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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF CEPHRADINE METAL COMPLEXES: PART II COMPLEXES WITH COBALT, COPPER, ZINC AND CADMIUM

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Cephradine, the first generation cephalosporin, is active against a wide range of Gram-positive and Gram-negative bacteria including penicillinase-producing *Staphylococci*. Since the presence of complexing ligand may affect the bioavailability of a metal in the blood or tissues, therefore, in order to study the probable interaction of cephradine with essential and trace elements present in human body, cephradine has been reacted with cobalt, copper, zinc and cadmium metal halides in L:M ratio of 2:1 in methanol and the products recrystallized from suitable solvents to pure crystals of consistent melting points.

Infrared and ultraviolet studies of these complexes were carried out and compared with ligand. Magnetic susceptibility studies of these complexes were also carried out showing their paramagnetic behavior. From the infra red studies and elemental analysis of the complexes, it has been shown that the drug molecule serves as a bidentate ligand coordinating through both its carboxylate at C-3 and β-lactam nitrogen and the metal having a square planar or octahedral geometry.

To evaluate the changes in microbiological activity of cephradine after complexation, antibacterial studies were carried out by observing the changes in MIC (minimum inhibitory concentration) of the complexes and compared with the parent drug by measuring the zone of inhibition of complexes and compared with the parent cephalosporin against both Gram-positive and Gram-negative organisms. For MIC observation, serial dilution method was employed and zone series were determined by disk diffusion method. Our investigations reveal that formation of complexes results in decrease in antibacterial activity of cephradine and MIC values are increased.

Keywords: Cephradine, metal complexes, antibacterial activity.

INTRODUCTION

Cephradine is synthesized by coupling 7-amino-desacetoxycephalosporanic acid (7-ADCA) with a protected derivative of dihydrophenylglycine, such as the tert - butoxylcarbonyl derivative which can be converted to a mixed anhydride with ethylchloroformate and reacted with 7-ADCA (Dolfini *et al*., 1971). Cephradine can also be made by forming the methyl acetoacetic ester an enamine derivative of dihydrophenylglycine which is converted to a mixed anhydride with benzoyl chloride prior to coupling with 7-ADCA. Cephradine is then crystallized from a biphasic MIBK-aqueous solution (Dursch 1976). It can also be crystallized from water alone, as well as from other solvents.

There is no difference in solubility of various crystal forms, the solubility of cephradine in buffer at different pH values is reported (Afzal 1998). It is practically insoluble in diethylether, chloroform, benzene and hexane, very slightly soluble in acetone & absolute ethanol and freely soluble in propylene. The intrinsic dissolution rates of cephradine and its dihydrate as well as cephalexin were found identical at an agitation intensity of 100 rpm (Dursch 1976). Solutions of cephradine may vary in color from light straw to yellow, but this is not an indication of potency. The stability of cephradine under various experimental conditions have been reported (Wang and Monkouse 1983; Yamana and Tsuji 1976; Cohen *et al.,* 1973).

There are number of methods reported for the analysis of cephradine. They include titrimetric (Korbl and Pospibilova 1983), iodometric (Amato *et al*., 1983), fluorimetric, colorimetric (Dursch 1976), polarographic, paper chromatographic (Zaki *et al*., 1974), HPLC (Mc Ateer *et al*., 1987; Lindgren 1987; Toothaker *et al*., 1987), TLC (Fahre *et al*., 1985), gas chromatographic, spectrophotometric (Abdalla *et al*., 1983; Issopoulos 1988), capillary electrophoresis, kinetic and molecular modelling analysis (Raquet *et al*. 1994) and microbiological assay methods (Dursch 1976; Traub & Leonhard 1995).

EXPERIMENTAL

Materials

Velosef batch 2A015 and valodin batch BH-05 (cephradine) were gifts from Squibb Laboratories Karachi and Hilton Pharma Ltd Karachi. These materials had expiry date not earlier than 365 days, at the time of these studies. The essential and trace elements used were in the form of following hydrated salts; cobalt chloride ($CoCl_2.6H_2O$), copper chloride ($CuCl_2.2H_2O$), zinc chloride and cadmium chloride ($CdCl_2.H_2O$).

