Circular Dichroism Studies of Complex Forming Ability of (+)-Benzylpenicillin with VO(IV), Cr(III), Co(II) and Cu(II) ions in Alcohol Water (70:30) Solution

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Summary: Formation of complexes of antibiotic (+)-benzylpenicilline ((+)-BP) with VO(IV), Cr(III), Co(II) and Cu(II) ions in EtOH/MeOH:H₂O (70:30) solution has been studied through circular dichroism. The optimum conditions of pH, stoichiometry and time for achieving maximum complexation in these systems are reported. Their formation constants are also determined. The CD spectra of these complex ions formed in solution are interpreted.

Introduction

A number of β-lactam antibiotics e.g. penicillines, ampicillin, cephalexin, cephradine, cefixin etc. are optically active and possess N, O and S donor atoms at appropriate positions in molecular framework. These antibiotics form reasonably stable complexes with essential metal ions like VO(IV), Co(II), Cr(III), Fe(III),Ni(II), Ca(II), Mg(II) [1-3]. However, isolated complexes of these drugs are usually insoluble in most of the solvents and difficulties are encountered in assess-ing their potential as antibiotics. These drugs may interact with metal ions in water or water like solvents (e.g. MeOH and EtOH) forming soluble complexes. The interaction of these antibiotics with metal ions may be monitored through circular dichroism (CD) bands in the crystal field region developed due to induced chirality in the resulting chelates. A number of workers have utilized CD studies to investigate complex forming ability of antibiotics like (+)-cycloserine, (+)-ampicillin, (+)-cephradine, (+)-cepalexine and (-)-tetracyclines [4-6] in aqueous or mixed solvent systems. Penici-llines being first member of β-lactam antibiotics and consisting of a thiazolidine ring fused with a β-lactam, form stable complexes of composition $Fe(C_{16}H_{17}H_2O_4S)_2Cl$ and $Zn(C_{16}H_{17}N_2O_4S)_2$ [7]. The metal ligand binding

sites in these complexes were ascertained through spectroscopy. In 1988, researchers synthesised, characterised and determi-ned antibacterial activity of complexes of cloxacilline and benzylpenicilline with a number of metal ions [8]. However, complex forming ability of penicillines with essential metal ions in aqueous or non-aqueous solutions are not investigated to our knowledge.

In this paper, complexation of sodium (+)-benzylpenicillinate ((+)-BP) (I) with VO(IV), Cr(III), Co(II) and Cu(II) ions in alcohol:water (70:30) solution is investigated by their CD spectra developed due to vicinal effect. The optimum condition of pH, stoichiometry, time and range of concentrations of complexes formed in solution have been determined. The stability constants of these complex ion species are reported and CD bands assigned to electronic transitions.

(1) (+)-BP
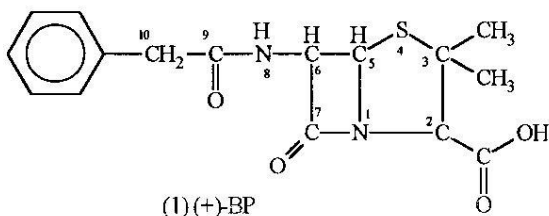
Results and Discussion

The complex forming ability of Na(+)-BP with VO(IV), Cr(III), Co(II) and Cu(II) was studied in alcohol:water (70:30 v/v) mixed solvent. When aqueous solutions of these metal ions were mixed with that of Na(+)-BP, immediate precipitation occured. The precipitated complexes were redissolved upon addition of acid (thereby decreasing pH) and clear solutions were obtained at a pH of 1.0, but characteristic CD bands due to complexes were not observed in these systems. The ligand and metal salts are freely soluble in the mixed solvent. Interaction of VO(IV) with Na(+)-BP in EtOH:H$_2$O mixed solvent was indicated with appearance of fairly strong positive Cotton band at 18,349 cm^{-1} and a negative band at 25,000 cm^{-1} while that in Cr(III)-(+)-BP system in the same solvent consists of two fairly strong positive bands at 17,699 cm^{-1} and 24,096 cm^{-1} and a weaker negative band at 20, 202 cm^{-1}. Similarly, a number of oppositely signed CD bands were developed in Co(II)-(+)-BP in EtOH:H$_2$O

solution, the most prominent positive band at 19,048 cm^{-1} was chosen for subsequent studies. In case of Cu(II)-(+)-BP in MetOH:H$_2$O (70:30 v/v) solution, a positive Cotton band at 17,241 cm^{-1} in d-d transition region was developed and chosen for further investigation.

