Circular Dichroism Studies on Complex Forming Ability of Antibiotic D-Cycloserine with VO(IV), Co(II) and Ni(II) ions in Aqueous Solution

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Summary: CD studies have been utilized to investigate complex forming ability of antibiotic (+)-cycloserine (CCS) with VO^{2+}, Co^{2+} and Ni^{2+} ions in aqueous solution. In solution, complex ions of the type $[M(CCS)_3]^{2+}$ are formed at pH 4.0 which show 2-3 Cotton bands between 16,129-28,011 cm^{-1} with molar ellipticity values 59.0-1838.6 dm^3 mo^{-1} $cm.^{-1}$ At optimum conditions intensity of CD bands is linearly increased with concentration of the components between $1\text{-}10 \times 10^{-2}$ mol dm^{-3}. CD bands are assigned to electronic transitions corresponding to lower symmetry of octahedral complexes.

Introduction

D-Cycloserine (CCS) (1), like many other antibiotics, contains a number of potential donor atoms [1]. It is an antibiotic with simplest structure and lowest molecular weight. In solution it exists as dipolar anion in equilibrium with its various forms [2].

In complexes, it acts as an ambidentate neutral ligand due to the presence of donor atoms in cyclic arrangement [3-4]. In the past, a number of complexes of Cr(III), Mn(II), Rh(III) and Ir(II) ions incorporating different number of (+)-cycloserine molecules have been characterized [5].

In biological systems, absorption of antibiotics chiefly depends upon their solubility in water. The most effective drugs are freely soluble in water and easily penetrate the cell membrane in the body. How-

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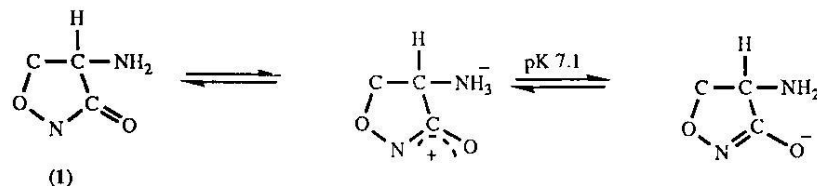
ever, metal complexes of (+)-cycloserine being insoluble in water, may be ineffective for theraupetic purposes. The formation of complexes of this ligand in aqueous medium have never been reported. In fact, literature is scarce on the complexation behaviour of metal ions with antibiotics in acidic aqueous solutions. This is an area which needs further exploration for developing an understanding of the role of metal ions in controlling the drug action particularly that of antibiotics in biological systems. The *dextro* rotatory enantiomer of cycloserine is most effective in therapeutics. In solution, complexing ability of (+)-cycloserine and other chiral ligands with transition metals is indicated by the appearance of Cotton bands in crystal field spectral region which can be easily monitored through circular dichroism (CD) studies [6-8].

In this paper, formation of VO(IV), Co(II) and Ni(II) complexes of (+)-cycloserine in aqueous solution at pH 4.0 as studied by circular dichroism is being reported. These studies indicate that each metal ion is coordinated with three molecules of (+)-cycloserine. In these systems, complexation linearly increases between concentration 1-10×10^{-2} mol dm^{-3} of the components. The observed Cotton bands have been assigned to electronic transitions between non-degenerate levels in low symmetry octahedral species.

Results and Discussion

The chiral ligand (+)-chycloserine is a colorless compound. In aqueous solution it is transparent in the visible region but has a strong positive Cotton band at 47,619 cm^{-1} in UV region. The free metal ions (VO^{2+}, Co^{2+} or Ni^{2+}) although coloured, do not exhibit CD bands in crystal field spectral region due to their achiral nature. However, complexation of these metal ions with (+)-cycloserine is indicated by the appearance of Cotton bands in crystal field region. Preliminary investigations were carried out by recording CD spectra of aqueous solution containing the metal ions and (+)-CCS in 1:6 molar ratio respectively. The complexation of these metal ions with the ligand was indicated with the appearance of 1-2 cotton bands between 16, 129- 23, 256 cm.$^{-1}$

