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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF CEPHRADINE METAL COMPLEXES: PART I COMPLEXES WITH MAGNESIUM, CALCIUM, CHROMIUM AND MANGANESE

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ABSTRACT

Cephradine is included among the first generation cephalosporins, which 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 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.

INTRODUCTION

Cephradine is a semisynthetic cephalosporin antibiotic developed at the Squibb Institute for Medical Research (Brithsh Pharmacopoeia 2002), chemically designed as 7-[D-2-amino-(1,4-cyclohexadiene-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azyabicyclo-octa-2-ene-2-carboxylic cid (Ryan *et al.,* 1969) or 7-[2-amino-2-(1,4-cyclohexadienyl)acetamido]-desacetyl-cephalosporanic acid (figure 1). It is defined as a hydrated form containing 3-6% of water, which is not a stoichiometric hydrate since the water moves freely in the crystal lattice.

Cephradine dihydrate, which crystallizes from aqueous solution under controlled conditions, is very stable and resistant to oxidation. However, on dehydration the dihydrate becomes very unstable. The structure of this was determined by a single crystal x-ray diffraction. Cephradine monohydrate recrystallized from acetonitrile: water is a true hydrate; whereas cephradine recrystallized from anhydrous methanol also appears to be a true monohydrate, although another one half mole of unbound water was also present. It is a white crystalline powder having the

molecular weight 349. 41 for anhydrous form; 367.43 of monohydrate and 385.45 of dihydrate. It melts with decomposition for cephradine dihydrate the m.p. is 183-185 whereas it has a varied range from 175-192°C. The IR spectra of cephradine monohydrate & dihydrate, NMR and mass spectrum are reported (Dursch 1976).

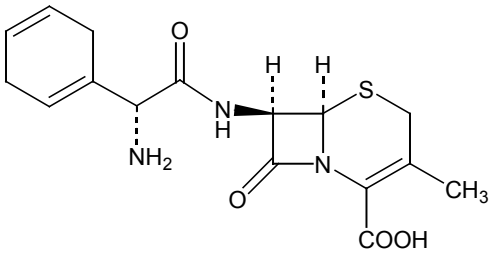


Fig. 1

Its spectrum is similar to that of cephalexin, with only minor differences (Moellering and Swartz 1976). Gram-positive bacteria are usually sensitive, *Staphylococcus aureus*, including most penicillin-resistant (Lambert and O'Grady 1992), but not methicillin-resistant strains, are sensitive. Cephradine is about as resistant as cephalothin to inactivation by Staphylococcal β-lactamase (Fong *et al.,* 1976; Basker *et al.,* 1980). Most other aerobic Gram-positive cocci, such as *Staphylococcus epidermis*, *Streptococcus pyogenes, Streptococcus pneumoniae* and *Streptococcus viridans*, are susceptible to cephradine. *Streptococcus faecalis* is resistant (Hamilton-Miller 1974). Anaerobic Gram-positive cocci, such as the *Peptococcus* and *Peptostreptococcus spp*., are usually cephradine sensitive. Most strains recovered from airway associated infections are relatively sensitive (MICs 8-16 g per ml), but other strains are less so (Busch *et al.,* 1976). Against Gram-positive bacilli, cephradine has a similar inhibitory action to cephalexin.

Gram-negative bacteria like E. coli, *Proteus mirabilis* and Klebsiella spp. (McGowan *et al.,* 1974; Bill & Washington 1977; Wise *et al.,* 1979) are susceptible. Cephradine is only moderately active against *N. gonorrhoeae* (Phillips *et al.,* 1976), but it retains this activity against β-lactamase producing strains (Selwyn & Bakhtiar 1977). Cephradine is relatively inactive against *H. influenzae*, many strains of which are completely resistant to this drug (Sinai *et al.,* 1978; Watanakunakorn & Glotzbecker 1979). The drug is moderately active against *Gardnerella vaginalis* (Goldstein *et al.,* 1983; Sirot *et al.,* 1982; Messmer *et al.,* 1983).