Methods

I Synthesis of cephradine metal complexes

The procedure for the preparation of complexes of cobalt, copper, zinc and cadmium with cephradine involved mainly of reacting a solution of the metal halide with the solution of a ligand in a common organic solvent, preferably methanol. In general, the synthesis of these complexes required a few hours of stirring at certain temperature with some easily reduced metal ions. These were recrystallized from suitable solvents to pure crystals of consistent melting points.

1 *Preparation of cephradine-cobalt complex*

Cephradine 2 millimole (0.800 gm) dissolved in methanol (10 ml) and cobalt chloride hexahydrate 1 millimole (0.2739 gm) was dissolved in 5 ml methanol. Both the solutions were mixed and constantly stirred. The pink solution turned brown on refluxing. The resultant mixture was allowed to crystallize at room temperature. Brown crystals were washed with chloroform to remove gum and dried m.p. 150°C.

2 *Preparation of cephradine-copper complex*

Cephradine 2 millimole (0.800 gm) was dissolved in 5 ml methanol. To this was added a solution of 1 millimole of copper chloride dihydrate (0.17048 gm) dissolved in 8 ml methanol with constant stirring. The resultant green solution was refluxed for 2-3 hours. The brown filtrate obtained was allowed to crystallize at room temperature. The crystals so formed were washed with methanol for purification, dried and melting point of pure dark brown powder was 117°C.

3 *Preparation of cephradine-zinc complex*

Zinc hydroxide 1 millimole (0.136 gm) was dissolved in 5 ml methanol. Cephradine 2 millimole (0.800 gm) was dissolved in 10 ml methanol. To this was added a solution of metal salt with constant stirring and refluxed for 2-3 hours. The clear solution on refluxing turned yellow and gave yellow crystals; these were filtered, washed with methanol and dried with ether m.p. 230°C.

4 *Preparation of cephradine-cadmium complex*

Cephradine 2 millimole (0.800 gm) was dissolved in 10 ml methanol and to this was added 1 millimole (0.1832 gm) solution of cadmium chloride dissolved in methanol. This was refluxed for 2-3 hours on a water bath and left for crystallization at room temperature. Yellow crystals were filtered off, washed with methanol and dried, m.p. 240° (d).

II Analysis

Unless otherwise stated, all analytical measurements of the synthesized cephradine metal complexes were carried out as follows.

Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra (potassium bromide discs) were measured using Perkin-Elmer 1430 IR Spectrophotometer. Ultraviolet and visible spectra were recorded using UV-Visible spectrophotometer (Shimadzu 2101-PC).

Carbon, hydrogen and nitrogen analysis were carried out using CHN analyzer and metals were estimated by Pye-Unicam AA Spectrometer at the Department of Chemistry, University of Karachi. Magnetic susceptibility measurements were made using Guoy Balance of Mettler H-20. Conductance measurements were carried out with conductivity measuring bridge type MC-3 Mark V, portable electrolytic conductivity measuring set in nitrobenzene.

Analysis of cephradine metal complexes by AA spectrometry

For the estimation of metals incorporated in these cephradine metal complexes, known weight of each of these samples was digested in concentrated nitric acid (Analar) for 2 - 3 hours or till the digestion was complete and only traces of the acid was left in the residue. The material was transferred quantitatively to volumetric flasks and diluted with 0.1 M HCl to the mark. Aliquots were diluted further till an approximate concentration of 1-10 μg/ml was reached. Ten reference standard solutions of each metal were also prepared having concentration 1-10 μg/ml.

The spectrometer was calibrated and set at the required wavelength of the metal (for Co 240.7 nm, Cu 324.8 nm, Zn 213.9 nm & Cd at 228.8 nm). Absorbances of the standard and unknown samples were measured using air-acetylene flame and concentration of the unknown was calculated by comparing with that of the standard solutions.

III Antibacterial studies

Samples

Cephradine metal complexes with cobalt, copper, zinc and cadmium synthesized as stated above in section I, were used in these studies.

