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Calcium (II) complexes with drug cirprofloxacin and leucine and phenylalanine : Equillibrium studies in $HClO_4$ & NaOH solution

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Abstract : The equillibrium studies of the mixed ligand complexes of calcium (II) ion with drug cirprofloxacin as primary ligand and the aminoacids viz. leucine and phenylalanine as secondary ligand were determined pH metrically at 27^0C and an ionic strength of 0.1 M $NaClO_4$ in 80% (v/v) ethanol-water medium. The calculations have been made using the stability constant of generalized species computer programme.

Key word : Equilibrium constant, ΔlogK and mixed ligand complexes.

Introduction

Ciprofloxacin is a antibacterial drug[1], it is the second-generation quinolones currently marketed in USA. The properties of ciplofloxacin, the market leader, are typical of those of the group. It is rapidly and nearly completely absorbed on oral administration and is not highly protein bound.

The ciprofloxacin is rapidly bactericidal[2-6] 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. Other quinolones inhibit these essential enzymes to different extents.

The ciprofloxacin chelate, with polyvalent metal ions such as Ca^{++}, Mg^{++}, Al^{3+} and Fe^{++} to form less water soluble complexes and thereby loose considerable potency. Thus, co-administration of certain antacids[7], hematinics, tonics and consumption of dairy product soon after ciprofloxacin administration is contraindicated.

Ciprofloxacin, are also used for prostatitis, upper respiratory tract infection [8], bone infection, septicemia, staphylococcal and pseudomonal, endocarditis, meningitis, sexual transmitted diseases (gonorrhea and chlamydial), chronic ear infections and purulent osteoarthritis.

Leucine[9] is neutral essential ketogenic amino acid and forms an acetoacetate and acetate. It is branched chain amino acid and taken up by brain and muscle. In leucine metabolism, transamination gives α-keto isocaproic acid, which is converted into corresponding CoA, this is similar to oxidative decarboxylation of alfaketoglutarate and pyruvate. The enzyme complex is very important in the body of living organism. A deficiency of the enzyme causes maple syrup urine disease. In this disease the urine gives odor of maple syrup or burnt sugar, deterioration is rapid and results in mental retardation.

Phenylalanine[10] is aromatic essential glucogenic and ketogenic amino acid. In metabolism phenylalanine is converted into tyrosine. In

metabolism homogenstic acid is formed which undergoes cleavage and form fumarate and acetoacetate. The hormones such as adrenaline, noradrenaline, thyrosine and melanin pigment formed from tyroxine. Several abnormalities observed in phenylanine metabolism such as phenylketonaria and alkaptonaria. In phenylketonaria, there is a black in hydroxylation of phenyl alanine to form tyrosine, this leads to mental retardation. Alkeptanaria, in this homogenstic acid is not further oxidised and excreted in urine. This lead to black urine.

Calcium occurs in the body in large amount than any other mineral elements. About 99% of the body calcium is in the skeleton, where it is present as deposit of Ca_3PO_4 in a soft, fibrous matrix. It plays an important role in the body of a living organism because it is well suited for binding to irregularly shaped crevices in proteins because calcium ion can form asymmetric complexes having a large radius, and binding of calcium is highly selective. Another characteristic of Ca++ that makes it a highly suitable intracellular messenger is that it can bind tightly to proteins. Negatively charged and uncharged oxygens bind well to Ca++. A capacity of Ca++ to be coordinated to multiple ligands six to eight oxygen atoms enables it to cross-link different segment of protein and induced large conformational changes. The intracellular level of Ca++ is kept low because phosphate esters are highly abundant are calcium phosphate and quite insoluble.

More than 99% of the total body of living organism, calcium is immobilized in bones and teeth as hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. A very little portion of calcium is present in extra cellular and intracellular fluids[11].

Milk is rich source of calcium where calcium is present largely as calcium caseinate. Absorption of Ca++ occurs mainly in the proximal small intestine and decrease in the more distal regions.

