Volume-4, Issue-4, April-2015 • ISSN No 2277 - 8160

Research Paper | Medical Science

Broad Spectrum Antibacterial Agent Ciprofloxacin Metal Complexes: Solution Studies and Stability Constants. Coordinate Through a Chromium Selective Binding Group.

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ABSTRACT

Equilibrium studies of ternary metal complexes of chromium (III) with drug ciprofloxacin and amino acids have been determined potentiometricaly in 80 % (v/v) ethanol-water medium at 27°C and fixed ionic strength 0.1M NaClO4 by SCOG computational programmed.

KEYWORDS : Equilibrium studies, amino acids, chromium and SCOG.

INTRODUCTION:

A substance used for cure of an ailment or alleviation of symptoms is called drug or medicine. There are several types of drugs[1] such as sulpha drugs, antibiotics, antipyretics, analgesics, tranquillizers, antiseptics etc widely used for the treatment of diseases.

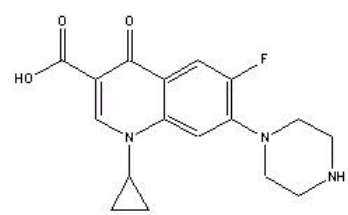
The drugs produced by the ethical pharmaceutical industry over the past century have irrevocably changed the fabric of society improving both the individual quality of life and life expectancy. The Bacterial infections[2], Polio, small pox, tuberculosis and related diseases and gastric ulcer that were once life threatening, have to a very major extent, become minor public health concern although the emergence of bacterial resistance due to the overuse of antibiotics has begun to reverse this trend.

The increase in life expectancy resulting from drug therapy has also resulted in a shift in population demographic toward a healthier, elderly population. As a consequence, disease like cancer and neuron degenerative, degenerative and autoimmune disease have become increasingly prevalent, resulting in an increase in health care needs and a greater consumption of the gross national product in providing health care. Drug regimens for birth control, compounds for erectile dysfunction and new treatments for incontinence are drugs that improve individual life choices and the quality of life. Similarly, HIV protease and reverse transcriptase inhibitors for the treatment of HIV infections in the space of few years have changed a disease with a fatal prognosis to a potentially chronic one. Cancer is also being viewed as potentially chronic, rather than fatal, disease with the potential for newer, non-cytotoxic approaches that include inhibition of the angiogenic events supporting tumor growth and proliferation.

It is observed that the drug action enhances due to the formation of metal complexes. Therefore, in the present study, we planned to study the metal complex equilibrium in solution involving currently used drug with amino acid. Hence it is necessary to explain the importance of these drugs.

Ciprofloxacin is an antibacterial drug[3].The term antibacterial is referred to a group of drugs which are capable of inhibiting the growth of and destroying disease causing bacteria[4]. Those which inhibit bacterial growth only are described as bacteriostatic and those which have the capacity to kill are called bactericides. The bacteria are a type of microorganism which causes diseases in man. The ciprofloxacin is the most potent first generation fluoro quinolones and rapidly bactericidal[5] largely as a consequence of inhibition of DNA gyrase and topoisomerase IV key bacterial enzymes that dictate the conformation of DNA so that it can be stored properly, unwound, replicated, repaired and transcribed on demand. These enzymes alter the conformation of DNA by catalyzing transient double strand cut staggered by four base pairs, passing the uncut portion of molecule through the gap and resealing the molecule back together. This alters the degree of twisting of DNA and releases torsional stress in the molecule. Inhibition of DNA gyrase and topoisomerase IV makes a cell's DNA in accessible and leads to cell death, particularly if the cells must deal with other toxic effects at the same time.

Ciprofloxacin is a very popular drug for many systemic infections such as urinary tract infection, bacterial gastroenteritis, typhoid fever, gonorrhea caused by penicillin's producing as well as non penicillinase producing gonococci, chancroid, skin and soft tissue infections, wound and gynecological infections, skeletal infections, etc. It is not a primary drug for respiratory tract infections. In combination with other antibiotics, ciprofloxacin has been used for serious infections like gram negative septicemias, meningitis, etc. It frequent component of combination chemotherapy for multidrug resistant tuberculosis.



