Circular Dichroism Studies of Co(II), Ni(II) and Cu(II) Complexes of Antibiotic (+)-Ampicillin Formed in Solution

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Summary: Circular dichrosim studies show that (+)-Ampicillin, a broad spectrum β-lactam antibiotic, forms soluble complexes with Co(II), Ni(II) and Cu(II) ions in aqueous solution. Complexation of these metal ions with (+)-ampicillin ((+)-amp) is indicated by appearance of CD bands in crystal field region. In solution, complexes of composition $[M((+)\text{-amp})_2]^{2+}$ are formed at pH 6.5-8.0 which have 2-3 Cotton bands in 14,706-23,697 cm^{-1} region. The CD band ellipticity is linearly increased with concentration of the components under optimum conditions between 0.002-1.0 mol L^{-1}. The Cotton bands in these systems are interpretted in terms of electronic excitations to unidimensional levels arising due to lower symmetry of these complex ions.

Introduction

(+)-Ampicillin (I) is a broad spectrum antibiotic widely used for therapeutic purposes. It belongs to second generation of β-lactam cephalosporin drugs and contains a number of potential donor atoms like N,O,S. However, metallic derivatives of ampicillin have been investigated to a lessor extent. The synthesis and antibacterial activity of a cobalt(II) complex of apicillin was reported from our laboratory [1]. It is interesting to note that $[Co((+)\text{-amp})_2(H_2O)_2]^{2+}$ is more effective antibiotic as compared to ampilcillin itself [1] while Fe(III), Ni(II), Zn(II) and Pd(II) complexes of this antibiotic were found ineffective against various gram positive and gram negative bacteria [2]. The difference in antibacterial activity of $[Co(+)amp)_2]^{2+}$ has been attributed to different metal ligand binding sites than other complexes of this series.

(I)
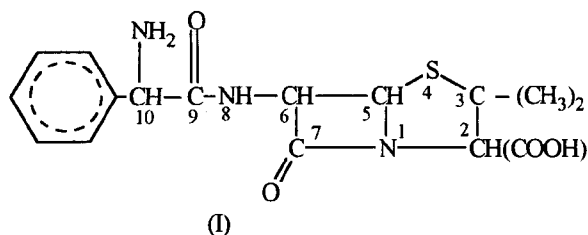
The metal complexes of (+)-ampillicin have low solubility in water and many organic liquids, rendering them unsuitable for antibacterial studies. In biological systems, solubility of drugs play important role in determining their effectiveness. Further, presence of trace amounts of a number of transition metal ions in biological systems may control the antibacterial effects of drugs through complexation. Obviously complex forming ability

of antibiotics with essential metal ions in solution is of utmost importance as model systems. Further, (+)-ampicillin, like a number of other antibiotics in this class is optically active and its complexation with metal ions may be indicated by appearance of CD bands in d-d transition region. Therefore, circular dichroism spectra of aqueous solutions of various transition metal ions in presence of (+)-ampicillin were studied, parameters like stoichiometry, pH, concentration range for maximum complexation are reported in this paper. These studies indicated that Co(II), Ni(II) and Cu(II) ions react with (+)-ampicillin between pH 6.5-8.0 to form complexes of stoichiometry $[M((+)\text{-}amp)_2]^{n+}$. The complexation is linearly increased with concentration between 0.002-1.0 mole L^{-1} of the components. The observed CD bands in these systems are assigned to electronic excitations to unidimensional upper levels within d-orbitals.

Results and Discussion

The CD spectrum of aqueous solution of (+)-ampicillin consists of only one strong positive Cotton band at 44,446 cm^{-1}. Complexation of this drug with bivalent ions Co(II), Ni(II) and Cu(II) is indicated with the appearance of Cotton bands in lower frequency crystal field region. The CD spectra of aqueous solutions of these metal ions in presence of (+)-amp. in 1:4 molar ratio invariably consist of 2-3 Cotton bands in 14,706-23,697 cm^{-1} region. Further studies on these systems were carried out with reference to their respective CD band maxima.

