

BIOLOGICALLY ACTIVE MIXED-LIGAND COMPLEXES OF Cu(II), Ni(II) AND Zn(II) AND THEIR EFFECT ON SOME BACTERIA AND FUNGI

R. C. SHARMA, R. S. SHARMA* AND S. P. TRIPATHI

Department of Chemistry, Agra University, Agra 282 002, India.

*Department of Pharmacy, G.S.V.M. Medical College, Kanpur, India.

ABSTRACT

Protonated mixed ligand species, formed by the addition of secondary ligand to the initially formed 1:2, M(II)-HQ binary complex, in the systems 1:1:1, M(II)-HQ-Cat/TRA/CTA has been studied. The resulting mixed species have been isolated and characterised by elemental and IR studies. A comparative study of their biocidal properties on *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhineurium* and *Escherichia coli* and on the fungi *Candida albicanes*, *Cryptococcus neoformanes*, *Tychophyton metagrophitis* and *Aspergillus niger* indicates that the mixed-ligand complexes are more active as compared to their constituting fragments and binary complexes.

INTRODUCTION

METAL chelates as biocidal agents has been extensively studied¹⁻⁵. It has been reported that the mixed-ligand complexes of metals are better anti-fungal or antibacterial agents than their constituting fragments. In the present communication we report the formation and biocidal activity of some biologically active ternary complexes of Cu(II), Ni(II) and Zn(II).

MATERIAL AND METHODS

The chemicals used were either AR (BDH) or GR (E. Merck) and their solutions were prepared in double-distilled water. HQ was used as its monohydrochloride and the chromatographic acid (CTA) and Tiron(TRA) as their dipotassium salt.

Philips pH-meter (PR 8405 M), with standard glass (PV 9012) and calomel (PV 9021) electrodes, was used to measure the pH. IR spectra of the mixed ligand complexes and the corresponding ligands were recorded on Perkin-Elmer 521 spectrophotometer in KBr pellet in the range 4000-200 cm⁻¹.

PROCEDURE

Biocidal studies of the isolated complexes on the biogenic species were conducted in slant and broth culture media⁶. The incubation period for the bacteria was 24 hr at 37° C while in the case of fungi, it was 48 hr for *C. albicanes* and *C. neoformanes* and 96 hr for *T. mentagrophites* and *A. niger* at 28° C. Broth Agar Slant⁶ (No. 1 and 13), two culture media, were prepared and used for cultivating the microorga-

nisms. The incubation process⁷ was carried out under Laminar Flow Clean Bench. The two-fold serial dilution method was applied for testing the complexes.

RESULTS AND DISCUSSION

The curves representing the corresponding systems for all the three metals [Cu(II), Ni(II) and Zn(II)] are similar in nature; hence only those representing Cu(II)-HQ-Cat/TRA/CTA are discussed.

Curve 3 (figures A, B and C) represents the pH-titration of 1:1, Cu(II)-HQ binary mixture. The formation of a yellow solid from the beginning of titration and the occurrence of two inflections at $m=2$ and $m=3$ may be ascribed to the formation of 1:2, Cu(II)-HQ binary complex and the metal hydroxide respectively.

A single well-defined inflection at $m=2.5$ on the curve 4 (figure A) may be correlated to the simultaneous formation of two different binary species during the titration of 1:1, Cu(II)-Cat system. The appearance of a well-defined inflection at $m=2$ on this curve (figures B and C) is probably due to the formation of 1:1, Cu(II)-CTA/TRA binary complex. Another inflection at $m\sim 3$ in 1:1, Cu(II)-TRA may be attributed to the formation of 1:1, hydroxo species whereas in 1:1, Cu(II)-CTA it is due to the metal hydroxide formation resulting from the disproportionation of the initially formed 1:1, complex into 1:2 species.