The formation of these complexes is dependent upon pH of the system. It is apparent from Fig. 1 that elipticity of CD bands is increased with increasing pH and is maximum at pH 4. These complexes are formed in lower pH range where the ligand is essentially present in undissociated form and coordinates with the metal ions as a neutral species. Complexation increases as pH of the system is gradually raised to 4. Relatively smaller changes in ellipticity values at band maxima are observed between pH 3-4. If pH of the system is raised above 4, precipitation occurs in-
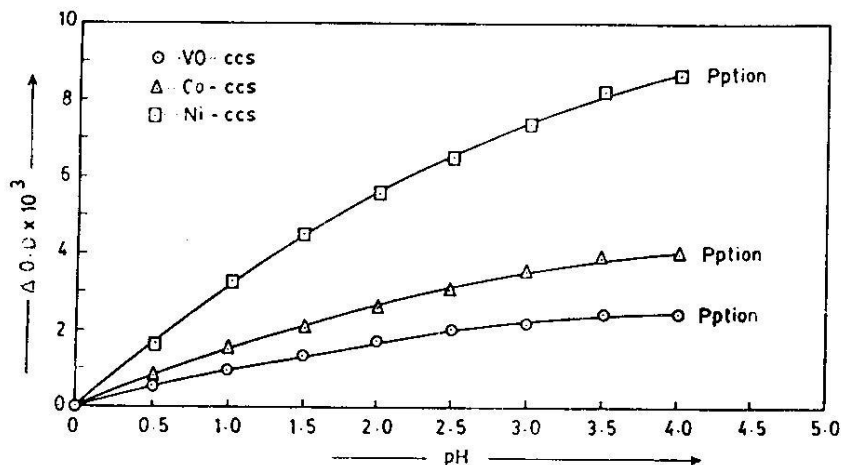

Fig. 1: Effect of pH upon differential optical density at Cotton band maxima of Metal-(+)-CCS systems.

dicating that the ligand has released a proton resulting in the formation of an insoluble non-ionic complex. Therefore, subsequent studies were carried out at pH 4.

Stoichiometry of the complexes formed in these systems were determined by varying mole fractions of the metal ions and ligand at pH 4 and measuring differential optical density at Cotton band maxima. It is evident from these plots (Fig. 2) that three molecules of (+)-CCS coordinate with each of the metal ions studied. Further, at optimum conditions, intensity of Cotton band is linearly increased by varying concentration of the components.

ambident ligand, bonding with metals through its exocyclic oxygen or nitrogen atoms [5]. However, complexes of an ambidentate chiral ligand induce very weak Cotton bands as observed by Bhatnager et al. [9]. The CD bands in present series of complexes are fairly strong and well defined indicating that asymmetric atom of the ligand is a part of chelate ring similar to that found in a number of complexes of chiral amino acids like (+)-alanine or (+)-serine [10]. A metal induced ring opening of the ligand is ruled out since very mild reaction conditions like room temperature, weakly acidic pH and low concentrations were used for establishing these equilibria. It is proposed that (+)-cycloserine acts as a bidentate ligand in these systems forming five membered che-
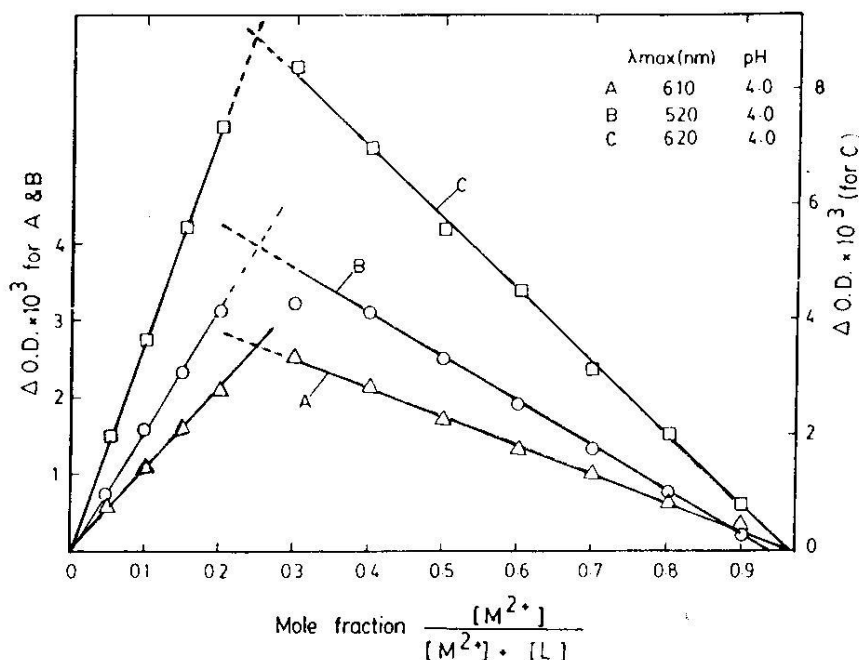

