	25	25
7.	1:1:1, Zn (II)-HQ- TRA	3.125	6.25	-	-
8.	1:1:1, Co (II)-HQ- TRA	6.25	12.5	3.125	-
9.	1:1:1, Cu (II)-HQ-CTA	6.25	6.25	-	-
10.	1:1:1, Ni (II)-HQ- CTA	6.25	6.25	25	25
11.	1:1:1, Zn (II)-HQ- CTA	3.125	3.125	-	-
12.	1:1:1, Co (II)-HQ- CTA	6.25	3.125	-	6.125

Table 4: Minimum Inhibitory Concentration (MIC, in µg/ml) Observed for the 1:1:1 M (II)-HQ-CAT/TRA/CTA Mixed Ligand Complexes Against Indicated Fungi Cultures

S.No.	Substance	Fungi			
		C.albicans	C.neoformens	T.mentagrophites	A.nigar
1.	1:1:1, Cu (II)-HQ-CAT	-	12.5	6.25	12.5
2.	1:1:1, Ni (II)-HQ-CAT	25	25	12.5	12.5
3.	1:1:1, Zn (II)-HQ-CAT	12.5	12.5	25	25
4.	1:1:1, Co (II)-HQ-CAT	6.25	12.5	12.5	-
5.	1:1:1, Cu (II)-HQ-TRA	-	-	25	6.25
6.	1:1:1, Ni (II)-HQ- TRA	25	25	12.5	12.5
7.	1:1:1, Zn (II)-HQ- TRA	3.125	6.25	6.25	12.5
8.	1:1:1, Co (II)-HQ- TRA	3.125	6.25	25	-
9.	1:1:1, Cu (II)-HQ-CTA	12.5	12.5	6.25	6.25
10.	1:1:1, Ni (II)-HQ- CTA	25	6.25	12.5	12.5
11.	1:1:1, Zn (II)-HQ- CTA	6.25	6.25	3.125	6.25
12.	1:1:1, Co (II)-HQ- CTA	6.25	12.5	6.25	-

Table 5: Minimum Inhibitory Concentration (MIC, in µg/ml) observed for the 1:1:1 M (II)-HQS-en/pn Mixed Ligand Complexes Against Indicated Bacterial Cultures

S.No.	Substance	Bacteria			
		B. subtilis	S.aureus	S. typhi	E.coli
1.	1:1:1, Cu (II)-HQS-en	6.25	25	-	-
2.	1:1:1, Ni (II)- HQS-en	6.25	12.5	25	25
3.	1:1:1, Zn (II)- HQS-en	3.125	6.25	-	-
4.	1:1:1, Co (II)- HQS-en	3.125	25	25	6.25
5.	1:1:1, Cu (II)- HQS-pn	3.125	6.25	-	-
6.	1:1:1, Ni (II)- HQS-pn	3.125	6.25	50	50
7.	1:1:1, Zn (II)- HQS-pn	1.56	3.125	-	-
8.	1:1:1, Co (II)- HQS-pn	3.125	1.56	25	-

Table 6: Minimum Inhibitory Concentration (MIC, in µg/ml) Observed for the 1:1:1 M (II)- HQS-en/pn Mixed Ligand Complexes Against Indicated Fungi Cultures

S.No.	Substance	Fungi			
		C.albicans	C.neoformens	T.mentagrophites	A.nigar
1.	1:1:1, Cu (II)-HQS-en	25	25	12.5	25
2.	1:1:1, Ni (II)- HQS-en	25	25	6.25	12.5
3.	1:1:1, Zn (II)- HQS-en	6.25	6.25	1.56	3.125
4.	1:1:1, Co (II)- HQS-en	12.5	12.5	25	25
5.	1:1:1, Cu (II)- HQS-pn	12.5	12.5	6.25	6.25
6.	1:1:1, Ni (II)- HQS-pn	25	25	6.25	6.25
7.	1:1:1, Zn (II)- HQS-pn	1.56	3.125	3.125	6.25
8.	1:1:1, Co (II)- HQS-pn	3.125	6.25	3.125	6.25

Endnotes

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