Organisms

The organisms employed in these studies were *Salmonella typhi, Shigella dysentery, Corynebacterium diptheriae, Streptococcus pyogenes, Proteus vulgaris, Staphylococcus aureus, Escherachia coli, Corynebacterium hoffmanni, Streptococcus faecalis* and *Klebsiella pneumoniae*.

The details of antimicrobial assay procedure, sample preparation, media, collection of cultures, preliminary identification of the cultures, sub culturing and maintenance of different organisms, turbidity standards, medium for inoculation, preparation of assay plates, streaking, disk placement and test procedures of antimicrobial susceptibility testing have already been reported earlier (Sultana *et al*., 2003).

After 18-24 hours of incubation, the diameter of zones of inhibition around each disk was measured with a venire caliper on the back of the plates, with reference light against a dark non-reflective background. The zone diameter for each antimicrobial agent was then interpreted as shown in table 4.

RESULTS AND DISCUSSION

As the biological activity of cephalosporins, as a result of interaction with minerals and trace elements is questionable, present studies deal with further assessing and comparing the biological activity of these drug metal complexes with their parent antibiotics.

I Synthesis of cephradine metal complexes

An attempt has been made to synthesize metal complexes of cephradine, with various essential and trace elements in 2:1 ligand to metal ratio. These complexes were then subjected to analytical studies for the elucidation of their structures. The possible interaction of β-lactam antibiotics *in vivo* and *in vitro* is of obvious interest. The reaction of various metal salts as cobalt, copper, zinc and cadmium with cephradine by refluxing them in methanol in different molar ratios on a water bath at 70°C led to the formation of complexes having variable characteristics and different stoichiometry as $[M(CPH)_2X_2]$, $[M(CPH)_2]X_2$ etc. The physical characteristics and solubility of these complexes are given in table 1.

II Infrared studies of cephradine metal complexes

Since IR is the most reliable tool of assessing the presence of substituents, all the synthesized complexes were studied spectroscopically in the IR region, as all the compounds contain cephradine, an aromatic ligand with a thiozolidine ring and a β-lactam group.

Aromatic C-H bending and stretching vibrations

The most prominent and informative bands in the spectra of aromatic compounds occur in the low frequency range between 900-675 cm-1 resulting from out of plane bending of the ring C-H bonds. These absorptions can be correlated with the number of adjacent hydrogen atoms on the rings (Bellamy 1958). The in-plane bending bands appear in the 1300 - 1000 cm^{-1} region.

The C - H aromatic stretching vibrations occur between 3100 and 3000 cm^{-1} (Pavia 2000) and the presence of an aromatic structure is best recognized by the presence of C - H stretching vibrations near 3030 cm^{-1} (Smith 1995; Volland 1999) which are generally three in number, but some times there are more bands and in multiple ring systems, very complex patterns are produced; many mono substituted aromatics give a characteristic triplet at about 3058 cm^{-1}. Nearly in all cases one of the band in this region is considerably more intense than the others, within the range 3079-3030 cm^{-1}, changes of state cause small shifts usually of the order of ± 10-15 cm^{-1}. The shift above 3000 cm^{-1} can also be due to various types of double bonds and of CH - X where X is halogen.

C = C Stretching vibrations

Skeletal vibrations involving carbon to carbon stretching within the ring, absorbs in the 1600-1585 cm^{-1} and in the 1500-1400 cm^{-1} regions. The skeletal bands frequently appear as doublets depending upon the nature of the ring substituents. In these regions, the absorption bands are very little affected by substitution. The characteristic C=C skeletal stretching vibrations lead to a group of four bands between 1650-1450 cm^{-1} region. The bands near 1600-1500 cm^{-1} regions are highly characteristic of the aromatic ring itself. Taken in conjunction with the C-H stretching mode (near 3030 cm^{-1}), one of these four bands (1650-1450 cm^{-1}) (Randle and Whitten 1955) is very weak and falls near 1580 cm^{-1}, while another near 1450 cm^{-1} is close to the deformation mode of CH_2 group and some times obscured. The intensity of all these bands is extremely variable and some times appear as a shoulder on the sides of other band. With the study of several aromatic compounds (Chakrawarti 1993) it was found that the 1625-1575 cm^{-1} band occurs within the range 1650-1585 cm^{-1} for the *para*-substituted aromatics, on the other hand *mono*-substituted show a shift within ± 5 cm^{-1} of 1600 cm^{-1}. The position of this band is somewhat dependent upon the electronegativity of the substituent groups.

