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STUDIES OF METAL COMPLEXES OF DRUG FUROSEMIDE AND AMINO ACIDS WITH COPPER (II).

KEYWORDS: Stability constant, ΔlogK and mixed ligand complexes.

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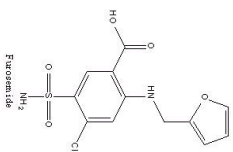
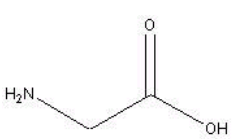
ABSTRACT *The stability constant of the mixed ligands complexes of copper (II) ion with drug furosemide as primary ligands and some amino acids were determined in 80 %*

(v/v) ethanol-water medium at 27°C fixed ionic strength 0.1M NaClO4 by computational programmed SCOGS.

Introduction:



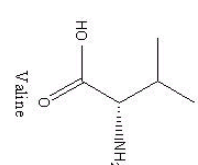
Furosemide, a sulfonamide type drug is an example of high-ceiling diuretic[1] & may be regarded as a derivative of anthranilic acid or o-aminobenzoic acid. It is used to cure hypertension and edema associated with congestive heart failure, cirrhosis and renal diseases.[2] Research on 5-sulfamoylanthranilic acid at the Hoechst laboratories in Germany showed them to be effective diuretics. The most active of a series of variously substituted derivative was furosemide. The chlorine and sulfonamide substitutions are features seen also in another diuretic such as thiazide. Because the molecule posses free carboxyl group, furosemide is a stronger acid than the thiazide diuretics. This drug is excreted primarily unchanged. A small amount of metabolism, however, can take place on the furan ring, which is substituted on the aromatic amino group. Furosemide has a saluretic effect[2, 3] ten times that of the thiazide diuretics



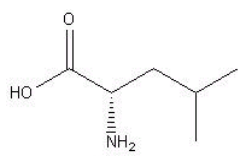
Glycine

All amino acids, commonly known as Magic 20 are polymer and regarded as building block of protein. Some amino acids are studied in this research [4]. **Glycine (α-amino acetate),** is the simplest, neutral, aliphatic, optically inactive non-essential, glycogenic aminoacid, [5-8]. It is isolated from protein and has characteristics sweet taste. Zwiter ionic structure some time called as betaine structure. It can be synthesized from CO_2 and NH_3 by glycine synthase or transamination of glyoxylate and in metabolism of serine and choline. It plays an important role in haeme synthesis.

Valine *(α- aminoisosovalerate)*, is essential amino acid[9-22]. It

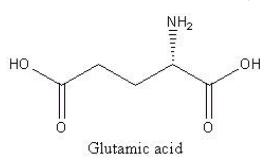


is widely distributed but rarely occurs in amount exceeding 10%. It is branched chain amino acid and can be derived from alanine by the introduction of two methyl group present on α - carbon atom. Ths is glycogenic. On deamination, it forms methyl-malonyl-CoA which can be converted to succinyl – CoA in place of two H atoms of the Methyl group.



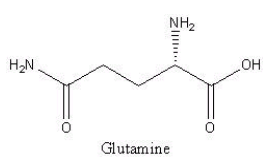
Leucine

Leucine[23] (*α-amino isocaproate*), is branched chain neutral essential ketogenic amino acid and forms an acetoacetate and acetate. It is taken up by brain and muscle.



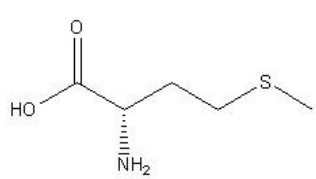
Glutamic acid

Glutamic acid[24] (*α-aminoglutarate*), is acidic non-essential glycogenic amino acid with one amino group and two carboxylic groups. It is parent compound of glutamine and widely occurs in protein.It takes part in transamination, transamidation and inter conversion of amino acids and also participate in ammonia transport and urea formation. Glutamic acid involve in glycogenic function.



Glutamine

Glutamine (*γ-amide of α-aminoglutarate*), is acidic non-essential glycogenic amino acid[25, 26]. It is homologue of aspargine and constituent of folic acid. Basically it is used in higher animal for conjugation, detoxification of phenyl acetic acid and plant tissue.