(+)-Ampicillin is insoluble in water. Its aqueous solution was prepared in dilute sodium hydroxide solution (pH 8-9). The metal ions dissolved in water have pH 4.00, while pH of the mixed solution of metal ions and the ligand is between 6-7. The complexation of (+)-amp. with these metal ions in solution is pH dependent. The effect of pH on ellipticity of Cotton band in these systems is shown in Fig. 1. It is observed that CD band ellipticity gradually increases as pH of these systems is raised. The ellipticity of Cotton band is maximum for systems (i) Co(II)-(+)-amp. at pH 7.5, (ii) Ni(II)-(+)-amp. at pH 8 and (iii) Cu(II)-(+)-amp. at pH 6.5. Further increase in pH of these systems resulted in precipitation of the complex. The ligand is a weak carboxylic acid which is soluble as its sodium salt. In basic medium two molecules of monobasic anionic drug interact with
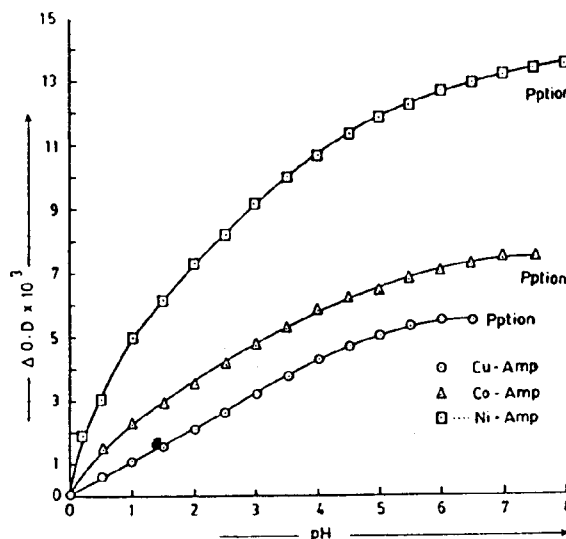

Fig. 1: Effect of pH upon differential optical density at Cotton band maxima of metal-(+)-ampicillin system.

bivalent metal ions to form neutral, water insoluble complexes. However, formation of soluble complexes indicates that at least one proton remains attached to the ligand maintaining ionic nature of these species. Subsequent complexation studies in these systems were carried out at their respective pH values.

The stoichiometry of complexes formed by these metal ions with (+)-amp. in aqueous solution was determined by varying mole fraction of components under optimum conditions of pH and temperature using CD spectra. The differential optical density (Δ O.D) at Cotton band maxima was plotted against mole fraction of the metal ions as shown in Fig. 2. The metal ion to ligand ratio was determined by extrapolating and joining the two lines from extreme positions of the curve. It is found that Co(II), Ni(II) and Cu(II) ions form complexes of 1:2 metal: ligand composition with (+)-amp. This is in agreement with our earlier reported composition of an isolated complex of this ligand as $[Co(+)\text{-}amp)_2 (H_2O)_2]Cl_2$ [1].

The effect of varying concentration of metal ions and ligand upon complexation equilibria in these systems was studied between 2 x 10^{-2}-1.0 mole L^{-1} maintaining optimum conditions of pH and metal ligand ratio. The plots of Δ O.D. vs concentration of complexes in these systems were
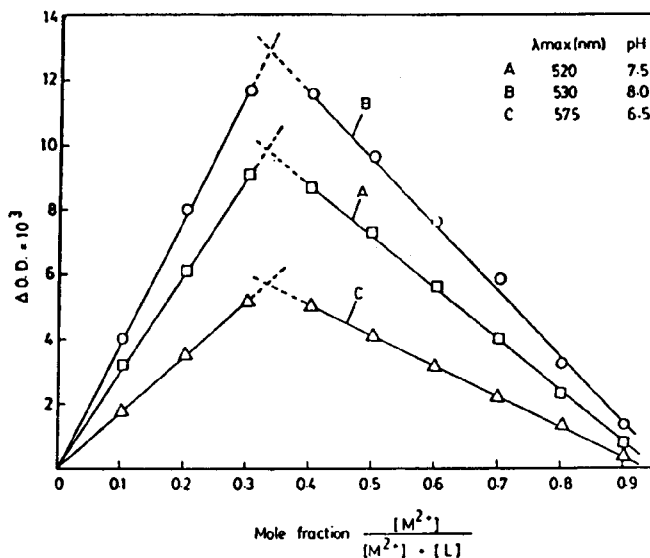
Fig.2:   Mole ratio plot for determining stoichiometry of metal complexes of (+)-ampicillin in water. (A).
Co(II)-(+)-amp. (B). Ni(II)-(+)-amp. (C). Cu(II)-(+)-amp.

essentially straight lines originating from zero. It may be inferred that complexation in these systems quantitatively increased with increasing concentration of the components in fixed ratio.