The complexation in these systems is dependent upon pH as apparent from Fig. 1. The differential optical density (ΔO.D.) at λ_{max} in VO(IV)-(+)-BP gradually increased with variation of pH between 1.5-2.5, then started decreasing as pH was further raised and finally rapidly increased between pH 4.0-5.0. The complex precipitated out at pH 5.2. Therefore, subsequent studies were carried out at pH 2.5 and 4.70. In case of Cr(III)-(+)-BP system, ΔO.D. gradually increased between pH 1.5-4.0, then rapidly increased between pH 4.0 - 5.0 and precipitation started above pH 5.2. The system was maintained at pH 5.0 during subsequent studies. The ΔO.D. at 19,048 cm^{-1} in Co(II)-(+)-BP slowly decreased upon raising pH between 0.5-2.0 and then rapidly increased between pH 2.5 - 7.0. The precipitation of complex started above pH 7.2 Therefore, solutions were maintained at pH 6.5 in subsequent studies of this system. The CD band ellipticity at 17,241 cm^{-1} in Cu(II)-(+)-BP in MeOH:H$_2$O (70:30) solution gradually increased between pH 1.0-4.0 and then started decreasing reaching to minimum at pH 6. Further studies of this system were carried out at pH 4.0.

The composition and quantity of complexes formed in these systems are also dependent upon time after mixing the components. The CD spectrum of VO(IV)-(+)-BP (Fig. 2) recorded within 2 hours after mixing the solutions, consists of a strong negative Cotton band at 18,182 cm^{-1}. After keeping the solution for about 48 hours, couple of fairly well defined bands i.e. a positive band at 18,182 cm^{-1} and a negative band at 23,256 cm^{-1} were developed in this system. The ellipticity of these bands attained maximum value after 55 hours and remained unaltered up to 203 hrs. Therefore, sample solutions of VO(IV)-(+)-BP maintained at pH 4.7, were kept for 48 hours before recording their spectra. The CD of Cr(III)-(+)-BP in EtOH:H$_2$O solution (Fig. 3) measured within 2 hours after mixing the solutions consists of a negative band at 17,699 cm^{-1} with two positive bands in higher frequency region. This
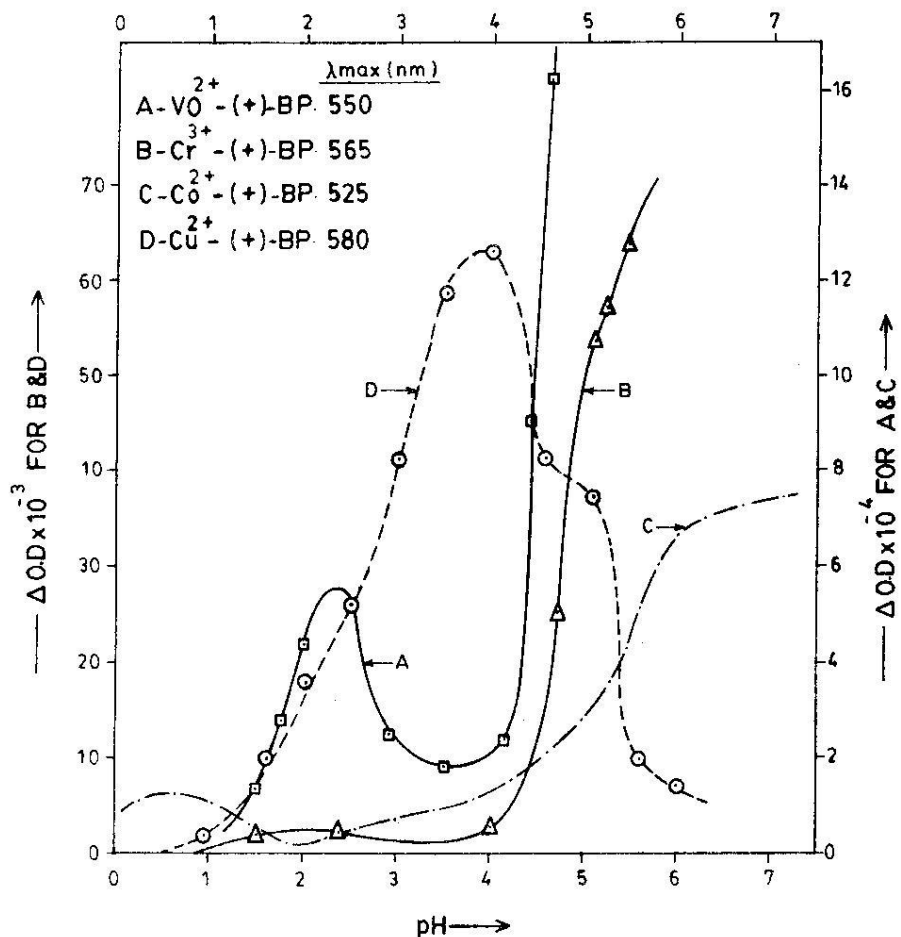
Fig. 1:   Effect of pH upon CD band height of Metal (+)-benzylpenicilline in alcohol water solutions.