Methionine

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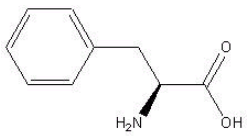
Methionine[27](*α-amino-β-methylmercaptobutyrate*), is essential glycogenic amino acid. It is the only common amino acid possessing an ether linkage. Cereals have sufficient quantity of methionine. Methionine is particularly important as a donor of methyl group in reaction known as transmethylation reactions.



Phenylalanine

Phenylalanine[28, 29] (α-amino-β-phenylpropionate), is aromatic essential glucogenic and ketogenic amino acid. Several abnormalities observed in phenylanine metabolism such as phenylketonaria and alkaptonaria. phenylketonaria and alkaptonaria are the inheritance abnormalities , 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 leads to black urine.

Copper is a transition metal ion and is used by various enzymes in the body in different biochemical reactions. These reactions may be creating energy, decreasing the body's inflammatory blood clotting[32] etc. Copper is absorbed by the body at two main sites such as small intestine and stomach. Copper does not float through the blood stream as copper ion but is carried by proteins. Two main carrier proteins especially for copper are ceruloplasmin[33] and albumin; these can carry many things including copper. Copper is stored in proteins called metallothione.[34, 35] Enzymes are proteins specialized to assist in a chemical function. Copper is needed by enzymes as a helper in a chemical reaction. This function makes copper essential for cytochrome C oxidase, essential for energy and superoxide dismutase essential oxidative tissue damage etc.

In recent years it has been proved that transition metals like copper is essential for normal development and function of human cells. Disruption of copper metabolism causes severe neurodegenerative disease, such as Wilson's disease[36-40], and Menken's disease[41-43] with symptoms that range from psychiatric abnormalities and motor dysfunction, to poor temperature control and liver & kidney abnormalities.

Rossetti and Rossotti[44] defined complex, as a species formed by association of two or more simpler species, each capable of independent existence.

The formation of complex is not restricted to association between two ions of positive charges. A metal cation, a proton or another positively charged species may form complex with an electron donor, whether it is negatively charged[45-46]; electrically neutral or even positively charged. The ligand is referred as electron donor atoms or groups. This term is sometimes applicable to the molecule as a whole, which contains donor atoms or groups by means of which the molecule is attached to the central metal atom. When a ligand contains two or more donor atoms close to each other, the metal complex formed is said to be a "chelate"[47]

The most obvious feature of metal–chelate structure is the formation of heterocyclic ring, usually of five or six membered. Owing to the range of normal covalent bond angle, five and six membered rings are more stable. For a ring of single bond only the five-membered ring is usually most stable[48] where as a six membered rings have maximum stability, when there are two double bonds in the ring. The chelates have been extensively studied in the solid state as well as in the solution by many workers[49] due to their remarkable properties and high stability.

The present investigation deals with the study of stability constants of various metal chelates in 80% (v/v) ethanol-water medium. It is therefore; appropriate to mention the salient features of solution study.

Material and methods

The nitrates of transition metal ions copper, of Analar quality 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[51]. The concentration of metal ions was estimated by the standard procedures[52-54].

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 filtered and kept in a pyrex vessel, free from carbon dioxide and was used as titrant for the pH 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 such as Glycine, arginine, tryptophan, Leucine, Glutamic acid, Glutamine, Methionine and Phenyl alanine obtained either from Merck (Germany) or Fluka (Germany) were prepared by dissolving Analar grade sample in 80% (v/v) ethnol – water medium.

Drugs such as Furosemide 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 chelates by the potentiometric titration technique, involves the titrations of carbonate free solution of

I	Free $HClO_4$ (A)
II	Free $HClO_4$ (A) + Ligand (D)
III	Free $HClO_4$ (A) + Ligand (D) + Metal ion (M)
IV	Free $HClO_4$ (A) + Ligand (R)
V	Free $HClO_4$ (A) + Ligand (R) + Metal ion (M)
VI	Free $HClO_4$ (A) + Ligand (D) + Ligand (R)+ Metal ion (M)

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 27^0C in an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrode to expel out CO_2.