It is apparent from these studies that two molecules of (+)-amp. are coordinated with each metal ion. At room temperature maximum complexation occurred between pH 6.5-8.0. (+)-Ampicillin has a number of potential donor atoms like N, O and S. Both S and N donors are part of a five membered bicyclic ring which cannot form a chelate. Further, complexation through S donor (soft base) with relatively harder metal ions Co(II), Ni(II) and Cu(II) is also unlikely under mild conditions used. However, chelation of these metal ions through carboxylic oxygen (C-2), N-1 or oxygen at C-7, N-8 or oxygen at C-9, nitrogen at C-10 of the ligand is conveniently possible. The positions of various donor atoms in (+)-amp. permit only bidentate chelation with metal ions. The ligand may form five membered chelate with metal ions either through carboxylate oxygen and cyclic nitrogen at position 1 or carbonyl oxygen (C-9) and primary amine attached to C-10 in the side chain. Further, in (+)-amp., a number of asymmetric carbon atoms i.e. C-2, C-5 and C-6 in the bicyclic ring and C-10 attached to the amide part of the side chain are present. The optical activity of the ligand arising from these asymmetric

centres should be affected differently upon complexation with the metal ions. If the ligand is coordinated through donor atoms present far away from the chiral centers, optical activity/CD is very little affected. In such cases, weak Cotton bands are induced in crystal field spectral region. On the other hand if asymmetric atom is a member of the chelate, marked changes in optical activity and CD spectra of complexes are observed [3,4]. CD spectra of complexes containing asymmetric centres in the chelate usually consist of well defined and relatively stronger Cotton bands in d-d transition region. In the present series of complexes, asymmetric atoms either C-2 or C-10 become part of five membered chelates if binding sites stated earlier are used by the metal ions. However, chelation through carboxylate oxygen and cyclic nitrogen (N-1) should immediately cause precipitation of a neutral species while chelation through primary amine nitrogen at chiral C-10 and oxygen of C-9 should give a soluble cationic species. The possibility of loss of proton through enol formation in this bonding position is ruled out since the complexes remain in solution at the working pH. Further, formation of enolate as a part of chelate should generate planar, symmetrical carbon atom at position 10. It is, therefore proposed that (+)-ampicillin behaves as a bindentate neutral ligand coordinating through oxygen attached to C-9 and amine nitrogen of asymmetric carbon (C-10)

in these systems. These metal-ligand bonding sites are similar to those proposed for pencilline-G complexes by earlier workers [5].

In aqueous solution, each metal ion is bonded with two ligands and two water molecules occupy trans position on an octahedran as shown below:

C_6H_5

R= —NH—CH—CH—S
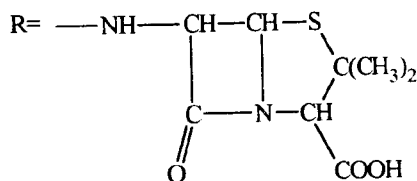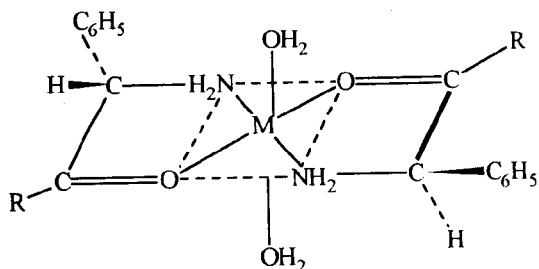
M = Co (II), Ni (II), Cu (II).

The absorption spectra of these solution systems consist of single broad bands between 14,599-23,923 cm^{-1} (ε 38-155.2 $L.mole^{-1}cm^{-1}$) in crystal field region. The intensity of these bands is typical of octahedral species in solution. The absorption spectrum of sodium (+)-ampicillinate consists of an intense band at 48,077 cm^{-1} (ε 11.93 x 10^3 $L.mole^{-1}cm^{-1}$) which is shifted to 44,444-45,872 cm^{-1} with the appearance of a number of shoulder bands in these complex ion systems.