Fig. 2: Mole ratio plots for determining stoichiometry of complexes of (+)-cycloserine at pH 4. (A) VO^{2+}-(+)-CCS (B) Co^{2+}-(+)- CCS and (C) Ni^{2+}-(+)-CCS, M^{2+} = 0.2 mol dm^{-3}, CCS = 0.2 mol dm^{-3}. The left hand scale of differential optical density is for curve A and B while right hand scale is for curve C.

The ligand contains four potential donor atoms and an asymmetric carbon atom in the ring system. The imino nitrogen or oxygen atoms present in the ring are likely to show non-chelate (ambidentate) behaviour while five membered chelates may be formed by coordination of exocyclic nitrogen and oxygen (carbonyl) atoms with the metal ions. The earlier workers have suggested that (+)-CCS behaves as an

lates as (II). The metal ligand ratio of 1:3 and molar absorptivity values of these complex ions in solution suggest that metal ions are surrounded by six donors in an octahedral environment in $[Co(CCS)_3]^{2+}$ and $[Ni(CCS)_3]^{2+}$ while two molecules of (+)-CCS act as bidentate and third as ambidentate in $[VO(CCS)_3]^{2+}$ retaining the very strong V = O bond intact in sixth coordination site as in III.
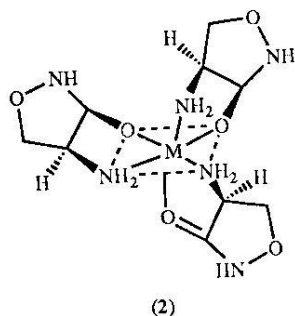
(2)
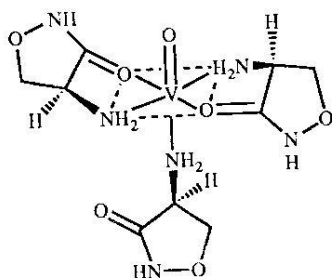
(3)

The solution absorption spectra of these systems consist of single, weak and broad bands between $15,337$-$21,276$ cm^{-1} with molar absorptivity values between 3.2-58.0 dm^3 mol^{-1} cm^{-1}. Another, relatively stronger band at $25,641$ cm^{-1} is observed in the spectra of VO(IV) and Ni(II) complexes. The solution CD spectra of these complex ions (as shown in Fig. 3) consist of 2-3 negative or oppositely signed Cotton bands between $16,129$-$31,056$ cm^{-1} with molar ellipticity values of 59.0-1838.6 dm^3 mol^{-1} cm^{-1}. The data on absorption and CD spectra is given in Table 1.

