Short Communication(NS-2)

ANTIBIOLOGICAL ACTIVITIES AND SPECTRAL STUDY OF PPIs WITH METALS

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ABSTRACT

PPIs are first generation drugs. Pantoprazole (PAN) is one of the important PPI drug. PPIs play an important role to suppress the Gastric acid which is responsible for Peptic ulcer. Again, Metal complexes also play an important role in biological activities of drugs. In many cases metal complexes of drugs are more potent than the parent drug. Some physico-chemical properties are helpful in biological activities. Stepwise formation constant and corresponding values of free energy change of complexes of Pantoprazole (PAN an important antiulcer drug, with transition metal ions [Vo(II), Cu(II) and Cd(II)] have been determined at 26^0C and 36^0C and 0.1M ($NaNO_3$) ionic strength. Probable structures of isolated chelates have been assigned on the basis of IR and NMR spectral data. Antibacterial activities of the complexes *"In vivo"* for sensitivity test has been screened.

Key Words : Proton pump inhibitors (PPIs), Pantoprazole (PAN), Antibacterial activities, Sensitivity test , physico-chemical property.

INTRODUCTION

Structural modification of organic molecule has considerable biological relevance.[1] Metal complexes play an important role in biological activity of drugs. In many cases metal complexes of drugs are more potent than the parent drug.,[2,3] Some physico-chemical property helpful in biological activity. With this view, in this study, some metal complexes of Pantoprazole drug[4-11] (synthesized in this study) have been screened for their activity towards *E. coli* and *Staphylococci aureus*. The Present communication report the thermodynamic stability constants and free energy change for the interaction of Pantoprazole (PAN), with transition metals [Vo(II), Cu(II) and Cd(II)] determined by Bjerrum Calvin pH titration technique as adopted by Irving Rossotti at 26^0C and 36^0C and 0.1M ($NaNO_3$) ionic strength. Probable structures of the isolated chelates have been assigned on the basis of IR and NMR spectral data. Literature survey indicates that no work has been done on metal complex of Pantoprazole (PAN), as the molecule posses chelating site.

AIMS AND OBJECTIVES

The main aim of this study is the physico-chemical properties of biologically interesting substance play an important role in the different fields. This study will help in understanding the probability of participation of mixed complexes during biological activity of the drugs.

MATERIAL AND METHODS

All the chemicals were used of A.R. or S.M. grade. All Metal salts were of Merck Chemicals. The solvents used were millipore water. The metal salt solutions were standardized by appropriate standard methods. Pure sample of Pantoprazole drug (PAN), molecular formula $C_{16}H_{15}F_2N_3O_4S$ and molecular weight 383.37, was obtained from Medley Pharmaceuticals Ltd, India & Apex drugs and International (B.No.:Pzs/0670104) and β-Alanine (ALN) from Reanal Budapest, India. Metal-ligand ratio

was calculated using Systronics digital conductivity meter, IR spectra were obtained from Department of Pharmacy, RGPV, Bhopal (Instrument used: Perkin Elmer FTIR Spectrophotometer in the range of 4000-400 cm^{1}). Electronic Spectra were obtained from food and drug department, Bhopal . ESR spectra were obtained from IIT, Powai, Mumbai. The NMR spectra were recorded on TC and HRD Division DRDE, Gwalior. Bacteriological study of various compounds were obtained from Department of Microbiology, Rajiv Gandhi College, Bhopal.

Preparation of the complexes

Pantoprazole metal complexes were prepared by mixing of Pantoprazole and the metal salt in 1:1 molar ratio and refluxing the mixture for 5-6 hours over water bath. The solution on concentration gave insoluble complex, which was filtered washed and dried (after recrystallisation) in vaccume. The complexes were stored in airtight bottles.

Physical measurements

The stoichiometry of the metal chelates have been established by conductometric and potentiometric (pH) titrations, utilising the monovariation method. Molar conductance was measured on a systronics digital (model type 306) conductivity bridge. The general experimental procedures employed were carried out with a systronic type 362 digital pH meter. A pH meter with combined glass calomel electrode was used. I.R. Spectra (KBr) were recorded on JASCO, FT/IR-470 PLUS, spectrometer. The antibacterial activity was carried out using by the disc diffusion technique.