The CD spectra of these systems under optimum conditions consist of a number of well defined Cotton bands between 14706-31,250 cm^{-1}. The data on absorption as well as CD spectra alogwith optimum conditions are given in Table-1 and CD spectra reproduced in Fig. 3.

These complex ions have C_{2v} or D_{2h} symmetry depending upon cis or trans attachment of two ligand molecules with metal ions. As (+)-amp is a chiral ligand, the local symmetry of these species never exceeds C_1.

Octahedral complexes of Co(II), d^7 and Ni(II), d^8 have same energy levels [i.e T_{1g}, T_{2g}, A_{2g}
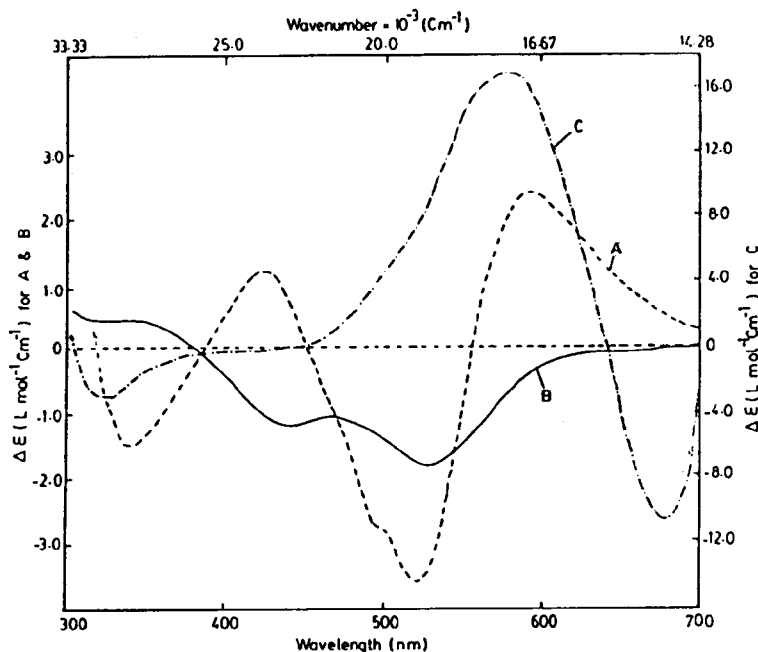

Fig.3: Circular Dichroism Spectra of: (A). [Co ((+)-amp.)$_2$(H$_2$O)$_2$]$^{2+}$ (B). [Ni(+)-amp.)$_2$ (H$_2$O)$_2$]$^{2+}$ (C). [Cu((+)-amp.)$_2$ (H$_2$O)$_2$]$^{2+}$ formed in aqueous solution (concentration 0.1 mol L^{-1}).

Table-1: Optimum conditions, absorption and CD spectra of metal complexes of (+)-ampicillin formed in aqueous solution