The adsorption spectrum of $[VO(CCS)_3]^{2+}$ system has a weak band at $16,129$ cm^{-1} which may be assigned to $^2B_1 \longrightarrow {}^2B_2$ transition. The CD spectrum of this complex ion consists of a couplet of bands, a negative Cotton band I at $16,393$ cm^{-1} ($[\theta]$ 151.8 dm^3 mol^{-1} cm^{-1}) and a positive band II at $19,048$ cm^{-1} ($[\theta]$ 151.8 $dm^3 mol^{-1}$ cm^{-1}). Another weaker negative band III at $24,509$ cm^{-1} ($[\theta]$ 59.0 dm^3 mol^{-1} cm^{-1}) is also observed. Band 1 may be assigned to excitation of electrons from $^2B_1$ state while band II arise from excitation of electrons from $^2A_1$ state to $^2B_2$ upper state. The weak Cotton band at $25,509$ cm^{-1} is probably due to a forbidden transition. These band assignments closely resemble to those made by other workers in low symmetry oxovanadium (IV) complexes [11]. Farmer *et al.* have studied CD spectra of a number of oxovanadium (IV) complexes of chiral Schiff bases and assigned bands between $16,800$-$17,400$ cm^{-1} and $21,000$ -$21,400$ cm^{-1} to similar transitions. The CD bands in $[VO(CCS)_3]^{2+}$ ion are comparable to those assigned by Farmer *et al.* [11a].

The adsorption spectrum of $[Co(CCS)_3]^{2+}$ ion consists of a broad band at $21,276$ cm^{-1} (ε 58 dm^3 mol^{-1} cm^{-1}) which may be assigned to excitation of electrons to $^4T_{1g}$ (P) upper state in octahedral environment. The CD spectrum of this complex ion consist of two negative Cotton bands at $19,231$ cm^{-1}($[\theta]$ 990 dm^3 mol^{-1} cm^{-1}) and II at $23,256$ cm^{-1} ($[\theta]$ 1386 dm^3 mol^{-1} cm^{-1}) respectively. Another stronger positive band at $31,056$ cm^{-1}($[\theta]$ 1838.6 dm^3 mol^{-1} cm^{-1}) is observed in UV region of this system. The molar ellipticity values of two negative Cotton bands are indicative of spin allowed transitions. Due to lower symmetry of these complexes, all degenerate states split to give unidimensional levels to which ex-

Table 1: Absorption and CD spectra of complexes of (+)- cycloserine in water

Complex	Absorption Bands		Circular Dichroism Band		
	$v(cm^{-1})$	$\varepsilon^{**} dm^3 mol^{-1} cm^{-1}$	$v(cm^{-1})$	$[\theta]^{**} dm^3 mol^{-1} cm^{-1}$	Assignments
$[VO(CCS)_3]^{*2+}$	16.129	9.9	16,393	- 135.3	$^2B_1 \longrightarrow {}^2B_2$
	25,641	120.0	19,048	+ 151.8	$^1A_1 \longrightarrow {}^2B_2$
			24,509	- 59.0	forbidden
$[Co(CCS)_3]^{2+}$	21,276	58.0	19,231	- 990.0	$^4A_1 \longrightarrow {}^4B_1(^4T_{1g}(P))$
			23,256	- 1386.0	$^4A_1 \longrightarrow {}^4B_1(^4T_{1g}(P))$
			31,056	+1838.6	MLCT
$[Ni(CCS)_3]^{2+}$	15,337	3.2	16,129	- 495.0	$^3B_1 \longrightarrow {}^3A_2(^3T_{1g}(P))$
	25,641	74.0	28,011	- 342.7	$^3B_1 \longrightarrow {}^3A_2(^3T_{1g} (P))$