The pharmacokinetics ((Klasterky *et al.,* 1973; Scholand *et al.,* 1974; Ginsburg and McCracken 1979; Caloza *et al.,* 1979; Macias & Eller 1975; Mogabgab 1976; Bennett *et al.,* 1977; Peffer *et al.,* 1977; Ginsburg *et al.,* 1982; Adam 1975; Maroske *et al.,* 1976; Kiss *et al.,* 1976; Parsons *et al.,* 1976; Craft & Foster 1978), toxicity (**191** Klasterky *et al.,* 1973**; 192** Scholand *et al.,* 1974; **195** Macias and Eller 1975; **196** Mogabgab 1976; **207** Bartlett *et al.,* 1979; **)** and clinical uses (Hart et al.,1981; Lacey *et al.,* 1983; Selwyn 1976; Daggett and Nathan 1975; Boeschoten *et al.,* 1985;) are extensively studied. There are 697 references to cephradine in Medline till March 2003.

EXPERIMENTAL

Materials

Velosef and Valodin (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; magnesium chloride ($MgCl_2.6H_2O$), calcium chloride ($CaCl_2$), chromium hydroxide ($Cr(OH)_3.H_2O$) and manganese chloride ($MnCl_2.2H_2O$).

Methods

I Synthesis of Cephradine Metal Complexes

The procedure for the preparation of complexes of magnesium, calcium, chromium and manganese 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-magnesium complex*

Cephradine 2 millimoles (0.800 gm) dissolved in methanol (10 ml) was added to a solution of magnesium chloride hexahydrate 1 millimole (0.203gm) in 5 ml methanol. The yellow solution thus obtained was refluxed for 2-3 hours on a water bath and allowed to stand for crystallization at room temperature, yellow needle like crystals formed were washed with diethyl ether to remove gummy material; m.p. 210°C.

2. *Preparation of cephradine-calcium complex*

Cephradine 2 millimoles (0.800 gm) dissolved in 10 ml methanol and calcium chloride dihydrate 1 millimole (0.147 gm) dissolved in 5 ml of methanol were mixed together in a refluxing flask with continuous stirring and the solution refluxed for 2-3 hours on a water bath. This clear solution was allowed to crystallize at room temperature, yellow crystals filtered washed with methanol and dried with ether m.p. 220°C (d).

3. *Preparation of cephradine-chromium complex*

Chromium chloride hexahydrate 1 millimole (0.266 g) was dissolved in 5 ml methanol and added into 2 millimole (0.800 g) of cephradine dissolved in 10 ml methanol. The resultant dark green solution was refluxed on a water bath for 2-3 hours, then filtered and allowed to crystallize at room temperature. Dark green crystals obtained were washed with methanol and dried in vacuum, m.p. 252°C (d).

4. *Preparation of cephradine-manganese complex*

Cephradine 2 millimole (0.800 g) was dissolved in 10 ml methanol. To this was added manganese chloride tetrahydrate 1 millimole (0.1979 gm) dissolved in methanol (5 ml). The resultant clear solution was refluxed on a water bath for 2-3 hours and then allowed to crystallize at room temperature. Golden crystals, m.p. 210°C was obtained.

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 CHN analyzer and metals were estimated using atomic absorption spectrophotometer at the Department of Chemistry, University

of Karachi, using Pye-Unicam AA Spectrometers. 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 Mg 285.2 nm, Ca 422.7 nm, Cr 357.9 nm & Mn 279.5 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

1. *Samples*

Cephradine and its metal complexes with magnesium, calcium, chromium and manganese synthesized as above, were used in these studies.

2. *Organisms*

The organisms were 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*.

3. *Media & antimicrobial disks*

Nutrient broth and Mueller Hinton Agar were used as media in these experiments. The antimicrobial impregnated filter paper discs of samples and standards were used. Both samples and standard discs having concentration of 32 μg/ml were used. Trypto-soy broth was used for determining the sensitivity of bacteria against different antimicrobial agents.

4. *Sample Preparation*

0.016 gm of standard sample of cephradine and each of its metal complex was individually dissolved in minimum amount of methanol in a 10 ml volumetric flask and volume was made up to the mark with the same solvent. The solution had concentration 1600 μg/ml. This standard solution was used for disc spiking and was diluted to 8 μg/ml for MIC.

5. *Collection of Cultures*

The cultures of the organisms were collected. They were further rechecked morphologically and culturally. The nutrient agar containing Bacto tryptone 15.00 gm, Bacto soytone 5.00 gm, sodium chloride 5.00 gm & Bacto agar 15. 00 gm (pH 7 ± 0.2) was used for the collection and primary isolation of the organisms. 20 gm of dehydrated medium was suspended in 1 liter of distilled water, heated on boiling water bath till dissolved completely and autoclaved for 15 minutes at 15 psi pressure at I21°C.