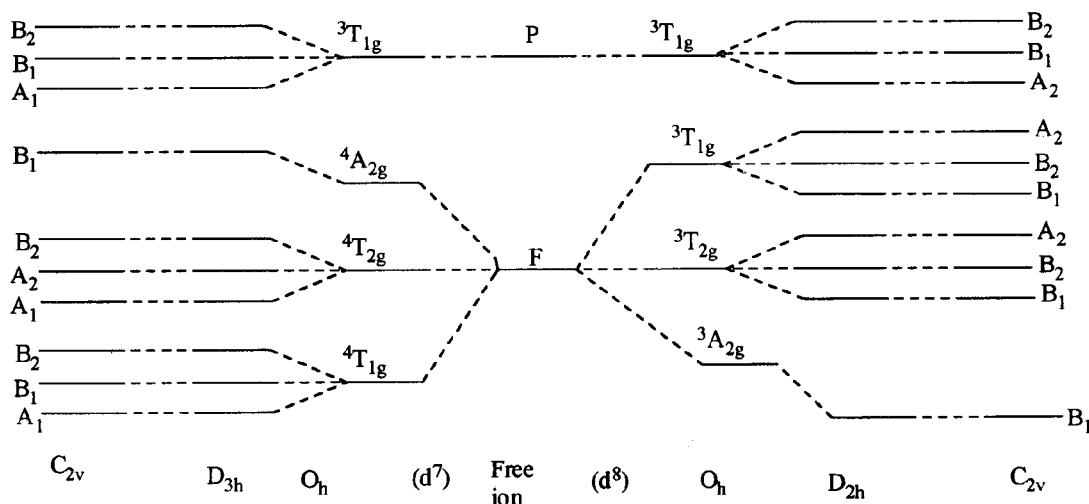|	Complex	Metal: Ligand Ratio	pH	Absorption $v(cm^{-1})$	Bands $\varepsilon {}^*M^{-1}cm^{-1}$	Circular $v(cm^{-1})$	Dichroism bands $\Delta\varepsilon\ {}^*M^{-1}cm^{-1}$	Band assignments
	(+)-Ampicillin			48,077	1.19×10^4	44,444		
1.	[Co((+)-amp)$_2$ (H$_2$O)$_2$]$^{2+}$	1:2	7.5	45,455	1.1×10^4			
				38,462	3.75×10^3	29,240	-1.51	MLCT
				29,586	1.12×10^3	23,697	+1.15	$^4A_1 \longrightarrow {}^4B^2({}^4T_{1g}(P))$
				20,408	155.2	20,408	-2.97	$^4A_1 \longrightarrow {}^4A_2\ ({}^4T_{1g}(P))$
						19,231	-3.94	$^4A_1 \longrightarrow {}^4B_1\ ({}^4T_{1g}(P))$
						16,949	+2.18	$^4A_1 \longrightarrow {}^4B_1\ ({}^4A_{2g})$
2.	[Ni((+)- amp)$_2$(H$_2$O)$_2$]$^{2+}$	1:2	8.0	45,872	1.08×10^4			
				38,462	6.0×10^3			
				29,412	2.55×10^3	31,250	+0.46	MLCT
				23,923	174.0	27,727	-1.5	$^3B_1 \longrightarrow {}^3B_2({}^3T_{1g}(P))$
				19,231	38.0	18,868	-2.2	$^3B_1 \longrightarrow {}^3A_2({}^3T_g(P))$
3.	[Cu((+)amp)$_2$(H$_2$O)$_2$]$^{2+}$	1:2	6.5	44,444	1.15×10^4	31,250	-3.1	MLCT
				40.323	$5.4 / \times 10^3$	17,391	+16.67	$^2A_1 \longrightarrow {}^2B_1$
				14,599	101.3	14,706	-9.6	$^2A_1 \longrightarrow {}^2B_2$

*Calculated on the basis of concentration of the metal ion

and T$_{1g}$(P)] of highest spin multiplicity, however these energy levels are inverted in d^8 case. The triply degenerate states split to give unidimensional levels like A$_1$, A$_2$, B$_1$, and B$_2$ in complexes of C$_{2v}$ or D$_{2h}$ symmetry as depicted below:

Absorption spectra of octahedral complexes of these metal ions consist of three bands, v$_1$, v$_2$, and v$_3$, due to excitation of electrons to various levels. However, separate absorption bands due to excitation of electrons to split levels are not observed and only band broadening occurs in lower symmetry complexes. The CD spectra of lower symmetry complexes usually consist of separate Cotton bands due to electronic excitations to unidimensional energy states. This is apparent from the CD of present series of complexes.

The absorption spectrum of [Co((+)-amp)$_2$]$^{2+}$ ion consists a broad weak band at 20, 408 cm^{-1} (ε 155.2 L. mole^{-1}cm^{-1}) which may be assigned to $^4T_{1g} \rightarrow {}^4T_{1g}$(P) transition. A number of very intense bands at 29,586 cm^{-1}, 38,462 cm^{-1} and 45,455 cm^{-1} are also observed in this system. The band at 45,455 cm^{-1} is also present in the spectrum of (+)-amp and arises due to π→π* transition. The remaining two bands arise from metal to ligand charge transfer transitions.

Energy level correlation diagram (left to right): C$_{2v}$ — D$_{3h}$ — O$_h$ — (d^7) — Free ion — (d^8) — O$_h$ — D$_{2h}$ — C$_{2v}$.