spectrum was gradually changed with time and after 48 hours, strong positive Cotton band at 17,699 cm^{-1} alognwith others was developed which remained unaltered afterwards. The CD spectrum of Co(II)-(+)-BP in EtOH:H$_2$O solution did not change with time even after 50 hours. The CD of Cu(II)-(+)-BP in MeOH:H$_2$O solution, initially consists of a positive band at 16,667 cm^{-1} and negative band at 31,250 cm^{-1}. The negative Cotton band in this system disappeared with time and positive band shifted to 17,241 cm^{-1} and its ellipticity attained maximum value after 58 hours. The absorption band in Cu(II)-(+)-BP system initially at 21,277 cm^{-1} was also shifted to 16,807 cm^{-1} in 48 hours and afterwards remained unchanged.

The stoichiometery of the complexes formed in these systems was determined by varying mole fraction of the two components. It is found, that each of VO(IV), Co(II) and Cu(II) coordinate with two molecules of the ligand forming [M(+)-BP)$_2$ (H$_2$O)$_2$]$^{n+}$ species while Cr(III) reacted with three ligand molecules to form [Cr(+)-BP)$_3$]$^{n+}$. At lower pH other species like [VO(+)-BP)$_4$]$^{2-}$ and [Cr(+)-BP)$_2$]$^-$ have been identified from these studies.