6. *Preliminary Identification of the Cultures*

The Brolacin agar (Bromothymol-blue-lactose cystine agar) was used for further confirmation of identified organisms. 33 g of Brolacin agar powder was suspended in 1 liter of distilled water,

heated on boiling water bath till dissolved completely and then autoclaved for 15 minutes at 15 psi pressure at 121°C.

7. *Sub culturing and Maintenance of Different Organisms*

The stock culture agar was used for the maintenance and sub-culturing of different organisms. It is a soft, almost semisolid medium. The success of the medium probably lies in the fact that the medium contains a small quantity of dextrose, which serves as a readily available source of energy. This medium, supports luxuriant growth of pathogenic bacteria and preserve their viability over a longer period of time.

8. *Turbidity Standards*

Once an actively growing broth culture or suspension of colonies was obtained, the turbidity was adjusted to match that of a $BaSO_4$ standard (a 1:2 dilution of a McFarland standard). Turbidity standard was prepared by adding 0.5 ml of 0.048 M $BaCl_2$ to 99.5 ml of 0.35 N H_2SO_4 and is agitated on a vortex mixer just before use. Its optical density is 0.08 - 0.10 at 625 nm.

Portions of each of four or five isolated colonies of same morphological type were touched with a sterile wire loop, suspended into tubes containing 5 ml of Trypto-Soy Broth. This medium was then incubated at 35°C for 2-8 hours until the turbidity reached. If turbidity exceeded than standard, it was diluted with broth until it was visually comparable with standard.

9 *Medium for inoculation*

The Mueller-Hinton agar composition was used for preparing inoculum (Mueller and Hinton 1941; National Committee for Clinical Laboratory Standards (NXXLS) 1973; Bauer *et al.,* 1966;), which is performance standard for antimicrobial disc susceptibility test, as used in clinical laboratories. The MHA was prepared by suspending 21 gm of MHA in 1 liter of distilled water, heated on boiling water bath till dissolved completely and then transferred into test tubes, each containing about 4-5 ml of MHA. It was then autoclaved for 15 minutes at 121°C.

10. *Assay plates*

Flat bottom petri dishes with internal diameter of 150 mm were used in the procedure. Bottles containing MHA were allowed to equilibrate to 50°C on a water bath. The medium was poured into sterile petri dishes on a level surface to a depth of 4 mm and allowed to harden. The pH of the medium was in between 7.2 and 7.4. The medium was allowed to dry for 15 to 30 minutes before use or storage at 4°C. Agar plates to be stored for longer than 7 days were wrapped in plastic to prevent excessive evaporation of moisture. Sterile swabs available commercially were used for the inoculation of the organism into the petri dishes.

11. *Test procedure of antimicrobial susceptibility testing*

Portions of each of four or five well-isolated colonies of same morphological type were touched with a sterile wire loop, suspended into tubes containing 5 ml of trypto-soy broth. This medium was then incubated at 35°C for 2-8 hours until the turbidity reached. If turbidity was exceeded than standard, it was diluted with broth until it was visually comparable with standard.

12. *Streaking and Disk placement*

A sterile swab was dipped into a broth suspension of organisms within 15 minutes of inoculum standardization. Excess inoculum was removed by rotating the swab several times against the wall of the tube above the fluid level. The entire surface of a Mueller-Hinton agar plate was then streaked evenly in three/two directions approximately 60 degree from each other. The lid was then replaced and was allowed to dry for 3 to 5 minutes. The antimicrobial-impregnated disks

were placed with sterile forceps on to the agar surface. The disks were distributed such that, they were at least 24 mm apart so as to avoid overlapping during incubation. The plates were then incubated at 37°C for 18-24 hours.

After 18-24 hours of incubation, the diameter of zones of inhibition around each disk was measured with a varnier 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 assessing and comparing the biological activity of these drug metal complexes with their parent antibiotics.

The interplay of β-lactam development and β-lactam resistance has been a major driving force in β-lactam research. The early clinical success of benzyl penicillin against *Staphylococcus aureus* in the forties was followed by the emergence of penicillinase associated resistance, leading to the development of penicillinase-resistant penicillins (e.g. methicillin, oxacillin etc.) (Hoover and Dunn, 1979).