Levels shown: $^3T_{1g}$ (P) splitting to B$_2$, B$_1$, A$_2$; $^4A_{2g}$; $^3T_{1g}$; $^4T_{2g}$; $^3T_{2g}$; $^4T_{1g}$; $^3A_{2g}$, with C$_{2v}$/D$_{2h}$ split levels A$_1$, A$_2$, B$_1$, B$_2$.

The solution CD spectrum of [Co((+)-amp)$_2$(H$_2$O)$_2$]$^{2+}$ (as produced in Fig. 3) consists of a number of Cotton bands, a positive band at 16,944 cm^{-1}, two negative bands IIa and IIb at 19,231 cm^{-1} and 20,408 cm^{-1}, a positive band IIc at 23,697 cm^{-1} and another negative band III at 29,240 cm^{-1}. The differential absorptivity ($\Delta\varepsilon$) values of these bands are between 1.15-3.94 L. mol^{-1} cm^{-1}. Further, band IIb is a prominent shoulder very close to band IIa indicating that these bands appear from electronic transitions to split level components of degenerate states. The band I may be assigned to $^4A_1 \rightarrow$ $^4B_1$ transition and compares with spin allowed v_2 in octahedral cobalt(II) complexes. The CD bands IIa, IIb and IIc are close enough to indicate that these arise from excitation of electrons to unidimensional levels (A,B) of $^4T_{1g}(P)$ state in octahedral symmetry. The shoulder CD band IIa is visible at 20, 408 cm^{-1} in absorption spectrum of this system. It is proposed that IIa, IIb and IIc arise from electronic excitations to $^4B_1$, $^4A_2$, and $^4B_2$ upper states respectively. The Cotton band III arises from allowed metal ligand charge transfer (MLCT) transition. This is also observed as a shoulder on the tail of very intense ligand absorption. The CD band assignments for IIa and IIc in this system are in good agreement with those of earlier reported CD spectrum of [Co((+)-CCS)$_3$]$^{2+}$ ion and 19,239 cm^{-1} to $^4B_1$ and 23, 256 cm^{-1} to $^4B_2$ [6].

The absorption spectrum of [Ni((+)-amp)$_2$ (H$_2$O)$_2$]$^{2+}$ consists of a number of very intense bands at 29,412 cm^{-1}, 38, 462 cm^{-1} and 45,872 cm^{-1} and weak broad bands at 19,231 cm^{-1} and 23,923 cm^{-1} in crystal field region. The CD spectrum of this system, under optimum conditions of pH and stoichiometry consists of two negative bands, I at 18, 868 cm^{-1} and II at 22,727 cm^{-1} and a weak positive band at 31,250 cm^{-1} with differential absorptivity values between 0.46-2.2 L mole^{-1} cm^{-1}. The two lower frequency CD bands are closely situated and arise from d-d transitions. The ground state in low symmetry nickel(II) (d^8) complexes is $^3B_1$. There are several closely spaced unidimensional upper states to which electronic transitions could occur. The first Cotton band at 18,868 cm^{-1} may be assigned to $^3B_1 \rightarrow$ $^3A_2(^3T_{1g}(P))$ and band II at 22, 727 cm^{-1} arises from excitation of electrons to $^3B_2(^3T_{1g}(P))$ upper level. These band assignments are comparable to the reported absorption bands in a number of lower symmetry nickel(II) complexes [7,8].

The absorption spectrum of [Cu((+)-amp)$_2$ (H$_2$O)$_2$]$^{2+}$ system consists of a weak broad band at 14,599 cm^{-1} (ε = 101.3 L.mol^{-1} cm^{-1}) and two very intense bands at 40,323 cm^{-1} and 44,444 cm^{-1}. The CD of this system consists of a couplet of bands, a negative band at 14,706 cm^{-1} and a positive band at 17,391 cm^{-1} in crystal field region. Another negative cotton band is observed at 31,250 cm^{-1}. All of these bands are associated with relatively high differential absorptivity values as 3.1-16.62 L.mol^{-1} cm^{-1} indicating that the complex ion has a square planar or highly distorted octahedral environment around the metal ion. The negative Cotton band at 31,256 cm^{-1} seems to arise from metal to ligand charge transfer (MLCT) transition. The negative Cotton band at 14,706 cm^{-1} may be assigned to d$_z$2 $\rightarrow$ d$_{xy}$ ($^2A_1 \rightarrow$ $^2B_2$) transition, while positive band at 17,391 cm^{-1} may arise from electronic transition between d$_x$2$_{-y}$2 $\rightarrow$ d$_{xy}$ ($^2A_1 \rightarrow ^2B_1$) orbitals. These band assignments closely agree with already reported CD bands in square planar [Cu(RR-chxn(BAA)$_2$)] as 14,837 cm^{-1} and 17,606 cm^{-1} taken in chloroform [9].