Ciprofloxacin

The metal ions play an important role in the biological system. Chromium is a transition metal ion are integral parts of enzymes and play an important role in the biological system, such as to trigger a reaction, control reaction mechanism, stabilize protein structure, maintain structure of cell walls etc. Latest information indicates regulation of metabolism and growth of animal cell is dependent upon the mobilization of divalent and trivalent metal ions. It is widely distributed throughout the body. Infants have a higher chromium concentration than adults. Brewer's yeast is rich in chromium and most grains and cereal products contain significant quantities. Significant amount of chromium is obtained in the diet by cooking foods in stainless steel cookware. It is transported to tissues, bound to 'transferrin' and appears in the liver mitochondria, microsomes and cytosol. Chromium is essential ultra trace metal and needed for potentiating of insulin action on carbohydrate and lipids; active as a bioorganic chromium complex. The deficiency of chromium causes insulin resistance.

Chromium plays an important role in carbohydrate, lipid and protein metabolism. It is a true potentiator of insulin and is known as glucose tolerance factor (GTF). Trivalent chromium has been claimed to be a constituent of glucose tolerance factor.Chromium supplementation in deficient diets decreases serum cholesterol levels and prevents athermanous plaque formation in aorta. When given with insulin in chromium deficiency state, it improves amino acid incorporation mainly with a- amino isobutyric acid, glycine, serine and methionine. In protein energy malnutrition states, chromium supplementation is beneficial for weight gain. Chromium functions in vivo as an organic chromium complex and biological role to potentiate insulin activity.

All amino acids are polymer and regarded as building block of protein. Some amino acids are studied in this research [5].

The present investigation deals with the potentiometric studies on chromium (III) metal complexes with antibacterial ciprofloxacin and amino acids in 80% (v/v) ethanol-water medium by SCOG method.

Computerized method of evaluation has become very important pro-

cedures in the calculation of the complex equilibrium. Specially, the identification and characterization of species present in biofluids, surface and underground waters effluents etc. constitute and important present day problem, which demands the use of sophisticated computer programmers that can be used to determine the formation constants of all the species present in the systems.

More importantly, these computational methods also permit the treatment of complex systems with known numerical & graphical methods, which cannot be evaluated only with difficulty. The procedures of computer evaluation also yield valuable information from the experimental data, which cannot be obtained by non-computerized methods.

Presently we used developed software package, 'stability constant of generalized species' (SCOGS') for the calculation of dissociation constants of ligands and stability constant of binary and ternary metal complexes.

MATERIALS AND METHODS:

The nitrates of chromium, of A.R. grade were obtained from B.D.H. (India). Metal ion was used in the form of their perchlorates to avoid the possibility of complex formation with anions. The perchlorates were prepared from the corresponding nitrates. The concentration of metal ions was estimated by the standard procedures [6-8].

Sodium porchlorate (E.Merck) was dissolved in carbon dioxide free distilled water.

The solution of sodium hydroxide was also prepared in carbonate free distilled water by allowing the solution to stand for a long time till any carbonate if present precipitated. The solution was used as titrant for the potentiometric titration. As a routine, the solution was standardized at least once every day by titrating with standard oxalic acid solution. Perchloric acid of Reidal (Germany) was used for the preparation of the stock solution. Its exact normality was obtained by titrating it conductometrically using standard sodium hydroxide solution. Amino acids from Merck (Germany) or Fluka (Germany) were prepared by dissolving A.R. grade sample in 80% (v/v) ethanol – water medium. Drugs such as ciprofloxacin were prepared by dissolving as received as sample in 80% (v/v) ethanol-water medium. Drugs samples in pure form were obtained from pharmacy industries.

The experimental procedure, in the study of ternary metal complexes by the potentiometric titration technique, involves the titrations of carbonate free solution of against standard sodium hydroxide, where D and R, are the two ligands. The ionic strength of the solutions was maintained constant i.e. 0.1 M by adding appropriate amount of 1M sodium perchlorate solution. The titrations were carried out at 27^{0}C in an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrode to expel out CO_2.The experimental procedure, in the study of ternary metal complexes by the potentiometric titration technique, involves the titration of carbonate free solution of in 80 % (v/v) ethanol-water, were corrected by method of Vansittart and Hass. The formation constant of ternary complexes were determined by computational programmed SCOGS to minimize the standard derivation.