The ligand, sodium (+)-benzylpenicillinate (I) has N, O and S donor atoms at various positions. It also possess three chiral centres (i.e., C-2, C-5 and C-6). It could form stable chelates with metal ions either through O of carboxylic acid and tertiary N in the bicyclic ring or carbonyl O of β-lactam ring and N of amide chain. Another possible chelation site through S of thiozolidine ring and N of linear amide chain is less probable because of softness of S atom. It is proposed that (+)-BP is bound to metal ions
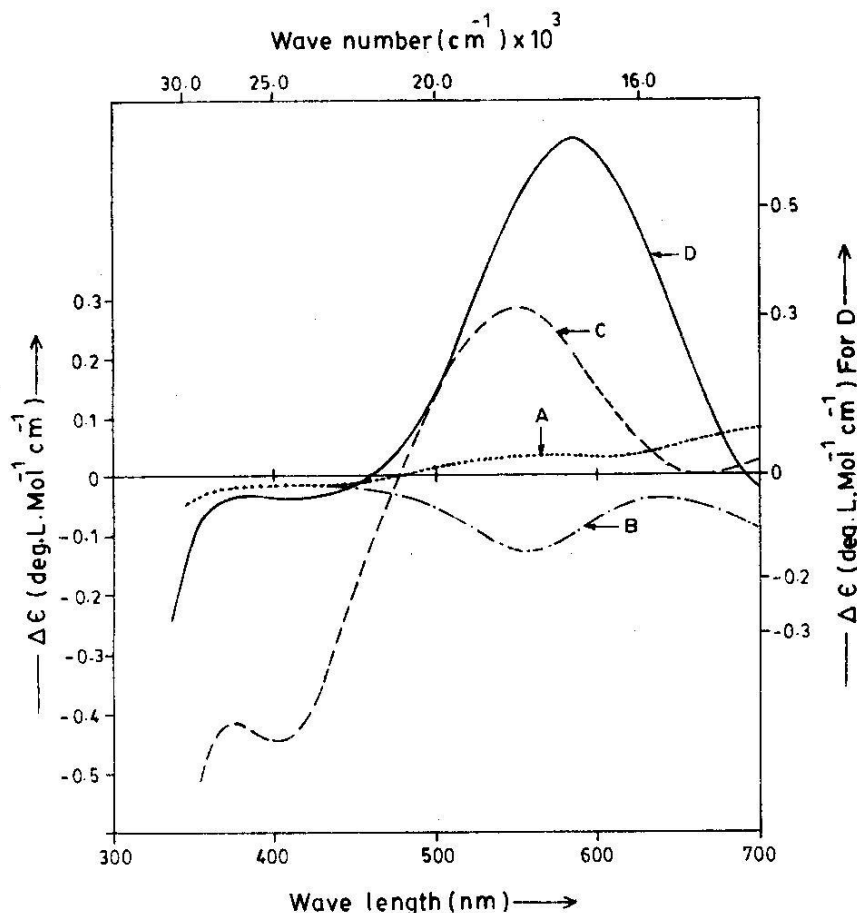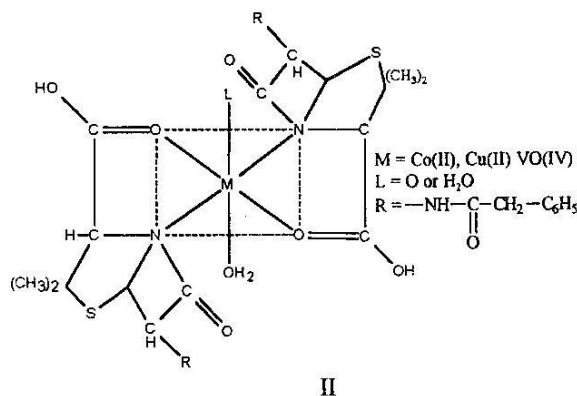
Fig. 2: Circular Dichroism spectra of (A) $[VO((+)-BP)^4]^{2-}$ at pH 2.5 recorded ater 2 hours (B) $[VO((+)-BP)_2H_2O]^{n+}$ at pH 4.7 recorded ater 2 hours (C) $[VO((+)-BP)_2H_2O]^{n+}$ at pH 4.7 recorded after 204 hours (D) $[Cu(+)-BP)_2(H_2O)_2]^{n+}$ at pH 4.0 recorded after 58 hours.

through its O of carboxylate and S of β-lactam ring forming five membered chelates as II. These bonding sites are in agrement with that proposed for isolated complexes like $[Fe(+)-BP)_2Cl]$ and $[Ni(+)-BP)_2]$ by earlier workers [9]. This bonding situation incorporates an asymmetric carbon (C-2) in the chelate and reasonably strong and well defined CD bands due to vicinal effect are observed. These complexes are formed in acidic conditions, therefore protons remain attached to carboxylic acid or carbonyl moeity of the ligand resulting soluble species. The metal ions in these complexes assume octahedral environment by coordinating solvent molecules in trans position. However, $[Cr((+)-BP)_3]^{n+}$ should have an octahedral geometry due to bidentate chelation of three ligands. These complex