In the literature (Kabayashmi 1954; Shoukry 1995; Hoover and Dunn 1979), there is much confusion at the occurrence and significance of 1600-1560 cm^{-1} band, but the conflict can be reconciled by the view that 1580 cm^{-1} band is a normal aromatic vibration which is always weak and can be

Table 1
Physical characteristics of cephradine metal complexes

S No.	Complex	Color	State	M.P	Solubility in		
				°C	CH_3OH	$CHCl_3$	DMSO
1	Cephradine cobalt	Brown	Crystalline	150	soluble	50%	50%
2	Cephradine copper	Dark brown	Powder	117	soluble	50%	50%
3	Cephradine zinc	Yellow	Crystalline	230	soluble	50%	75%
4	Cephradine cadmium	Yellow	Crystalline	240d	soluble	75%	75%

Table 2
Infra red absorptions of cephradine metal complexes

S. No.	Code	Compound	Frequency in Cm^{-1}
1	CPH	Cephradine	450sm, 690m, 800m, 830m, 860m, 890s, 1000m, 1160sm, 1260-1280db,m, 1380s, 1460s, 1630s, 1725s, 2920-2980 trp,s 3400-3500s
2	CPH-Co	Cobalt complex	690s, 880s, 960m, 1030s, 1160s, 1280m, 1600-1660trp,s, 1700b, 1760s, 2850m, 2920-2980s.
3	CPH-Cu	Copper complex	500m, 650sm, 700sm, 960m, 1100s, 1420m, 1650br, multiplet, 3200 trp, broad.
4	CPH-Zn	Zinc complex	670m, 710m, 750m, 850m, 965m, 1080m,1240m, 1335m, 1385m, 1440 dbm,1510m, 1540m, 1560sm, 1650s trp,1760 dbm, 3000m, 3450-3500db s.
5	CPH-Cd	Cadmium complex	450m, 690s, 790 sm, 1080s, 1325s, 1400-1440 m,br, 1600 -1680 br,s, 3200m, 3400-3500 br.

detected by external conjugation. The band around 1525-1475 cm^{-1} always occur in the range usually close to 1500 cm^{-1}, except in *para*-substituents and unsymmetrical tri-substituents which cause a shift towards higher frequency, while the vicinal tri-substituents towards lower frequency. The fourth band due to skeletal C–C vibration falls in 1450 cm^{-1} region as suggested (Chakrawarti 1993). This band is observed in 1470-1439 cm^{-1} region in mono-substituents usually as moderate to strong intensity and is overlaid by strong CH_2 deformation.

Substituents
The presences of substituents were studied in the regions 1250-1000 and 1000-650 cm^{-1}; of these regions the first is generally the most definite and usually gives a clear indication of the type of substitution (Pavia 2000). Since they are weak, the overtone and combination bands are most readily observed in spectra obtained from thick samples. All the bands in this region were assigned with high precision to summation bands of C–H out of plane fundamentals, which occur between 1000-700 cm^{-1} region. These bands were frequently intense and characteristic of polynuclear aromatic compounds. This accounts for the variation in characteristic patterns, the importance of general shapes, over absolute frequencies and the simplification of the band with more symmetrical structures in which a smaller number of out of plane C–H deformation occur. Fundamental and other bands in this region were easily recognized and appear simply as an additional band superimposed upon the original (Chakrawarti 1993).