*Metal ions and ligand solutions were mixed in 1:3 molar ratio and pH maintained at 4.
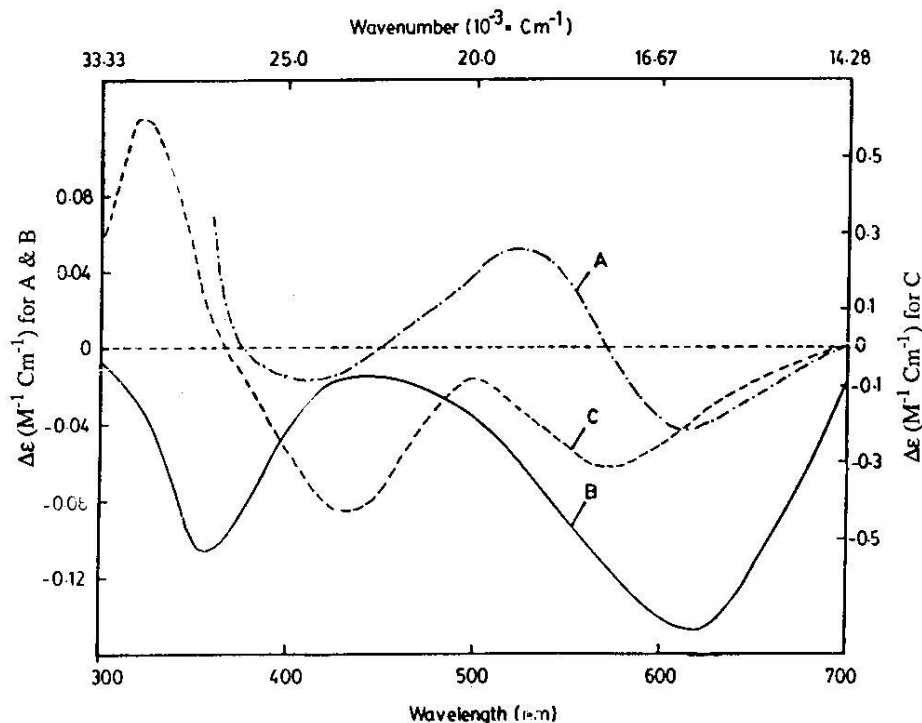**Calculated on the basis of metal ion concentration.

Fig. 3: Circular dichrosim spectra of (A) $[VO(CCS)_3]^{2+}$, (B) $[Ni(CCS)_3]^{2+}$ and (C) $[Co(CCS)_3]^{2+}$ ions in aqueous solution at pH 4. Concentration of complexes 0.1 mol dm^{-3}

citation of electrons may occur. The two Cotton bands in this system belong to spin allowed v_3 (i.e. $^4T_{1g} \longrightarrow ^4T_{1g}$ (P)) transition of an octahedral field. The broad adsorption band at 21,276 cm^{-1} has given rise to two negative Cotton bands. Therefore, band I may be assigned to $^4A_1$ ($^4T_{1g}$) $\longrightarrow ^4B_1$ $^1T_{1g}$ (P) and II to $^4A_1$ ($^4T_{1g}$) $\longrightarrow ^4B_2$ ($^4T_{1g}$) (P) transitions respectively. The positive Cotton band observed at 31.056 cm^{-1} in this system arise from metal to ligand charge transfer transition. The absorption spectra of a number of tetragonal complexes of Co(II) have been reported and bands between 18,600- 20,840 cm^{-1} and 20,200-23,600 cm^{-1} were assigned to excitation of electrons to split levels of $^4T_{1g}$ (P) upper state [12]. The CD band assignments in $[Co(CCS)_3]^{2+}$ ions are comparable to the reported assignments.

The solution spectrum of $[Ni(CCS)]^{2+}$ ion also consists of a broad band at 15,337 cm^{-1} (ϵ 3.2 dm^3 mol^{-1} cm^{-1}). The CD spectrum of this complex ion consists of two negative Cotton bands at 16,129 cm^{-1} ([θ] 495.0 dm^3 mol^{-1} cm^{-1}) and II at 28,011 cm^{-1} ([θ] 342.7 dm^3 mol^{-1} cm^{-1}). These bands have same sign and comparable molar ellipticity values suggesting that these arise from excitation of electrons to higher

levels of similar symmetry designation. The absorption and lower energy CD bands are very close and originate from $^3B_1 \longrightarrow ^3A_2$ ($^3T_{1g}$ (P)) transition. The second negative Cotton band at 28.011 cm^{-1} may be assigned to $^3B_1 \longrightarrow ^3A_2$ ($^3T_{1g}$ (P)) transition. These band assignments agree well with already reported absorption bands between 14,105-16,810 cm^{-1} and 25,910-28,300 cm^{-1} in a number of tetragonal complexes of Ni(II) ion [13].