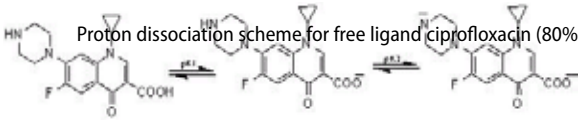
I	Free $HClO_4$ (A)
II	Free $HClO_4$ (A) + Ciprofloxacin(D)
III	Free $HClO_4$ (A) + Ciprofloxacin (D) + Chromium ion (M)
IV	Free $HClO_4$ (A) + Amino acids (R)
V	Free $HClO_4$ (A) + Amino acids (R) + Chromium ion (M)
VI	Free $HClO_4$ (A) + Ciprofloxacin (D) + Amino acids (R)+ Chromium ion (M)

RESULT AND DISCUSSIONS:

Binary metal complexes

The proton ligand constant and metal ligand stability constant of drug Ciprofloxacin and amino acids with chromium (III) determined in 80 % (v/v) ethanol-water mixture at 27°C and ionic strength m= 0.1 M $NaCl0_4$ are **Table No.1.**

The pK and logK value of drug Ciprofloxacin already published in journal [9-20]. This is here important for the explanation of stability constant of metal drug complexes.



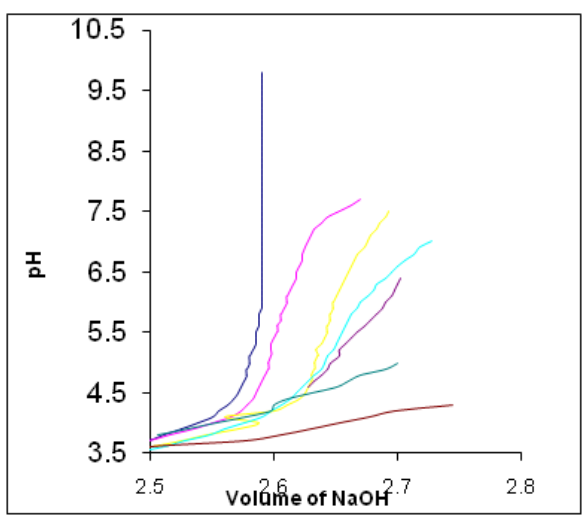
Proton dissociation scheme for free ligand ciprofloxacin (80% ethanol- water medium)

Table No.1

Ligands	PK_1	PK_2	Chromium	
			$Logk_1$	$LogK_2$
Ciprofloxacin	8.0016	9.3549	10.1320	
Glycine	2.7700	9.7400	6.5100	3.9400
Arginine	4.2659	12.200	8.5166	-
Tryptophan	3.8000	10.3900	8.4701	6.4134
Leucine	3.8100	10.3400	7.7078	4.3500
Glutamic acid	3.1360	5.8987	3.5087	3.0419
Glutamine	3.0100	9.2800	7.2486	6.0816
Valine	3.2100	9.8024	5.6122	3.5901
Methionine	3.1200	9.6000	3.1000	-
Phenylalanine	3.1400	9.3000	6.4405	5.3616
Alanine	3.7000	10.1800	10.6990	8.7200

(Standard deviation in pk and logk values (0.01-0.03))

The pH metric titration curve for Cr (III) – ciprofloxacin – Glycine System is represented in Figure 1.



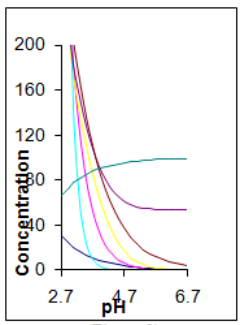
(Figure1.)

It is seen from the figure, that the mixed ligand curve coincide with A+D complex curve up to the pH~2.5 and after this pH, it deviates. Theoretical composite curve remains toward left of the mixed ligand complex curve. After pH~2.5, the mixed ligand curve drifts towards X-axis, indicating the formation of hydroxide species.