Several calcium-binding proteins have been identified. The skeleton is a huge reservoir of insoluble complexes of calcium which are in dynamic equilibrium with physicochemically soluble forms of circulating calcium that are maintained at a remarkably constant level. During states of calcium deprivation, calcium homeostasis is maintained at the skeleton, even to the point of producing severe bone disease. When calcium raises above normal, the C cells of thyroid secrete a hormone, calcitonin, which blocks mobilization of calcium from bone and stimulate calcium excretion in kidney thus restoring calcium to normalcy. Mobilization and deposition of calcium in biological system is controlled by parathyroid hormone, vitamin D, Calsequestrin, calcitonin and osteocalcium. Calcium is stored in the sarcoplasmic reticulum membrane on calcium binding proteins.

The contraction of muscle is associated with the release of Ca++ ions from sarcoplasmic and binding of Ca++ ions to different sites of muscle fibers.

Calcium plays a vital role in various essential physiological and biochemical processes. Calcium serve as the principal component of skeletal tissue, imparting to it the structural integrity essential to support the increasing body size of the individual during growth. It is used in the construction of cell walls, bones, teeth, some shells and other structural constituents. The biological functions include its influence on biological calcification, structural role, muscle contraction, nerve impulse transmission, release of hormone, activation of blood clotting enzymes, rhythm of heartbeats and permeability of gap junctions.

Survey of literature reveals that no work has been reported on complex tendencies of drug cirprofloxacin with transition metal ion calcium (II) in ethanol-water solution. Therefore in order to understand the complex formation tendencies of cirprofloxacin it was though worthwhile to determine the formation constant 1:1:1 ternary complexes of cirprofloxacin with calcium (II) in the presence of aminoacids in 80%(v/v) ethanol-water medium at 27^0C at a fixed ionic strength 0.1 M $NaClO_4$.

Experimental

Drug sample of cirprofloxacin in pure form were obtained from pharma industries and used as received. Ethanol was purified as described in literature[12]. Double distilled water was used for the preparation of ethanol-water mixture and stock solution of cirprofloxacin.

All chemicals used were AnalaR grade. $NaClO_4$ (0.1M) and NaOH solution was prepared in carbondioxide free double distilled water. Carbonate free NaOH was standardized by titrating with oxalic acid. $HClO_4$ Reidal (Germany) was used for the preparation of the stock solutions of calcium (II) to prevent hydrolysis and standardized by using standard EDTA solution[13].

The experimental procedure, in the study of ternary chelated by the potentiometric titration technique, involves the titration of carbonate free solution of

1) Free $HClO_4$(A)
2) Free $HClO_4$ + Ligand Cirprofloxacin Drug
3) Free $HClO_4$ + Ligand Cirprofloxacin + Metal ion
4) Free $HClO_4$ + Ligand Aminoacids
5) Free $HClO_4$ + Ligand Aminoacids + Metal Ion
6) Free $HClO_4$ + Ligand Drug + Ligand Aminoacids + Metal Ion

Against standard solution of sodium hydroxide, were drug cirprofloxacin and aminoacid are two ligands. The ionic strength of the solutions was maintained constant i.e. 0.1M by adding appropriate amount of 1M sodium perchlorate solution. The titration were carried out at 27^0C in an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrode to expel out CO_2. pH meter reading in 80%(v/v) ethanol-water were corrected by method of Vanuitert and Hass[14]. The formation constant of ternary complexes were determined by computational programme SCOGS[15] to minimize the standard derivation.

Table 1

The proton ligand constant and metal ligand stability constant of cirprofloxacin and amino acids with calcium (II) determined in 80%(v/v) ethanol-water mixture at 27^0C and ionic strength μ = 0.1M $NaClO_4$ are given in Table 1[11]

Ligand	pK			
	pK_1	pK_2		
Cirprofloxacin	8.0016	9.3549	$LogK_1$	5.4170
			Log K_2	--
			Log β	5.4170
Leucine	3.8100	10.3400	$LogK_1$	7.2478
			Log K_2	--
Phenylalanine	3.1400	9.3000	$LogK_1$	3.1611
			Log K_2	

Table 2

Parameters based on some Relationship between the formation of Ternary Complexes of Calcium (II) Metal ion with Ciprofloxacin in the presence of Aminoacids (1:1:1) System