The addition of the amino group at α-carbon of benzyl penicillin gave rise to ampicillin, extending the activity spectrum to Gram-negative bacteria. The success of aminopenicillins in the 1960's led to the appearance of plasmid mediated enzymes in the enterobacter and pseudomonas and the spread of these enzymes to other pathogens (N. gonorrhoeae and H. influenzae) in the following decade. This in turn provided the impetus for a massive synthetic effort, resulting in the first generation cephalosporin, such as cephalexin and cephradine.

The 6α-acylamino side chain characteristic of most penicillins was replaced in the 1970's by an acyl group with an N, N-dialkyl formamidine, giving rise to mecillinam (Lund and Tybring, 1972). This compound is very active against many Gram-negative bacteria, though relatively inactive against Gram-positive bacteria. It illustrates a non-acyl type of modification on the 6-position of penicillin that broadens the antibacterial profile (due to its limited use, resistance to this compound has not occurred). The development, success and non-spread use of β-lactam resistant cephamycins and oxyimino cephalosporins in recent years have led to the emergence of methicillin-resistant *Staphylococci* and Gram-negative organisms that overproduce chromosomal and plasmid mediated, extended spectrum, β-lactamases.

Differing C-7 acyl groups produce significant activity changes in both potency and spectrum of activity (Roberts, 1992). Since the 7-ACA molecule can be modified at two sites, the C-3 and C-7 amino positions, an enormous number of semi-synthetic derivatives were produced. In general, altering the substituents at the C-3 position affects pharmacokinetic behavior and metabolic stability, while changes at the 7-position affect antibacterial activity and β-lactamase stability (Kliebe *et al.,* 1985 and Williams, 1987).

The cephradine have widespread use for the treatment of upper respiratory, urinary soft tissue and a variety of other infections (Tan and Salstrom, 1984 and Weinstein, 1980). There are possible alternatives to benzylpenicillin in allergic patients for the treatment of streptococcal, pneumococcal and staphylococcal infections; because of the prevalence of resistant strains they

are not recommended as penicillins substituents in the prophylaxis or treatment of clostridial infections (Nord *et al.,* 1989 and Norrby, 1986).

Cephradine is less effective than established agents as single dose therapy for uncomplicated urinary infection, but may be useful for the long-term treatment of selected patients (Neu, 1984). With persistent or recurrent infection due to sensitive organisms, it is inactive against enterococci which may emerge in the course of treatment. It has also been used for the prophylaxis and treatment of bone and joint infections. The drug however, should not be used for the treatment of infections in which H. influenzae is known or likely to be implicated (Yogev, 1986).

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 are 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 magnesium, calcium, chromium and manganese with cephradine by refluxing them in methanol in different molar ratios in a water bath at 70°C led to the formation of complexes having different characteristics. Various physical characteristics like melting points, color, state and solubility in solvents like methanol, chloroform and dimethylsulfoxide are given in *table 1*. Infrared absorption spectrum of cephradine in the region 3500 – 600cm^{-1} is given in Fig. 2 and the details of absorption showing frequencies (cm^{-1}) of cephradine metal complexes are given in Table 2, while the CHN and metal analysis of these complexes are reported in Table 3.

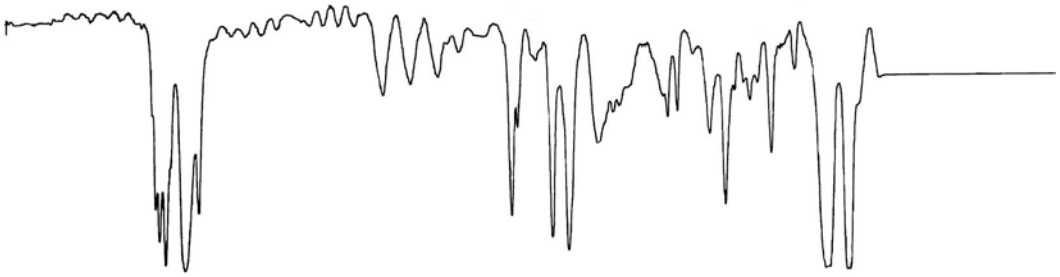


Fig. 2

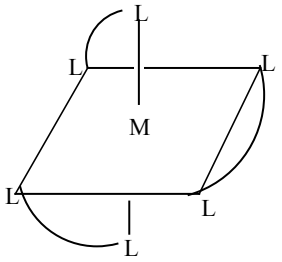
Infrared Absorptions of Cephradine Metal Complexes

β-lactam C=O and COO⁻ Stretching Frequencies

The β-lactam infrared stretching frequency (υ 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.