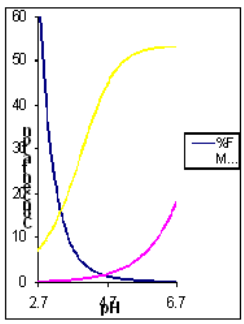
Since the mixed ligand curve coincide with individual metal complex titration curve, the formation of 1:1:1 complex by involving stepwise equilibrium.

The primary ligand drug ciprofloxacin forms 1:1 and secondary ligand amino acid glycine form 1:1 and 1:2 complexes with Cr (III). It is evident from the figure of percentage concentration species of Cr (III)-ciprofloxacin- glycine system that the percentage distribution curves of free metal decreases sharply with increasing pH. This indicates involvement of metal ion in the complex formation process. Percentage concentration of free ligands FD and FR increases and practically negligible as compared with that of free metal. This increase in percentage concentration may be due to the dissociation of excess ligand present in the system.

Species distribution studies:
To visualize the nature of the equilibrium and to evaluate the calculated stability constant of ternary complexes Cr(III)-ciprofloxacin- glycine, species distribution curves have been plotted as a function of pH at temperature 27°C and μ = 0.1 M $NaClO_4$. The system Cr (III)-ciprofloxacin- glycine is given in Figure 2 and 3.



(Figure 2)



(Figure 3)

It can be seen from the figure that, the concentration of Cr(III)-ciprofloxacin-glycine increases from pH~2.7, whereas the concentration for the formation of D and HR represented by C_1 and C_2 show continuous decrease with increasing pH which indicates the formation of Cr-D-R and represented by C_7. The concentration of this species continuously increases; confirm the formation of ternary complexes.

Species distribution curve of Cr (III)-ciprofloxacin-glycine ternary system showed that the formation of ternary complex started at pH~2.7 when Cr (III) at pH~5.9. Ternary complexes attain their maximum concentration in the pH ~5.7. From the species distribution curve, it is concluded that the formation of ternary complex started only after the metal-primary ligand complex has attained its maximum concentration. This indicates that the metal-primary ligand complex Cr (III)-ciprofloxacin is formed first and then the secondary ligand glycine coordinated to it, resulting the formation of ternary complex.

Moreover, the maximum percentage of the formation of ternary complexes is less than that of the Cr (III)-glycine binary complex; and more than Cr (III)-ciprofloxacin binary complex, this indicates that the ternary complex is less stable as compared to Cr (III)-glycine binary complex and more stable than Cr (III)-ciprofloxacin binary complex.

According to this method, in the present work three types of the concentration species distribution are observed.

Ternary complexes with glycine, leucine, glutamic acid, glutamine, valine and phenyl alanine show the following types of the concentration species distribution.

$C_1 = HD \rightleftharpoons D + H$

$C_2 = H_2R \rightleftharpoons HR + H$

$C_3 = HR \rightleftharpoons R + H$

$C_4 = M+R \rightleftharpoons MR$

$C_5 = MR+R \rightleftharpoons MR_2$

$C_6 = M+D \rightleftharpoons MD$

$C_7 = MD + D \rightleftharpoons MD_2$

$C_8 = M+D+R \rightleftharpoons MDR$

Ternary complexes with arginine, tryptophan, leucine and methionine show the following types of the concentration species distribution.

$C_1 = HD \rightleftharpoons D + H$

$C_2 = H_2R \rightleftharpoons HR + H$

$C_3 = HR \rightleftharpoons R + H$

$C_4 = M+R \rightleftharpoons MR$

$C_5 = MR+R \rightleftharpoons MR_2$

$C_6 = M+D \rightleftharpoons MD$

$C_7 = M+D+R \rightleftharpoons MDR$

Where M = Chromium, R = Amino acids & D = drug ciprofloxacin.

Moreover the maximum percentage of the formation of ternary complexes is more than that of the Cr (III) amino acids and Cr(III) ciprofloxacin binary complex, this indicates that the stabilization of ternary complex.

The Stability Constants of Ternary Complexes:
The relative stabilities of the binary and ternary complexes are quantitatively expressed in term of β_{11}, β_{20}, β_{02}, K_D, K_R, K_r and ΔlogK values which are presented in Table2.