Curve 5 (figures A, B and C) depicts the titration of 1:1:1, Cu(II)-HQ-Cat/TRA/CTA ternary system. This curve runs almost parallel or superimposes to the curve 3 showing the priority of chelation of HQ with the metal ions which is further supported by the formation of a heterogeneous phase from the beginning followed by an inflection at $m=2$. Another inflection

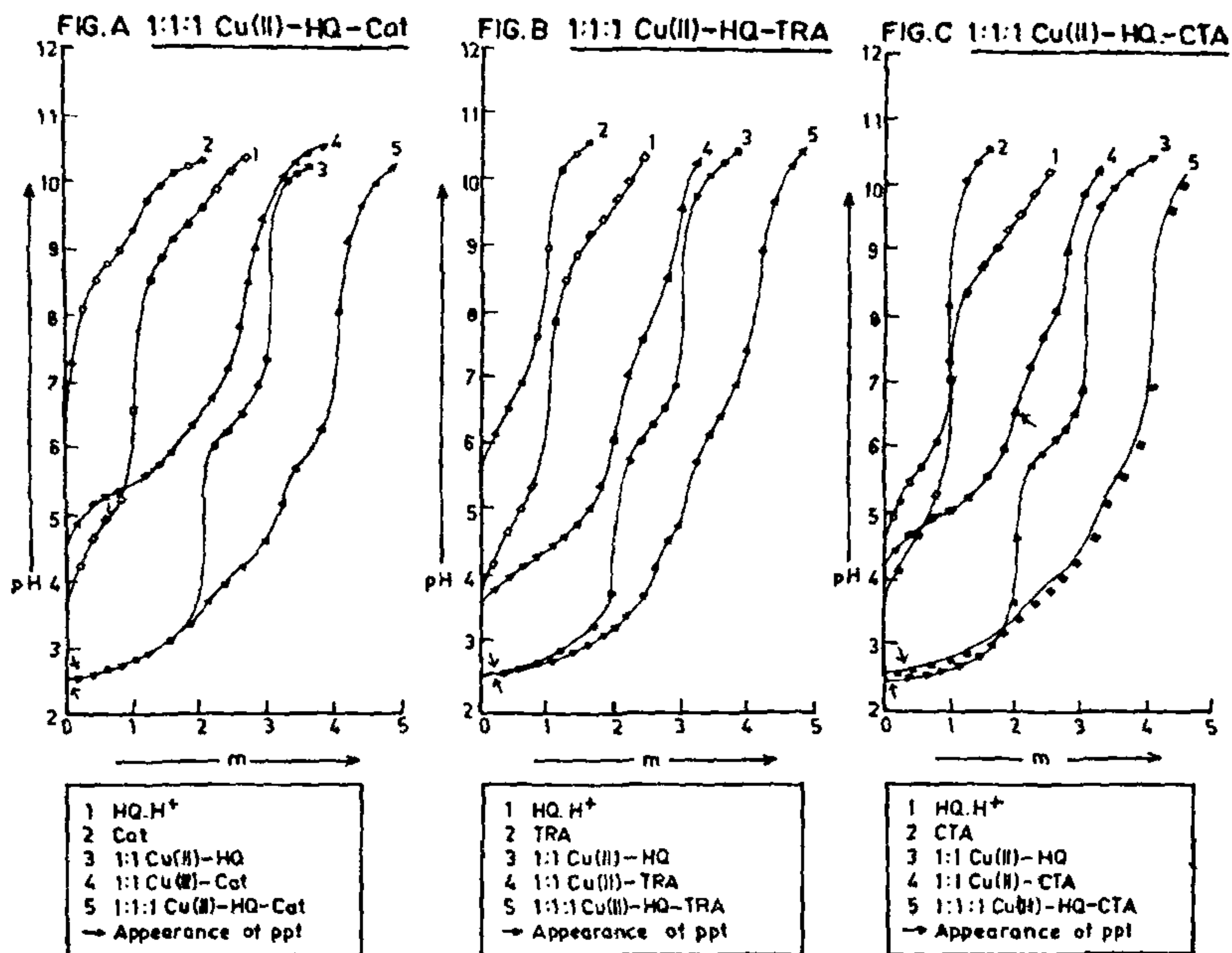


TABLE I

(MIC) in $\mu\text{g/ml}$ for bacteria

Substance	A	B	C	D
Cu/Ni/Zn	5/50/-	50/50/-	50/50/-	-/50/-
HQ/Cat/TRA/CTA	12.5/-/-/50	25/-/-/25	-/-/-/-	-/-/-/-
Cu(II)-HQ-Cat/TRA/CTA	3.125/25/6.25	12.5/6.25/6.25	-/-/-	-/-/-
Temperature (Time)	37° C (24 hr)	37° C (24 hr)	37° C (24 hr)	37° C (24 hr)
	Fungi			
	A ₁	B ₁	C ₁	D ₁
Cu/Ni/Zn	-/50/-	-/50/-	-/50/50	50/-/50
HQ/Cat/TRA/CTA	25/50/-/-	12.5/25/-/25	25/50/50/50	25/-/-/25
Cu(II)-HQ-Cat/TRA/CTA	-/-/12.5	12.5/-/12.5	6.25/25/6.25	12.5/6.25 6.25
Temperature (Time)	28° C (48 hr)	28° C (48 hr)	28° C (48 hr)	28° C (48 hr)

A = *B. subtilis*; B = *S. aureus*; C = *S. typhi*; D = *E. coli*.

A₁ = *C. albicans*; B₁ = *C. neoformans*; C₁ = *T. mentagrophites*; D₁ = *A. niger*.

MIC = minimum inhibitory concentration.

at $m=3$ corresponds to the addition of the secondary ligand (Cat/TRA/CTA) to the initially formed 1:2, Cu(II)-HQ complex resulting in the formation of 1:1:1, Cu(II)-HQ-Cat/TRA/CTA protonated insoluble ternary species. One more inflection at $m=4$ with the partial dissolution of solid phase may probably be ascribed to the titration of the coordinated hydroxy proton forming 1:1:1, Cu(II)-HQ-Cat/TRA/CTA normal complexes. The formation of such protonated species may further be supported by the appearance of different coloured solids during titrations and also by the elemental analyses and IR studies.

Elemental analyses:

The results of chemical analyses agreed with the calculated values within the limits of experimental errors.

IR Studies:

For the sake of brevity, only shifted or altogether new peaks appearing in the spectra of metal chelates, have been discussed. A sharp absorption band at 3270 cm^{-1} due to OH stretching vibrations in the free ligand (HQ) disappears in the spectra of metal chelates probably due to the involvement of OH group in chelation. The appearance of a new band in the reported region⁸ $640\text{--}420\text{ cm}^{-1}$ is probably due to M—O stretching vibrations. The M—M stretching vibration in the metal complexes has been observed in the reported region⁸ $400\text{--}300\text{ cm}^{-1}$. However, the presence of some strong bands at ca. 710 cm^{-1} and in the region of $1170\text{--}1100\text{ cm}^{-1}$ indicates the presence of coordinated oxine molecule⁹.

Biocidal Studies:

The MIC values given in table I indicate that although metal ions and ligands are almost biologically inactive to bacteria (A, B, C and D) and fungi (A_1 , B_1 , C_1 and D_1) they become effective in the form of their mixed-ligand complexes.

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ANNOUNCEMENT

HARI OM AWARDS

The Hari Om Ashram Alembic Research Awards for 1982, totalling to Rs. 20,000 for basic research in medical sciences has been awarded to the following four scientists:

Prof. A. Krishnamurti, Head of the Anatomy Department, Post Graduate Institute of Basic Medical Sciences, Madras University; Prof. Ramakrishnan, Head of Biochemistry Department,

JIPMER, Pondicherry; Prof. V. K. Vinayak, Department of Experimental Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh and Dr. (Mrs.) Surinder Kaur, Head of Biochemistry Department, PIMER, Chandigarh. Each award carried a medal and Rs. 5000 in cash.