It is concluded that at pH 4, VO(IV), Co(II) and Ni(II) ions efficiently form soluble complex ions with (+)-cycloserine. The composition of these complex ions is $[M(CCS)_3]^{2+}$ as determined by varying mole fractions of the components and measuring differential optical density at Cotton band maxima. The CD spectral bands of these systems can easily be assigned to various electronic transitions.

Experimental

Materials

D-Cycloserine as free base was purchased from Fluka, Switzerland. All other reagents used were of

highest purity Analytical Reagent grade obtained from E. Merck (Germany) or Fluka, Switzerland and used without further purification. The purity of (+)-cycloserine was checked by infra-red and specific rotation measurements. All solutions were prepared in double distilled water.

Solutions

(a) Stock solution of (+)-cycloserine

An accurately weighed amount of (+)-cycloserine (1.02 g. 0.01 mole) was suspended in a small volume of water and carefully titrated with 1M acetic acid with stirring until all solid particles were dissolved. The clear solution was diluted to 100 ml. in a volumetric flask. The contents were thoroughly mixed and pH of the solution measured.

(b) Buffer solution

A stock buffer solution of pH 4 was prepared by mixing 18.5 ml of 2M sodium acetate solution with 81.5 ml of 2M acetic acid and diluting to 1 litre in a flask.

(c) Metal salt solutions

Aqueous solutions of metal chloride (Co(II) or Ni(II)) and vanadyl sulphate were prepared by dissolving requisite amounts of hydrated salts in water and diluting to 100 ml so as to give 0.05 mol dm^{-3} solution.

(d) Solutions for complexation studies

A series of 10 samples were prepared by mixing different volumes (1-10 ml) of stock solutions of the metal ion with that of 10 ml of the ligand and adjusting their pH with dil HCl/NaOH solution. These solutions were diluted to 25 ml. in volumetric flasks, thoroughly mixed, kept in the dark for 30 minutes and finally 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 metal ion and the ligand solutions maintaining 1:6 molar ratio. Their pH was adjusted between 1-5 with dropwise addition of 0.1M HCl/NaOH solution. The samples were diluted to 25 ml and their final pH values were measured before recording CD spectra.

For determination of stoichiometry of the complexes formed, different volumes of stock solutions of the metal ion and chiral ligand were mixed so as to obtain variation in mole fraction of the components. To each of the resultant solution, 5 ml of buffer was added and samples were diluted to 25 ml. The contents were thoroughly mixed and their CD spectra were recorded. The differential optical density (Δ OD) at Cotton band maxima was plotted as a function of mole fraction of the metal ion. Stoichiometry of the complex ions formed was determined by extrapolating the two lines and obtaining corresponding mole fraction for various systems.

A number of samples were prepared by mixing varying volumes of stock solutions of the metal ions and ligand maintaining 1:3 metal ligand ratio, adding 5 ml of the buffer and diluting to 25 ml. These samples were kept in dark for 30 minutes and their CD spectra recorded. The differential optical density (Δ O D) at Cotton band maxima was plotted as a function of concentration of the metal ion (which is proportional to the concentration of the complex in the solution). A straight line plot emerging from the origin was obtained in each system.

Instrumentation

pH measurements were carried out on F-8 Horiba pH meter using a glass and calomel electrode system. The solution absorption spectra were recorded on Hitachi model 220-S spectrophotometer using pair of 10 mm quartz cuvettes. Circular Dichroism spectra between 300-700nm were recorded on JASCO-20A spectropolarimeter using a 10 mm cylindrical quartz cell. The base line of these spectra were recorded using aqueous solutions of the ligand maintained at pH 4.

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