Parameters based on some relationship between the formation of ternary complexes of chromium (iii) metal ion with ciprofloxacin in the presence of amino acids (1:1:1) system

Temp = 27°C I = 0.1 M $NaClO_4$ Medium = 80% (V/V) Ethanol-Water.

Table No.2.

AMINOACIDS	β_{11}	β_{02}	β_{20}	K_D	K_R	K_r	ΔlogK
Glycine	16.6347	10.4500	10.1320	6.5027	10.1247	1.6164	-0.0073
Arginine	17.3945	8.5166	10.1320	7.2625	8.8779	1.8655	-1.2541
Tryptophan	18.3944	14.8835	10.1320	8.2624	9.9243	1.4706	-0.2076
Leucine	17.3416	12.0578	10.1320	7.2096	9.6338	1.5630	-0.4982
Glutamic acid	13.6304	6.5506	10.1320	3.4984	10.1217	1.6340	-0.0103
Glutamine	15.8597	13.3302	10.1320	5.7277	8.6111	1.3519	-1.5209
Valine	15.4486	9.2023	10.1320	5.3166	9.8364	1.6405	-0.2956
Methionine	12.4788	3.1000	10.1320	2.3468	9.3788	1.8861	-0.7532
Phenyl alanine	15.5728	11.8021	10.1320	5.4408	9.1323	1.4199	-0.9997

In Cr (III)-ciprofloxacin-glycine system, primary ligand ciprofloxacin form only 1:1 and secondary ligand glycine form both 1:1 and 1:2 binary complexes. Therefore this system favors the following disproportion reactions

$MR_2 + MD \rightleftharpoons MRD + MR$

$MD_2 + MR \rightleftharpoons MRD + MD$

The comparison of β_{11} with β_{20} and β_{02} of this system show that preferential formation of ternary complexes over binary complex of primary as well as secondary ligand. The considerably low positive value of K_D and K_R indicate less stability of ternary complexes with respect to that of primary as well as secondary ligands. The K_r value of this complex is positive but less which indicates lower stability of ternary complexes.

Results of the present investigation show that the ternary complexes formed are less stable. The negative ΔlogK value of this system is due to statistical consideration, stability of binary complexes, reduced number of coordination sites, steric hindrance, electrostatic consider-

ation, difference in bond type and geometrical structure etc.

The values obtained for all systems such as Glycine, Arginine, Tryptophan, Leucine, Glutamic acid, Glutamine, Valine, Methionine and Phenyl alanine for the formation of Cr (III) ternary complexes are depicted in Table 2 to discuss the effect of displacement of the ligand by the other for the formation of ternary complexes, other useful parameters like K_D, K_R, K_r and ΔlogK along with the values of β_{02} and β_{20} are presented in the same table.

Primary ligands ciprofloxacin form 1:1 complexes and secondary ligands amino acids form 1:1 and 1:2 complexes with Cr (III).

CONCLUSIION

The conclusion drawn from the pattern of the different species distribution curves for same representative systems are discussed earlier. The stability of ternary complexes is governed by the nature of both, the primary and secondary ligands. The ligand first bound to metal ion influence the bonding properties of secondary ligands to be bound. The stabilisation of ternary can be governed by bonding properties of secondary ligand.

The value of Kr (Statistical relationship) is presented in table, which indicates the measure of relative stability of a mixed-ligand complex with respect over all stabilities of binary complexes.

From Table 2, it is observed that ΔlogK values for all the systems are negative, as already discussed that the negative ΔlogK values for ternary systems indicates less stability of complexes

The orders of stability of ternary complexes of Cr (III) with respect to secondary ligand Glycine, Arginine, Tryptophan, Leucine, Glutamic acid, Glutamine, Valine ,Methionine and Phenyl alanine for respective primary ligand are,

Ciprofloxacin = Gly>Glut acid >Trypto.>Vali.>Leu.>Methi.>Phenyl ala. >Argi. >Gluta.

Acknowledgement

The author very much thankful to the principal Dr. P.L. More, The Head, Department of Chemistry, Dr. W.N. Jadhav and Dr.B.R.Arbad for providing necessary facilities.

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