The C–H in plane deformation vibration of the aromatic compounds fall between 1225-950 cm^{-1} region as relatively weak bands. As the position of this band varies with the type of substitution arrangement, this gives information of the presence of aromatic substituents and their substitution pattern (Pavia 2000; Smith 1995; Volland 1999). The mono substitution showed absorptions from 1075-1065 cm^{-1}, the *ortho* falls in 1125-1085 cm^{-1}, the meta absorbs within 1170-1140 cm^{-1} region and the para substitution between 1120-1090 cm^{-1} region.

During the studies of spectra of the prepared metal complexes with cephradine, it must be noted that the band shapes and relative intensities may be of importance equal to or greater than band positions. Aromatic bands are equally sharp for example; the relative depth of the 1600-1450 cm^{-1} absorption may give a clue as to the type of the substituents on a phenyl group. The C=C absorption near 1650 cm^{-1} is sharp and not likely to be confused with amide C=O absorption which falls in the same region (Bellamy 1958).

Cephradine
The IR spectrum of cephradine showed absorptions characteristic of the particular groups present. It showed absorptions at 3050 cm^{-1} (due to C-H and C=N), 3000-3500 cm^{-1} (series of broad bands, characteristic of OH from H_2O and NH (amide), 1760 cm^{-1} (β-lactam, C=O), 1730 cm^{-1} (COOH, C=O), 1650 (amide HNC=O stretching), 1540 cm^{-1} (O=C=N), 1600-1400 cm^{-1} (very broad), 1050 cm^{-1} (C–O stretching) and 690-820 cm^{-1} (aromatic H bending, mono-substituted aromatic ring).

Metal Complexes
The site of complexation is observed to be a function of side chain and also of pH. The effect of varying pH on cephradine-metal complexation has been discussed elsewhere.

β-lactam C=O and COO^- Stretching Frequencies
The β-lactam infrared stretching frequency (ν C=O) has been regarded as an important index both for inhibition of amide resonance and for investigating structure activity relationships of the β-lactam antibiotics. In monocyclic infused β-lactams, carbonyl stretching frequency occurs in the 1730-1760 cm^{-1} and about 1600-1680 cm^{-1} for amides.

In general the non-polar 3 cephems show higher stretching frequencies (1786-1790 cm^{-1}) than the planar 2-cephems, which absorb at 1750-1780 cm^{-1}. The frequency in cephalosporins increases by ca. 5 cm^{-1} when the ring sulfur is replaced by oxygen but decreases by a similar amount when the 7-α-hydrogen is substituted by a methoxy group. It is difficult to make generalizations about the observed β-lactam frequency, since different conditions may cause variations comparable with those produced by structural changes.

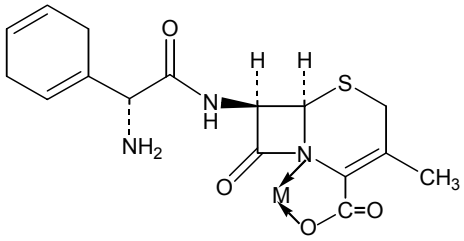
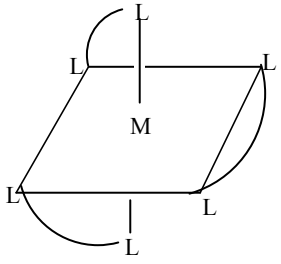


Figure 1

The infrared spectra of the metal complexes are given in table 2. Copper(II) ion is thought to coordinate to the carboxylate group and the β-lactam nitrogen as shown in figure 1. Esterification of this group decreases the rate enhancement by a factor of ca $5x10^3$. Although it has been suggested (Gensmantel 1981) that Cu(II) ion coordinates to the 7-acylamino side chain and the β-lactam carbonyl group, replacement of the side chain by a more basic amino group has little effect upon the binding constant. The later suggestion seems unlikely due to the steric hindrance of the α-amino group present on the side chain and secondly, if copper at all bonds to acyl amino nitrogen, then cephradine will act as monodentate ligand under present circumstanes. Furthermore, penicillanic acid, in which the amino side chain has been removed, also gives similar binding constants and rate enhancements. It is apparent that Cu(II) ion do not bind to the amido side chain in penicillins and that coordination probably occurs between the carboxylate oxygen and β-lactam nitrogen (Sultana *et al.,* 2003). Precipitation of the β-lactam/metal ion complex in the presence of excess of ligand gives solid with interestingly different characteristics.