Methodology

The formations constant were determined by Bjerrum Calvin[12] pH titration technique as adopted by Irving and Rossotti.[13] The Bjerrum Calvin pH titration technique was used to determine proton ligand constant of Pantoprazole ((PAN). The following mixture (total volume 50 ml) were titrated with a carbonate free 0.1M NaOH solution:

(a) 5 ml of 0.01M HNO_3
(b) Mixture(a) + 10 ml 0.002M (PAN)
(c) Mixture(b) + 5 ml 0.01M metal

The ionic strength of above solutions were maintained to 0.1M By adding required quantity of 1.0M $NaNO_3$ solution and the total volume of solution to be titrated was made 50 ml by the addition of required volume of millipore water. The Proton ligand formation constant and the value of stability constants are recorded in **Table 1**. The value of log K_1 were recorded during directly from the formation curves and refined by the method of least square. The antibacterial activity of the Pantoprazole ((PAN) and its metal complexes was checked by disc diffusion technique. This was done on E. coli and staphylococci aureus at 26^0C. The disc formed by whatman filter paper No.1 and diameter 6mm was soaked in the aqueous solution of compound. Minimum Inhibitory Concentration (MIC) of PAN drug 34 µg/ml taken. Binary complexes of PAN are in the range of 50 µg/ml to 100 µg/ml are taken.

RESULTS AND DISCUSSION

Metal - PAN (Drug) interaction :

Conductometric studies of the metal - PAN (drug) equilibria in solution (binary system) utilising Nair and Pande's mono-variation method[14-20] indicated formation of two complexes in these systems with metal- PAN molar ratio method. Analysis of the titration curve was carried out according to the procedure of Irving and Rossotti. The pH titration curves in case of PAN ligand before the acid curve indicating that in acidic solutions each molecule of the ligand is in association with one equivalent of proton. This proton should be attached with the lone pair of electrons present on the nitrogen of the ring in the case of PAN. PAN show one step of acid dissociation due to deprotonation of one secondary amine group.

$$LH \leftrightarrow L^- + H^+$$

Representative set of experimental titration curves, obtained according to the sequence for different M(II)- PAN reveals that below pH 6.1 formation of different M(II)-drug(PAN) binary

complexes take place. This is clear from the appeared divergence of each of the 1:1 binary M(II)- PAN titration curve from that of the corresponding free drug curve. Binary complexes are stable upto pH 5-8 in case of different metal ions. Hydrolysis of the complexes leads to the formation of hydroxocomplex species. The nature of the pH titration curves indicates that stepwise complex formation take place through deprotonation of secondary amine group.

Stability Constants

The Proton ligand stability constants of the drug (PAN) have been calculated at 26^0C and 36^0C at 0.1M ($NaNO_3$) ionic concentration utilising the Irving Rossotti pH titration technique. The value of proton ligand formation constant K are given in **Table 1** (PAN log K value 6.1). With increasing temperature ligand (PAN) show dissociation of the species. The metal ligand formation constants are given in **Table 2**. The values were directly obtained from the formation curve. The themodynamic parameters (ΔG, ΔH and ΔS) calculated at 26^0C and 36^0C at 0.1M ($NaNO_3$) ionic strength indicate the interaction enthalpy characterised **(Table 3)**. The reaction is exothermic in nature as supported by the negative value of free energy change.

Table 1 : Proton Ligand Formation Constant at 26ºC and 36ºC and at 0.1M ($NaNO_3$) ionic strength

Drug	Method	Log K_1^H		Log K_2^H		Log β_2	
		26ºC	36ºC	26ºC	36ºC	26ºC	36ºC
PAN	Half Integral method	6.01	5.51	-	-	6.01	5.51
	Point-wise calculation	6.01	5.51	-	-	6.01	5.51
	Least square method	6.31	5.80	-	-	6.31	5.80

Table 2 : Stability constants PAN-complexes, at 26ºC and 36ºC and at μ = 0.1M ($NaNO_3$)

Metal		log K_1
Vo(II)	26ºC	4.70
	36ºC	4.50
Cu(II)	26ºC	5.25
	36ºC	5.00
Cd(II)	26ºC	5.60
	36ºC	5.25

Antibacterial activity

The PAN show moderate inhibition with E.coli species with 1.9 mm inhibitory zone (**Table-4)**. The binary complex of PAN with Vo(II), Cd(II) metal ions show less activity as compared to parent drug PAN, whereas binary complex of Vo(II) show almost similar effect as PAN with E.coli. But binary complex of Cu(II) show higher antibacterial activity. The binary complexes of PAN with Staphylococci aureus also show such antibacterial activity. PAN inhibit the Staphylococci aureus species with 2.0 mm inhibition zone. Cu(II) show high inhibition activity against

Table-3 Ligation free enthalpy and Entropy Change of PAN - Complexes, at 26ºC and 36ºC and at μ = 0.1M ($NaNO_3$)

Metal	ΔG (K.cal/mole)		ΔH (K.cal/mole)		ΔS (K.cal/mole)	
	26ºC	36ºC	26ºC	36ºC	26ºC	36ºC
Vo(II)	0.919	0.923	198.70	190.25	19.77	18.93
Cu(II)	0.985	0.988	221.96	211.39	22.09	21.04
Cd(II)	1.023	1.018	236.75	221.96	23.57	22.09

Table 4 : Antibacterial activity of Pantoprazole and its Complexes against *E.coli* culture and *Staphylococci aureus* culture

Inhibition Area **Unit (mm)**

	E.coli	***S. aureus***
PAN	1.9	2.0
Vo(II)- PAN	1.6	1.4
Cu(II) - PAN	2.1	2.3
Cd(II) - PAN	1.5	1.5

Staphylococci aureus species, whereas other complexes show moderate inhibition activity as drugs do.The results of antibacterial screening indicate that the metal complex show more activity than drug. This may be due to higher stability of the metal complex than the drug.