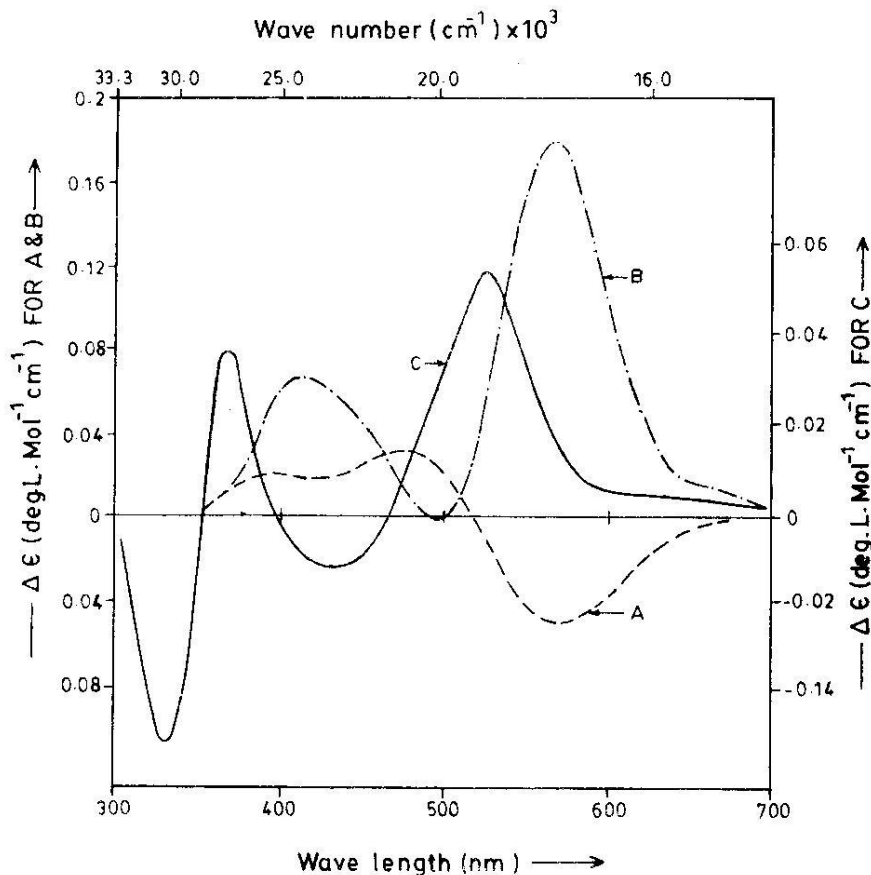ions have low symmetry due to the presence of asymmetric carbon in the chelates.

M = Co(II), Cu(II) VO(IV)
L = O or H_2O
R = —NH—C—CH_2—C_6H_5
              ‖
              O

II

Fig. 3:   Circular Dichroism spectra of (A) $[Cr(+)-BP)^4]^-$ at pH 5.0 recorded after 2 hours. (B) $[Cr((+)-BP)_3]^{n+}$ recorded after 48 hours and (C) $[Co(+))-BP)_2(H_2O)_2]^{n+}$ at pH 6.5 recorded after 2 hours.

The effect of varying concentration of metal ions and ligand upon complexation in these systems was studied btween 2.5-25.0 x 10^{-3} mol/dm^3 maintaining optimum conditions of pH, metal ligand ratio and allowing sufficient time to achieve equilibrium. The plots of ΔO.D. vs concentration were essentially straight lines indicating that complexation in these systems linearly increased with concentration. Lambert Beers law is obeyed over a reasonable range of concentration in these systems. From these studies, equilibrium concentration of complexes formed and other components were estimated and finally stability constants determined. The stability of complexes decrease as $[Cu((+)-BP)_2 (H_2O)_2]^{n+} > [Cr((+)-BP)_3]^{n+} > [VO((+)-BP)_2H_2O]^{n+}$.