Temp = 27^0C I = 0.1 M $NaClO_4$ Medium = 80% (V/V) Ethanol-Water

Aminoacids	β_{11}	β_{02}	β_{20}	K_D	K_R	K_r	ΔlogK
Leucine	12.1648	7.2478	5.4170	6.7478	4.9170	1.9210	-0.5000
Phenyl Alanine	8.4386	3.1611	5.4170	3.0216	5.2775	1.9675	-0.1395

Results and Discussion

a. Binary metal complexes

The proton ligand constant and metal ligand stability constant of cirprofloxacin and amino acids with calcium (II) determined in 80%(v/v) ethanol-water mixture at 27^0C and ionic strength μ = 0.1M $NaClO_4$ are given in Table 1[16]

b. Ternary metal complexes.

In the ternary systems, the mixed ligand titration curve coincide with acid + drug complex curve up to the pH ~ 2.5 and after this pH, it deviates. Theoretical composition curve remains toward left to the mixed ligand titration complex curve. After pH~ 2.7, the mixed ligand curve drift towards X axis, indicating the formation of hydroxide species. Since the mixed ligand curve coincide with individual metal complex titration curves, the formation of 1:1:1 complex by involving stepwise equilibria.

The Primary ligand cirprofloxacin form 1:1 and secondary ligand amino acids such as leucine & phenylalanine form 1:1 and 1:2 complexex with Ca(II). It is evident from the figure of the percentage concentration species Ca(II)- cirprofloxacin amino acids system, that the percentage distribution curve of free metal decreases sharply with increasing pH. This indicate involvement of metal ion in the complex formation process. Percentage concentration of free ligand cirprofloxacin and aminoacids increases and this increase may be due to the dissociation of ligand present in the system, as a function of pH.

Species distribution studies.

To visualize the nature of the equilibria and to evaluate the calculated stability constant of ternary complexes Ca(II) - cirprofloxacin- aminoacids, species distribution curves have been plotted as a function of pH at temperature 27^0C & μ = 0.1 M $NaClO_4$ using SCOG programme.

It can be observed that the concentration of Ca(II)-cirprofloxacin aminoacids such as leucine increases from pH 2.9 where as phenylalanine from pH~ 3.8. The concentration for the formation of D(drug) and HR (aminoacid) represented by C_1 and C_2 show continuous decrease with increasing pH which

indicates the formation of Ca (II) – ciprofloxacin (D)-aminoacid (R) such as leucine and phenylalanine, represented by C7. The concentration of this species continuously increases, confirm the formation of ternary complexes. Ca(II) – ciprofloxacin (D) – aminoacid(R) such as leucine and phenylalanine represented by C_7. The concentration of this species continuously increases, confirm the formation of ternary complexes. From the SCOG 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 indicate that metal primary ligand complex Ca(II)-cirprofloxacin is formed first then the secondary ligands such as leucine & Phenylalanine coordinated to it, resulting the formation of ternary complex.

According to this method in this system ternary complex of cirprofloxacin with leucine and phenylalanine 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+R+D $\rightleftharpoons$ MDR

Where M = Metal, R= Aminoacids & D = drug cirpfloxacin.

Moreover the maximum percentage of the formation of ternary complexes of cirprofloxacin is more than that of the Ca(II) aminoacids leucine and Ca(II) ciprofloxacin binary complex, this indicates that the more stabilization of ternary complex. While the percentage of the formation of ternary complexes of ciprofloxacin is less than that of the Ca(II) amino acid phenylalanine and Ca(II) ciprofloxacin binary complex, this indicate the less stabilization of ternary complex.

The stability constant 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 value which are represented in table 2. The stability constants of ternary systems are represented in table 2. The stability of ternary complexes is conveniently characterizes by two ways, one based on difference of stability constant Δlog K and second disproportion constant.