Cephradine metal complexes
in d^2sp^3 confiugration

Figure 2

On the basis of infra red spectral characteristics (table 2), elemental analysis, (table 3) and magnetic susceptibility measurements, cobalt, zinc and cadmium complexes have been assigned an octahedral geometry having d^2sp^3 hybridization (figure 2), while copper cephradine complex have a square planar structure with a dsp^2 hybridization. Earlier, magnesium and calcium cephradine complexes had been assigned a tetrahedral geometry, having sp^3 hybridization, whereas chromium and manganese complexes an octahedral geometry having d^2sp^3 hybridization.

Cephradine forms complexes with transition metal ions in which not only is the asymmetric stretching frequency of the carboxylate decreases, but also the β-lactam carbonyl stretching frequency by 10 - 30 cm^{-1} depending upon the nature of the metal ion. For metals such as chromium(III) and cobalt(III) the band is in the region 1700 - 1725 cm^{-1}; whereas it is in the region 1650 cm^{-1} as a broad triplet for copper (II) and zinc (II). Since the free COO^- stretching band is at 1730-1760 cm^{-1}, it is possible to distinguish the coordinated and free COO^- stretching bands if a metal as Cu(II) is chosen for complex formation.

The site of metal ion coordination for cephradine is thus, not different from other cephalosporins, which involve carboxylate oxygen in metal coordination. In our newly synthesized complexes of copper the shifting of the COO^- frequency to 1650 - 1680 cm^{-1} is also a clear indication of coordination at this site.

A number of workers have studied complexes of cephalosporins with Mg, Mn, Fe, Ni, Co and Zn etc. and showed that these metals form 1:1 with cephalosporins (Chakrawarti *et al*., 1993; Sultana *et al*., 2003). Spectral studies indicate that the linking of the drug molecule with the metal ions is through the nitrogen of the β-lactam thiozolidine ring and carboxylate ion forming a five membered ring (figure 1). The same has ensued in our complexes. IR spectrum shows that most of the bands of the ligand remain unchanged on complexation. There is a considerable shift in the frequencies of tertiary nitrogen and of the carboxylic group of the ring. The β-lactam amide bands in the drugs appearing at 1770 cm^{-1} is shifted to 1700 cm^{-1} in the complexes, while the frequency of the ternary N-atom appearing at 1384 cm-1 is shifted to 1130 cm^{-1}. The asymmetric and symmetric COO^- frequencies are shifted from 1614 to 1630 cm^{-1} and from 1395 to 1375 cm^{-1} respectively. The differences between asymmetric and symmetric frequencies of COO^- suggest that the complexes have considerable degree of covalency in the M-oxy bond. The structures of number of complexes of cephradine with uranyl (II) ion also suggest that the antibiotic acts as a bidentate ligand and is bound to the metal ion through the carbonyl and the amino group of the side chain (Shoukry *et al*., 1995).

Table 3
Elemental analysis of cephradine metal complexes

S. No.	Compound	Elements—>	C	H	N	S	X	M
1	$[Co(CPH)_2Cl_2]$	Calculated	46.38	4.62	10.14	7.74	8.56	7.11
		Found	46.62	4.35	10.15	7.75	8.61	7.16
2	$[Cu(CPH)_2]Cl_2$	Calculated	46.13	4.60	10.09	7.70	8.51	7.63
		Found	46.24	4.65	10.25	7.76	8.64	7.65
3	$[Zn(CPH)_2(OH)_2]$	Calculated	48.15	5.05	10.53	8.03	—	8.19
		Found	48.24	5.42	10.45	7.98	—	8.40
4	$Cd(CPH)_2Cl_2]$	Calculated	43.57	4.34	9.53	7.27	8.04	12.74
		Found	43.43	4.43	9.72	7.24	8.00	12.92

Table 4
Antimicrobial susceptibility of cephradine cobalt, copper, zinc and cadmium complexes