The absorption spectrum of $[VO((+)-BP)_2 H_2O]^{n+}$ and $[Cu((+)-BP)_2 (H_2O)_2]^{n+}$ in ROH: H$_2$O

solution consist of single broad bands at 16,978 cm^{-1} (ϵ, 30.0 L mole^{-1} cm^{-1}) and 16,779 cm^{-1} (ϵ 26.0 L mole^{-1} cm^{-1}). The CD spectrum of $[VO(+)$-$BP)_2H_2O]^{n+}$ recorded after 204 hours consists of a positive band at 18,549 cm^{-1} ($\Delta\epsilon$ 0.29 L mole^{-1} cm^{-1}) and a negative band at 25,000 cm^{-1} ($\Delta\epsilon$ 0.45 L mole^{-1} cm^{-1}). The absorption band at 16,978 cm^{-1} in this system may arise from $^2E_g \rightarrow ^2T_g$ transition in an octahedral environment. However, due to John Teller distortion and lower symmetry of the complex, increased number of bands may be observed. The positive Cotton band at 18,349 cm^{-1} may be assigned to $^2B_2 \rightarrow ^2B_2$ and second negative band at 25,000 cm^{-1} to $^2B_2 \rightarrow ^2A_1$ transition. These band assignments agree well with already reported bands for a number of low symmetry oxovanadium (IV) complexes [10].

The CD spectrum of $[Cu((+)-BP)_2 (H_2O)_2]^{n+}$ consists of a positive band at 17,242 cm^{-1} which is

Table-1: Optimum conditions, stability constants, absorption and CD spectra of metal complexes of (+)-benzylpenicilline formed in alcohol:water (70:30) solution.

Complex Ion	pH	Time to Achieve Equilibrium (hours)	Stability Constants log β	Absorption Spectra ν (cm⁻¹)	ε L.mo⁻¹ cm⁻¹	Circular Dichroism Spectra		Band Assignment
						ν (cm⁻¹)	Δε L.mol⁻¹ cm⁻¹	
1. [VO((+)-BP)$_2$H$_2$O]$^{n+}$	4.7	2.0	4.35 ± 0.12	16,978	30	18,349	+ 0.29	$^2B_2 \rightarrow {}^2B_1$
						25,000	- 0.45	$^2B_2 \rightarrow {}^2A_1$
2. [Cr((+)-BP)$_3$]$^{n+}$	5.0	48.0	7.50 ± 0.32	18,282	62.0	17,699	+ 0.2	$^4A_2 \rightarrow {}^4B_1$
				25,000	60.0	20,202	- 0.002	$^4A_2 \rightarrow {}^4B_2$
						24,096	+ 0.07	$^4A_2 \rightarrow {}^4B_2$
3. [Co((+)-BP)$_2$(H$_2$O)$_2$]$^{n+}$	6.5	instant	xxx			16,000	+ 0.004	$^4A_2 \rightarrow {}^4B_1$
				19,646	9.0	19,048	+ 0.054	$^4A_2 \rightarrow {}^4B_2$
						22,727	- 0.012	$^4A_2 \rightarrow {}^4B_1$
						27,174	+ 0.038	MLCT
						30,488	- 0.051	MLCT
4. [Cu((+)-BP)$_2$(H$_2$O)$_2$]$^{n+}$	4.0	55.0	7.70 ± 0.29	16,779	26.0	17,241	+ 0.6	$^2B_1 \rightarrow {}^2A_1$

comparable to its absorption band at 16,778 cm⁻¹ and may be assigned to $^2A_1 \rightarrow {}^2B_1$ transition. A number of workers have made similar assignments to bands observed for [Cu(RR-(chxn(BAA)$_2$)] at 17,606 cm⁻¹ and [Cu(R-pn(BAA)$_2$)] between 16,667-17,241 cm⁻¹ measured in different solvents [11].