MR+D $\rightleftharpoons$ MRD (I)

M+D $\rightleftharpoons$ MD (II)

Δlogk $\longrightarrow$ $\log K_{ML2} - \log K_{ML1}$

The first equation mentioned above is similar to the reaction

MD + D $\rightleftharpoons$ MD_2

With respect to the availability of coordination sites for ligand D in MR or MD. Generally $K_{ML1} > K_{ML2}$ because more coordination positions are normally available for bonding first ligand to a metal ion than the second ligand. Evidently $K_{ML1} > K_{ML2}$ or Δ log K is negative. Δ log K can be calculated by the expression.

$$\Delta \log K \longrightarrow \log \beta_{MRL} - (\log K_{MR1} + \log K_{MD1})$$

The negative Δ log K for ternary systems indicates that the primary ligand anion and secondary ligand anions preferentially form ternary complexes to their binary ones. It follows from above expression that the difference, Δ logK results from the substraction of two constants and therefore, a constant which corresponds the equation,

MR + MD $\rightleftharpoons$ MRD + M

The positive value of Δ logK indicates the equilibrium is more on its right side. The other characterization is based on the disproportion reaction represented by the following equilibrium ,

$MR_2 + MD_2$ $\rightleftharpoons$ 2MRD

The disproportion reactions for the system containing the ligands which form 1:1 and 1:2 complexes individually with the metal ion are as,

MR_2 + MD $\rightleftharpoons$ MRD+ MR

MR + MD_2 $\rightleftharpoons$ MRD+ MD

MR + MD $\rightleftharpoons$ MRD+M

Above two reactions are for the system containing one ligand which form only 1:1 and other form both 1:1 and 1:2 binary complexes. The last reaction is for the system containing ligands which form only 1:1 binary complexes. The magnitude of the constant is the measure of stability of mixed ligand complexes. Watter and Kida calculated statistically expected value 0.6 log unit by considering with probabilities for a variety of reason discussed by Sigel. Δ logK value can be calculated by using first or second approach. The calculated ΔlogK values for all systems are given in table 2

In Ca (II)- cirprofloxacin-aminoacids, Primary ligand cirprofloxacin form only 1:1 and secondary ligand form both 1:1 and 1:2 binary complexes. Therefore this system favour the following disproportion reactions.

$$MR_2 + MD \rightleftharpoons MRD + MR$$

$$MR_2 + MR \rightleftharpoons MRD + MD$$

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 value of K_D & 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 investigations show that the stability constant of ternary complexes formed are less stable. The negative Δ logK value of this system indicates that the ternary complex is less stable than the binary 1:1 metal –cirprofloxacin & metal – aminoacids complex. This is in accordance with statistical considerations. The negative value of Δ logK does not mean that the complex is not formed. The negative value may be due to the higher stability of its binary complexes, reduced number of coordination sites, steric hindrance[17-20], electronic consideration[21-22], difference in bond type, geometrical structure etc.

Sigel concluded that in the case of bidentate ligand cirprofloxacin & aminoacid, there are twelve edges of a regular octahedron available to the first entering ligand. But only five for the second. Then the statistical factor would be 5/12 and accordingly Δ logK= -0.4, -0.6 & -0.9 for square planer & distorted octahedral complexes. Hence the experimentally determined value Δ logK < -0.6 indicate less stabilization in ternary complexes.

The Δ logK value of this system is higher than the statistically expected value except leucine & phenylalanine, showing the stabilized nature of the ternary complex. The primary ligand cirprofloxacin having smaller size. Therefore its Δ logK value is less negative.

Thompson & Lorass pointed out that more negative Δ logK value of ternary complexes is due to the electrostatic repulsion between the negative charges on cirprofloxacin & amino acids. Steric hindrance consideration is the most important factor because in the present studies of ternary complex, primary ligand cirprofloxacin coordinates with the metal ion in the lower pH range and form 1:1 complex. In solution, ternary complex forms as the titration curve run below the Ca (II) –cirprofloxacin titration curve. So, it is evident that the entry of the secondary ligand amino acids faces steric hindrance due to bigger size of the Ca(II) cirprofloxacin complex as compared to aquo ion, which tries to restrict the entry of the secondary ligand in the coordination spehere of the Ca (II) metal ion & thus reduces the stability of ternary complexes. The order of stability of ternary complexes of Ca(II) with respect of secondary ligand for respective primary ligands is

Cirprofloxacin = Phenylala > Leu

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