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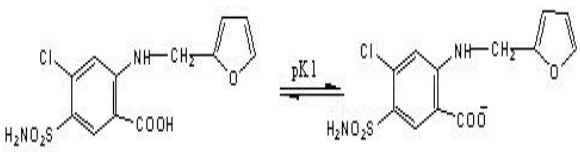
The experimental procedure, in the study of ternary chelated by the potentiometric titration technique, involves the titration of carbonate free solution of in 80 %(v/v) ethanol-water, were corrected by method of Vanuitert and Hass[55]. The formation constant of ternary complexes were determined by computational programmed SCOGS[56, 57] to minimize the standard derivation.

Results and Discussion

Binary metal complexes

The proton ligand constant and metal ligand stability constant of drug furosemide and amino acids with copper (II) determined in 80 % (v/v) ethanol-water mixture at 27°C and ionic strength μ= 0.1 M $NaClO_4$ are given in **Table I** , already published in research journal[58-60].



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

These values are important for the determination of stability constant of mixed ligand complexes therefore mentioned here.

Table I

Ligands	PK_1	PK_2	Chromium	
			$LogK_1$	$LogK_2$
Furosemide	5.6315	--	2.8309	-
Glycine	2.7700	9.7400	6.5100	3.9400
Leucine	3.8100	10.340	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

Ternary metal complexes.

The pH metric titration curves of ternary systems shows that the mixed ligand curve coincide with A+D complex curve up to the pH ~ 3.7 and after this pH, it deviates. Theoretical composite curve remains toward left of the mixed ligand complex curve. After pH ~ 4.5, 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 curves, the formation of 1:1:1 complex by involving stepwise equilibrium.

The primary ligand drug furosemide form 1:1 and secondary ligand amino acid glycine form 1:1 and 1:2 complexes with Cu(II). It is evident from the figure of percentage concentration species of Cu (II) - furosemide –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 furosemide and glycine 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 Cu (II)-furosemide-glycine, species distribution curves have been plotted as a function of pH at temperature 27°C and μ = 0.1 M $NaClO_4$ by using SCOG programme.

It can be seen that, the concentration of Cu(II)-furosemide-glycine increases from pH~2.5, whereas the concentration for the formation of D(Furosemide) and HR(Glycine) show continuous decrease with increasing pH which indicates the formation of Cu(II)-furosemide-glycine. The concentration of this species continuously increases; confirm the formation of ternary complexes.

The distribution curve of ternary system Cu (II) - furosemide-glycine showed that the formation of ternary complex started at pH ~ 2.5 when Cu (II) at pH ~ 5.8. Ternary complexes attain their maximum concentration in the pH ~3.8. From the 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 Cu (II)-furosemide is formed first and then the secondary ligand glycine coordinated to it, resulting in the formation of ternary complex.

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

The Stability Constants of Ternary Complexes:

The relative stabilities of the binary and ternary complexes of Drug furosemide (D) and amino acids (R) 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 Table 2.

The stability of ternary complexes are conveniently characterised by two ways, one based on difference of stability constant ΔlogK and second disproportion constant.

$MR + D \rightleftarrows MRD$

$M + D \rightleftarrows MD$

$\Delta LogK \longrightarrow Log\ K_{ML2} - logK_{ML1}$

The first equation mentioned above is similar to the reaction

$MD + D \rightleftarrows MD_2$

With respect to the availability of co-ordination sites for ligand D in MR or MD. Generally $K_{ML1} > K_{ML2}$ because more coordination positions are normally available for bonding

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first ligand to a metal ion than the second ligand. Evidently $K_{ML1} > K_{ML2}$ or ΔlogK is negative. ΔlogK can be calculated by the expression,

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

negative ΔlogK for ternary systems indicates that the primary ligand anions and secondary ligand anions preferentially form ternary complexes to their binary ones. It follows from above expression that the difference, ΔlogK, result from the subtraction of two constants and therefore a constant which corresponds the equation,

$$MR + MD \rightleftarrows MRD + M$$

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

$$MR_2 + MD_2 \rightleftarrows 2MRD$$

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

$$MR_2 + MD \rightleftarrows MRD + MR$$

$$MR + MD_2 \rightleftarrows MRD + MD$$

$$MR + MD \rightleftarrows MRD + M$$

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 units by considering the probabilities of finding the metal ions with the ligands for a variety of reason discussed by Sigel. ΔlogK value can be calculated by using first or second approach. The calculated ΔlogK values for all systems are given in table 2.