The CD spectrum of [Cr(+)-BP)$_3$]$^{n+}$ in EtOH:H$_2$O solution (Fig. 3) consists of three bands, two positive Cotton bands 1 at 17,699 cm⁻¹ (Δε 0.2 L mol⁻¹ cm⁻¹) and III at 24,096 (Δε 0.07 L mol⁻¹ cm⁻¹) and a very weak negative band II at 20,202 cm⁻¹. The absorption spectrum of this complex ion consists of two bands at 18,202 cm⁻¹ (ε 60.0 L mol⁻¹ cm⁻¹) and 25,000 cm⁻¹ (ε 62.0 l mol⁻¹ cm⁻¹) which may arise due to spin allowed $^4A_{2g} \rightarrow {}^4T_{2g}$ and $^4A_{2g} \rightarrow {}^4T_{1g}(P)$ transitions in an octohedral environment. However, $^4T_{1g}$ and $^4T_{2g}$ states in this species split to give A$_1$, B$_1$, B$_2$ unidimensional levels due to low symmetry. The two positive Cotton bands I and III are comparable with its absorption bands while weak negative band II seem to arise frrom excitation of electrons to split level of $^4T_{2g}$ state. The CD bands I and II may arise from excitation of electrons to $^4B_1$ and $^4B_2$ upper levels resulting from splitting of $^4T_{2g}(F)$ states of an octahedral species. The bands III may be assigned to $^4A_2 \rightarrow {}^4B_2$ ($^4T_{1g}$) transition. These band assignments are close to those already reported for a number of low symmetry Cr(III) complexes [12].

The absorption spectrum of [Co((+))-BP)$_2$ (H$_2$O)$_2$]$^{n+}$ consists of a weak band at 19,646 cm⁻¹ (ε 9.0 L mole⁻¹ cm⁻¹) while its CD consists of three i.e. a positive weak shoulder band I at 16,000 cm⁻¹

stronger positive band II at 19,048 cm⁻¹ and a negative band III at 22,727 cm⁻¹. In high frequency region, a couplet of bands i.e. a positive band at 27,149 cm⁻¹ and a negative band at 30,488 cm⁻¹ due to MLCT transition are observed for this system. The positive Cotton band II is very close to its absorption band and seems to arise from the same transition. The CD bands, I at 16,000 cm⁻¹ may arise from excitation of electrons to $^4B_2$ while band II and III may be assigned to excitation to $^4B_1$ and $^4B_2$ upper levels respectively arising due to splitting of $^4T_{1g}(P)$ state in an octahedral environment. These band assignments agree well with those already reported for analogous [Co((+)-Amp)$_2$(H$_2$O)$_2$]$^{n+}$ and [Co((+)-Ceph)$_2$(H$_2$O)$_2$]$^{n+}$ solution systems [3,5].

It is worth mentioning that positive components of couplet of CD bands in [Co((+)-BP)$_2$ (H$_2$O)]$^{n+}$ and analogous [Co((+)-Amp)$_2$ (H$_2$O$_2$]$^{n+}$, [Co((+)-Ceph)$_2$(H$_2$O$_2$]$^{n+}$ all lie on lower frequency while negative bands lie on higher frequency. Further, CD spectra of [Cr((+)-BP)$_3$]$^{n+}$ and [Cr((+)-Ceph)$_2$ (H$_2$O)$_2$]$^{n+}$ are similar. These observations indicate that chiral centres of the same absolute configuration of these ligands are part of chelates formed.

It is apparent from the proceeding discussion that in alcohol:water mixed solvent system, VO(IV), Cr(III), Co(II) and Cu(II) coordinate with (+)-benzylpenicilline to form complexes of composition [M((+)-BP)$_2$ (H$_2$O$_2$)], (M=VO(IV), Co(II) and Cu(II) and [Cr(+)-BP)$_3$]$^{n+}$ maintained between pH 4.00-6.5. At lower pH 2.5, [VO((+)-BP)$_4$]$^{2-}$ and [Cr((+)-BP)$_4$]⁻ are formed in solution. In these systems maximum complexation is achieved in 2-55 hours at room

temperature. The CD spectra of these systems may be interpreted in terms of electronic transitions to unidimensional levels resulting from lower symmetry of complexes.

Experimental

All reagents and chemicals were of highest purity, Analytical Reagent grade and obtained from commercial sources. Sodium (+)-benzylpenici-llinate was generously donated by M/S Antibiotics (Pvt) Ltd. Sikandarabad, Pakistan and used without further purification. The purity of sod. (+)-benzyl-penicillinate was checked by its infrared spectrum and specific rotation. The drug was stored in a refrigerator maintained below 4°C.