It may be concluded from these studies that chiral antibiotic (+)-ampicillin easily form water soluble complexes of composition [M((+)-amp.)$_2$)]$^{n+}$ with Co(II), Ni(II) and Cu(II) ions between pH 6.5-8.0 under room temperature conditions. The CD spectra of these systems may be fruitfully interpreted in terms of electronic excitations to unidimensional energy levels arising from lower symmetry of these species in solution.

Experimental

Chiral (+)-ampicillin as a free base was purchased from sigma biochemicals Co, U.S.A. The purity of (+)-ampicillin was checked by its infra-red spectrum and specific rotation measurements. The drug was stored in a refrigerator at temperature below 4°. All other metal salts of highest purity (analytical reagent grade) were obtained from E. Merck (Germany) and used without further purification. All solutions were prepared in double distilled water.

Solutions

Stock solution of (+)-Ampicillin

Accurately weighed sample of 4.03 g ampicillin trihydrate (0.01 mole) was suspended in a small volume of water and carefully titrated with

standard 1 mol L^{-1} solution of sodium hydroxide with shaking till all solid particles were completely dissolved. The clear solution was diluted to 100 mL. The contents of the flask were thoroughly mixed and kept in dark. The pH of this stock solution (8-8.5) was measured before its use for complexation studies.

Metal salt solutions

Aqueous solutions of metal chlorides (Co(II) or Ni(II) and copper (II) sulphate were prepared by dissolving requisite amounts of their hydrated salts in water and diluting to 100 mL so as to give 0.01-0.05 mol L^{-1} solutions.

Solutions for complexation studies

A series of 10 samples were prepared by mixing 1-10 mL stock solution of the metal ion with that of 10 mL of the ligand and adjusting their pH to 6 with dilute solutions of HCl/NaOH. These solutions were diluted to 25 mL, thoroughly mixed, kept in dark for 30 minutes and their CD spectra were recorded.

For determining the pH at which maximum complexation takes place, a series of samples were prepared by mixing appropriate volumes of the stock solutions of the metal ion, and the ligand maintaining 1:4 molar ratio. pH values of these solutions were varied in the range 1-10 with dropwise addition of 0.1 mol L^{-1} solution of NaOH/HCl. The samples were diluted to 25 mL and their final pH determined before recording CD spectra.

For determination of stoichiometry of the complex ions, different volumes of stock solutions of the metal ion and (+)-ampicillin were mixed so as to obtain gradual variation in mole fraction of the components. These samples were brought to optimum pH and diluted to 25 mL. The contents of the flasks were thoroughly mixed and their CD spectra were recorded. The differential optical density (Δ O.D) at Cotton band maxima were plotted as function of mole fraction of the metal ion. Stoichiometry of the complexes formed was determined by extrapolating the two lines from extremes and obtained corresponding mole fraction for various systems as shown in Fig. 2.

Another set of samples in varying concentration of complexes maintaining metal: ligand ratio at 1:2.1 were prepared by mixing appropriate volumes of stock solutions of the two components, bringing them to appropriate pH with dilute solutions of HCl/NaOH and diluting them to 25 mL. The samples were kept in dark for 30-60 minutes and their CD spectra recorded. The Δ O.D. at Cotton band maxima of these samples were plotted as a function of concentration of the complex, giving a straight line plot emerging from the origin.

Instrumentation

pH measurements were carried out on Horiba model F-8 pH meter using a combination electrode system. The solution absorption spectra were recorded on Hitachi model 220-S spectrophotometer using a pair of 10 mm quartz cuvettes. Circular Dichroism spectra in aqueous solution between 300-700 nm were recorded on JASCO model 20A spectropolarimeter using 1-10 mm cylindrical quartz cells. The base line spectra were recorded using aqueous solution of the chiral drug maintained at appropriate pH.

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