On the basis of infrared spectral characteristics (Table 2), elemental analysis, (Table 3) and magnetic susceptibility measurements, magnesium and calcium cephradine metal complexes have been assigned a tetrahedral geometry, having sp^3 hybridization, while chromium and manganese cephradine complexes an octahedral geometry having d^2sp^3 hybridization (Fig. 3).



Cephradine metal complexes
in d^2sp^3 confiugration

Fig. 3

Cephradine forms complexes with transition metal ions in which not only 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. 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.

Conductometric and potentiometric studies of metal complexes with cephaloridine have been studied (Chakrawarti *et al.,* 1993) and this indicates that cephaloridine forms 1:1 complex with the metal ions as Mg, Mn, Fe, Ni, Co and Zn, which takes place in stepwise manner but with copper there is the simultaneous formation of complex. The analytical data indicate that the composition of the complexes is $ML.2H_2O.2R$. 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 (fig. 4). IR spectra show that most of the bands of the ligands 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-lactone 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} in cephaloridine 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 difference between asymmetric and symmetric frequencies of COO^- suggests that the complexes have considerable degree of covalency in the M-Oxy bond. The presence of water finds conformation (Kabayashmi *et al.,* 1956) in the band at around 1600 cm^{-1}.

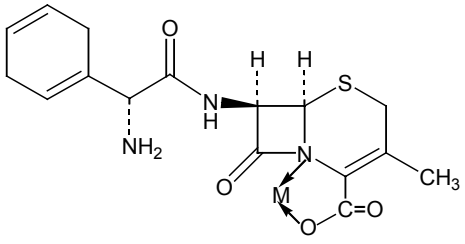


Fig. 4

A number of complexes of cephradine with uranyl (II) ion have been investigated using potentiometric titrations (Shoukry *et al.,* 1995). These have been synthesized and characterized by elemental analysis, conductivity and IR spectroscopy and from these studies it was proposed 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.

The metal complexation behavior of cephradine have also been studied by Krimpen *et al.* (Krimpen *et al.,* 1988). The emphatic metal ions of class B as Ag(I), Hg(II), and $C_6H_5Hg(I)$ form the most stable complexes with the drug.

C=N and C=N-O stretchings

IR spectra show that while most of the bands of the ligand remain unchanged on complexation, there is a considerable shift in the frequencies of -N-C=O and C=N (tertiary nitrogen of the carboxylic group of the ring) appearing at 1540 cm^{-1} and 3075 cm^{-1} in cephradine to approximately 1520-1560 cm^{-1}.

Divalent 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.

II Antibacterial Studies of Cephradine Metal Complexes

Cephradine showed antimicrobial activity against various human pathogens. 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.

Salmonella typhi and *Escherachia coli* were resistant to cephradine magnesium complex, while *Staphylococcus aureus*, *Corynebacterium diptheriae*, *Streptococcus pyogenes*, *Proteus vulgaris* and *Streptococcus faecalis* showed susceptibility.

Cephradine on complex formation with calcium showed moderate activity against Corynebacterium *diptheriae*, *Streptococcus pyogenes*, *Corynebacterium hoffmanni*, *Klebsiella pneumoniae* and *Streptococcus faecalis*, while *Salmonella typhi*, *Proteus vulgaris* and *Staphylococcus aureus* were resistant to complex.

Salmonella typhi, *Klebsiella pneumoniae Proteus vulgaris* and *Streptococcus faecalis* were resistant to cephradine chromium complex, while the activity of complex on other organisms such as *Escherachia coli*, *Corynebacterium hoffmanni*, *Corynebacterium diptheriae*, *Streptococcus pyogenes* and *Staphylococcus aureus* was moderate.