Structure of the complexes

The structures of complexes were determined by spectral data. ^{1}H-NMR spectra of PAN and its binary complexes are recorded in D_2O solvent and CD_3OD **(Table 5)**. Chemical shift of ArNH in pure drug PAN exhibit at 3.5 δ, whereas binary complex with Cd of PAN show ArNH chemical shift at 3.5 δ towards lower field (deshielding of proton occur) due to complexation with metals. NMR studies confirm the structure of metal complex. The integrated proton ratio also corresponds to the proposed formula. The Infra Red data of isolated complexes shows that ligand has been act as bidentate ligands. While PAN molecules chelates with the metal ions using its -NH group and S=O group. Most of the bands of PAN remain unchanged on chelation. In the case of PAN molecule the N-H (Aro. Sec. Amine) stretching and banding occurs at 3556 and 1644 cm^{-1} whereas aromatic tert. amine C-N occurs at 1464 cm^{-1}.In the case of binary complex of PAN and metals aromatic amine N-H stretching and bending occur at 3556 and 1644 cm^{-1} whereas aromatic sulfoxide stretching occur at 1073 cm^{-1} will shift towards higher frequencies near 3636 and 1700 cm^{-1} and aromatic tert. amine C-N occurs at 1464 cm^{-1}. In the case of binary complex of PAN and metals aromatic amine N-H stretching and bending frequency change occurs, whereas frequency of tert. C-N remains unchanged and sulfoxide stretching may change from 1073 cm^{-1} to lower frequencies. The characteristic frequencies of the ligands and its metal complexes are given in **Table 6**.

A perusal of table clearly shows that the sec. N-H in the ligand (PAN) appears at 3556 and 1644 cm^{-1} are changed in binary complexes, whereas S=O (sulfoxide stretching) also shifted towards lower frequencies from 1073 cm^{-1} to 1031 cm^{-1}.

Table-5 Chemical shift of ^{1}H-NMR of Complexes

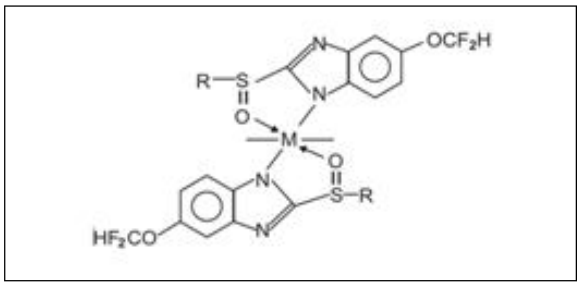
Complexes	**Protons**	**Chemical shift**
PAN	ArNH	3.5 δ
PAN -Cd(II)	ArNH	3.7 δ

Table 6 : I.R. Frequencies of PAN and their Complexes (cm^{-1})

	Aro. Sec. Amine N-H		**Aro.Sulfoxides S=O**	**Aro.Tert.Amine C-N**	**Co-ordinated H_2O**
	Stretching	**Bending**	**Stretching**	**Stretching**	
PAN	3556(s) 3453	1693 1644 1548	1153 1053	1498 1464 (s)	3743 842
Vo(II)- PAN	3563 3252	1692 1531 1515	1077 1029	1465 1427	3742 832
Cu(II) - PAN	3575 3283	1706 1693 1531	1064	1428	3743
Cd(II)-PAN	3557 3283	1645 1548 1516	1155 1033	1464	3742

The difference in the frequencies in the complexes show considerable degree of co-ordinate valency in M- ligand bond. The presence of co-ordinated water finds absorption band at ~ 820 cm^{-1}. A broad band near 3600 cm^{-1} to 3200 cm^{-1} in the complexes indicates the presence of co-ordinated water.

The proposed structures of these metal complexes are as follow :



CONCLUSION

In view of the foregoing discussions, the high melting points and insolubility in common organic solvents, we have assigned following probable structure for the complexes of Pantoprazole. From the analytical and spectral data, it can be concluded that the synthesized complexes are stable with probably octahedral environment around it. The results of antibacterial screening indicate that the metal complexes may be due to higher stability show more activities than drug.

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