S. No.	Organism	Zone of Inhibition				
		Standard	Co-complex	Cu-complex	Zn-complex	Cd-complex
1.	*Salmonella typhi*	10.40 - 12.00	00.09 - 16.20	0.07 - 11.00	16.40 - 32.00	12.00 - 21.00
2.	*Shigella dysentery*	04.90 - 10.40	19.60 - 39.00	08.00 - 16.00	12.40 - 19.70	19.60 - 39.00
3.	*Corynebacterium diptheriae*	16.60 - 34.50	15.40 - 34.90	13.00 - 16.00	15.70 - 32.00	16.70 - 28.70
4.	*Streptococcus pyogenes*	09.30 - 13.00	16.40 - 35.70	08.00 - 14.00	19.30 - 35.40	17.00 - 22.00
5.	*Proteus vulgaris*	16.00 - 36.40	16.40 - 35.70	14.00 - 20.00	14.30 - 29.70	16.40 - 35.70
6.	*Staphylococcus aureus*	04.60 - 11.70	13.20 - 29.40	14.00 - 16.00	17.00 - 39.00	13.20 - 29.40
7.	*Escherachia coli*	25.00 - 38.00	12.40 - 24.90	10.00 - 19.00	13.60 - 24.00	07.00 - 10.00
8.	*Corynebacterium hoffmanni*	15.00 - 39.60	06.70 - 28.70	13.00 - 29.00	16.20 - 23.40	05.40 - 13.60
9.	*Streptococcus faecalis*	14.30 - 29.50	04.90 - 12.00	04.00 - 07.00	04.50 - 11.30	17.90 - 36.40
10.	*Klebsiella pneumoniae*	12.00 - 14.70	15.90 - 33.00	04.00 - 09.00	03.40 - 07.00	03.60 - 07.90

Many metal ions also play important role in the activity of transpeptidase enzyme. It has been proposed that metal bridged enzyme drug complex is formed during inhibition activity. The results indicate that a maximum of two molecules of drugs link with the biologically active metal ion. Hence at least two coordination positions (out of six) of the metal ions are left uncoordinated. These may be utilized in binding the metal drug complex with the enzymes resulting in the observed bactericidal action.

III Antibacterial studies of cephradine metal complexes

Cephradine showed antimicrobial activity against various human pathogens (table 4). Although cephradine had aloft potent activity and bigger zones were formed for *Escherachia coli*, *Corynebacterium hoffmanni*, *Streptococcus faecalis*, *Corynebacterium diptheriae* and *Proteus vulgaris* than *Salmonella typhi*, *Staphylococcus aureus* and *Klebsiella pneumoniae* but these pathogens were also susceptible to it.

Corynebacterium diptheriae, *Streptococcus pyogenes*, *Proteus vulgaris* and *Klebsiella pneumoniae* were susceptible to the cephradine cobalt complex. Complex formation did not produce much effect on *Staphylococcus aureus*, *Escherachia coli*, *Streptococcus faecalis* and *Salmonella typhi* and were found to be resistant.

On forming complex with copper, the antibacterial activity of cephradine was reduced more for *Escherachia coli*, *Corynebacterium hoffmanni*, *Streptococcus faecalis* and *Corynebacterium diptheriae,* whereas for *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus,* activity was reduced moderately. *Salmonella typhi* and *Klebsiella pneumoniae* formed very small zones with the complex and were found more resistant to it.

Just like above complexes, cephradine zinc complex also reduced the activity of the organisms. *Corynebacterium* diptheriae, *Streptococcus faecalis*, *Proteus vulgaris* and *Klebsiella pneumoniae* were mildly susceptible. *Salmonella typhi*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Escherachia coli* were found moderately susceptible.

Klebsiella pneumoniae Escherachia coli, *Corynebacterium* hoffmanni and *Staphylococcus aureus* showed smaller zone sizes in case of cephradine cadmium complex. *Salmonella typhi*, *Corynebacterium diptheriae*, *Streptococcus pyogenes*, *Proteus vulgaris* and *Streptococcus faecalis* showed moderate susceptibility.

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