All solutions were prepared in double distilled water. The solvents methanol and commercial ethanol were dried and redistilled before use.

Solutions

(a) Stock solution of Sodium (+)-benzylpenici-llinate

An accurately weighed amount of Na(+)-BP (3.56 g, 0.01 mole) was dissolved in the mixed solvent alcohol:water (70:30) and thoroughly shaken to obtain a clear solution. The solution was diluted to 100 ml. Since there are reports that solutions of Na(+)-BP are unstable, fresh stock solutions were prepared for each experiment.

(b) Metal salt solutions

Accurately weighed amounts (0.1-0.05 mol) of hydrated metal sulphate or chlorides were dissolved in water and requisit volume of alcohol was added to obtained 100 ml of solution in alcohol:water (70:30). These stock solutionss were kept in dark and used accordingly.

12.2 g (0.1 mol) of sodium perchlorate was dissolved in water and then diluted to 100 ml with alcohol to obtain appropriate ratio of ROH: H_2O (70:30) and thoroughly mixed. This solution was used to adjust ionic strength in these systems.

(c) Solutions for complexation studies

For determining the pH at which maximum complexation between metal and Na(+)-BP takes place, a number of samples were prepared by mixing

1 ml of metal ion and 3.0 ml of (+)-BP stock solutions. The pH of these solutions was varied between 0.5-7.0 or up to the value where precipitation occured. Their pH was adjusted with dropwise addition of 0.1M HCl/NaOH solution. The samples were diluted to 25 ml and their final pH values measured. The samples were keept in dark for 2-3 hours before recording CD spectra. The differential optical density (Δ O.D) at Cotton band maxima was plotted as a function of pH (Fig. 1). The optimum pH for each system was selected from these plots and maintained in subsequent studies.

The method of continous variation of mole ratios was applied to determine metal:ligand ratio of complex ions formed in solution. A number of samples were prepared by mixing gradually increasing volumes of stock solutions of metal ion and decreasing volumes of ligand maintaining the sum of moles of the two components constant. These solutions were adjusted to optimum pH and kept in dark for sufficient time before recording their spectra. A plot of Δ O.D. at CD band maxima vs mole fraction of the metal ion was obtained for each system. These curves were extrapolated and from the points of intersection of two straight lines, stoichiometery of the complexes formed in solution was determined.

A number of sample solutions were prepared by mixing 1.00 ml of stock solution of metal ions with gradually increasing volumes of (1.0-15.0 ml) ligand solution. Ionic strength of background electrolyte was maintained with 1M solution of $NaClO_4$ in these samples. The samples were brought to appropriate pH, diluted to 25 ml and kept for appropriate time before recording their spectra. From the maximum values of ΔO.D., Δε was calculated for these systems. The Δε values at Cotton bands were subsequently used to estimate equilibrium concentration of the metal complex, free metal ion and free ligand in a series of samples of varying concentrations. From these values, stability constants in 5-7 samples of each system were determined and reported as log β.

Another series of samples was prepared by mixing different volumes of stock solutions of metal ions and (+)-BP so as to maintain their stoichiometic ratio and varying concentration between 1.00-

25×10^{-3} moles/lit. The ionic strength of background electrolyte $NaClO_4$ was maintained at 0.2 M in the samples. These solutions, were brought to appropriate pH and diluted to 25 ml. The samples were kept in dark for appropriate time before recording their spectra. The plots of $\Delta O.D.$ at λ_{max}, vs concentration are straight lines. These systems obey Lanbert-Beer law between concentration range $1.0 - 25.0 \times 10^{-3}$ mol/lit.

Instrumentation

The pH measurements were carried out on Horiba F-8 pH meter equipped with a combination electrode system. The circular dichroism spectra of these solution systems were recorded on Jasco-20A spectropolarimeter using 10 mm cylindrical quartz cells. The base line of these spectra were recorded using Na(+)-BP solutions prepared in the mixed solvent and maintained at appropriate pH. The solution absorption spectra were measured on Shimadu UV-265 spectrophotometer using a pair of 10 mm quartz cuvettes.

Acknowledgement

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