In Cu(II)-furosemide-glycine system, primary ligand furosemide form only 1:1 and secondary ligand form both 1:1 and 1:2 binary complexes. Therefore this system favours the following disproportion reactions.

$$MR_2 + MD \rightleftarrows MRD + MR$$

$$MD2 + MR \rightleftarrows MRD + MD$$

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 investigations show that the stability constant of ternary complexes formed are less stable. The negative ΔlogK value of this system indicates that the ternary complex is less stable than the binary 1:1 metal-furosemide and metal-glycine complex. This is in accordance with statistical considerations. The negative value of ΔlogK 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[61-64], electrostatic consideration[65], difference in bond type[66], geometrical structure etc.

Sigel concluded that in the case of bidentate ligand D and R, 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 ΔlogK = - 0.4,

-0.6 and –0.9 for square planer and distorted octahedral complexes. Hence the experimentally determined value ΔlogK < -0.6 indicate less stabilisation in ternary complexes. The ΔlogK value of this system is substantially lower than the statistically expected value, showing the destabilised nature of ternary complex.

The primary ligand furosemide having larger size therefore its ΔlogK value is more negative.

Thompson and Lorass pointed out that more negative ΔlogK value of ternary complexes is due to the electrostatic repulsion between the negative charges on furosemide and glycine. Steric hindrance consideration is the most important factor because in the present studies of ternary complex, primary ligand furosemide 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 Cu(II)-furosemide titration curve. So, it is evident that the entry of the secondary ligand glycine faces steric hindrance due to bigger size of the Cu (II)-furosemide complex as compared to aqua ion, which tries to restrict the entry of the secondary ligand in the coordination sphere of the Cu (II) metal ion and thus reduces the stability of ternary complexes.

Simillarly the stability constant of mixed ligand complexes of furesemide and secondary ligand such as leucine, glutamic acid, glutamine, valine, methionine and phenylalanine are studied and mentioned in table 2.

Parameters based on some relationship between the formation of ternary complexes of copper (II) metal ion with furosemide in the presence of amino acids (1:1:1) system at Temp = 27^{0}C I = 0.1 M $NaClO_4$ Medium = 80% (V/V) Ethanol-Water.

Table No.2

Amino acids	β_{11}	β_{02}	β_{20}	K_D	K_R	K_r	ΔlogK
Glycine	12.5042	19.67	2.8575	9.6467	2.8142	1.1616	-0.0433
Leucine	10.6455	08.07	2.8575	7.7880	2.5752	1.9483	-0.2823
Glutamic acid	13.8066	19.64	2.8575	10.9491	2.8266	1.2284	-0.0309
Glutamine	12.3649	17.43	2.8575	9.5074	2.8249	1.2189	-0.0326
Valine	11.3345	18.49	2.8575	8.4770	1.3245	1.0619	-1.5330
Methionine	11.9671	18.31	2.8575	9.1096	2.3271	1.1307	-0.5304
Phenyl alanine	11.5664	16.66	2.8575	8.7089	2.5767	1.1852	-0.2811

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Conclusion.

It is observed that, the stability constants of ternary complexes are negative in all cases this indicates that, the ternary complexes are less stable as compared to binary complexes of the corresponding systems. Different parameters like K_L, K_R, Kr and

ΔlogK are used to explain stability of mixed ligand complexes.

The order of formation of ternary stability constant of drug furosemide with secondary ligand such as amino acids with metal copper(II) mentioned as follows

Furosemide = glut. acid > glut. > gly. > phe. ala. > leu. > methi. > val.

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