Cephradine manganese complex showed activity on most of the organisms. *Corynebacterium diphtheria*, *Streptococcus pyogenes*, *Corynebacterium hoffmanni* and *Proteus vulgaris* were found susceptible. *Salmonella typhi* and *Klebsiella pneumoniae* showed moderate activity. *Escherachia coli*, *Staphylococcus aureus*, showed mild activity but *Streptococcus faecalis* was resistant to complex.

Table 1
Physical characteristics of cephradine metal complexes

S.No.	Complex	Color	State	M.P °C	Solubility in		
					CH_3OH	$CHCl_3$	DMSO
1	Cephradine magnesium	Yellow	Crystalline	210	soluble	75%	soluble
2	Cephradine calcium	Yellow	Crystalline	220	soluble	60%	soluble
3	Cephradine chromium	Dark green	Crystalline	252d	soluble	75%	soluble
4	Cephradine manganese	Golden	Crystalline	210	soluble	75%	soluble

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-Mg	Magnesium complex	660sm, 700sm, 850m, 965m, 1080s, 1280s, 1325s, 1420-1450s, 1670dbs, 1760 db,m, 3000m, 3450-3500db,s.
3.	CPH-Ca	Calcium complex	450, 690s, 750m, 960m, 1250br, 1420-1450br, 1680db,s, 1750m, 3300br.
4.	CPH-Cr	Chromium complex	680m, 840s, 960m, 1070s, 1170s, 1260-1280m, 1380s, 14401460db,m, 1520m, 1630s, 1670s, 1760m, 3400-3500br.
5.	CPH-Mn	Manganese complex	660sm, 700sm, 750m, 960m, 1250br, 1440db, 1670db,s, 1750m, 3300br.

Table 3
Elemental analysis of cephradine metal complexes

S. No.	Compound	Elements →	C	H	N	S	X	M
1.	$[Mg(CPH)_2]Cl_2$	Calculated	48.41	4.82	10.58	8.08	8.93	3.06
		Found	48.49	4.52	10.63	8.00	9.01	3.05
2.	$[Ca(CPH)_2]Cl_2$	Calculated	47.46	4.73	10.38	7.92	8.76	4.95
		Found	47.41	4.83	10.53	7.87	8.80	5.03
3.	$[Cr(CPH)_2Cl_2]$	Calculated	46.77	4.66	10.23	7.80	8.63	6.33
		Found	46.56	4.81	10.10	7.56	8.78	6.45
4.	$[Mn(CPH)_2Cl_2]$	Calculated	46.61	4.64	10.19	7.78	8.60	6.66
		Found	46.51	4.33	10.45	7.54	8.74	6.67

Table 4
Antimicrobial susceptibility of cephradine magnesium, calcium, chromium and manganese complexes

S. No	Organism	Zone of Inhibition				
		Standard	Mg-complex	Ca-complex	Cr-complex	Mn-complex
1	*Salmonella typhi*	10.40 - 12.00	07.60 - 15.00	0.09 - 16.60	00.04 - 07.00	16.09 - 29.50
2	*Shigella dysentery*	04.90 - 10.40	06.40 - 07.90	16.00 - 21.00	05.50 - 06.00	25.00 - 42.00
3	*Corynebacterium diptheriae*	16.60 - 34.50	14.00 - 30.20	13.00 - 18.00	13.00 - 16.00	19.00 - 38.00
4	*Streptococcus pyogenes*	09.30 - 13.00	15.90 - 37.40	14.00 - 15.00	08.00 - 14.00	14.10 - 21.40
5	*Proteus vulgaris*	16.00 - 36.40	12.00 - 27.40	00.50 - 04.00	04.00 - 09.00	16.30 - 37.00
6	*Staphylococcus aureus*	04.60 - 11.70	23.00 - 44.40	04.00 - 09.00	07.70 - 10.20	13.90 - 30.20
7	*Escherachia coli*	25.00 - 38.00	08.00 - 17.00	08.56 - 10.33	10.00 - 13.00	13.00 - 25.00
8	*Corynebacterium hoffmanni*	15.00 - 39.60	13.00 - 29.30	13.00 - 17.00	12.00 - 18.00	19.40 - 40.20
9	*Streptococcus faecalis*	14.30 - 29.50	16.00 - 34.40	10.00 - 12.12	04.00 - 09.00	04.00 - 07.00
10	*Klebsiella pneumoniae*	12.00 - 14.70	15.40 - 32.60	16.00 - 21.00	03.00 - 06.20	17.50 - 39.40

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