International Journal of Universal Science and Technology
ISSN: **ISSN: 2454-7263** Copyright © Universal Print
Volume No. 03, Issue No. 01, Page No. 47-51 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Studies on Metal Complexes of Some……

Studies on Metal Complexes of Some Non Essential Amino Acids With Copper (II)

Bhimrao C. Khade[1] and Pragati M. Deore[2]

[1]Research Laboratory, Department of Chemistry, Dnyanopasak College, Parbhani 431401, (M.S.), India

[2]Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431001, (M.S.), India.

E-mail: bckhbade@yahoo.com

Abstract

The formation constant of copper (II) ion with some non essential amino acids viz. arginine, glutamic acid, glutamine, glycine & alanine as ligand were determined pH metrically at 27°C and an ionic strength of O.1M $NaClO_4$ in 80% (v/v) ethanol-water medium. The calculations of stability constant of have been made using the computerized programmed

Keywords: Stability constant, logK and pK

1. Introduction

The metal ions 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. Short resume of relevant biological importance of metal ions is discussed here.

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 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 and albumin; these can carry many things including copper. Copper is stored in proteins called metallothione. 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 Willson's disease and Menke's disease with symptoms that range from psychiatric abnormalities and motor dysfunction to poor temperature control a liver & kidney abnormalities.

Arginine [1] is essential amino acids. It is basic and in addition to the amino group in the α-position, arginine has a guanido group. It is glycogenic amino acid. Arginine is required for polyamine biosynthesis in bacteria, fungi and higher eukaryotes. Arginine is an important source for the formation of nitric oxide (NO). Nitric oxide synthase is the enzyme, which cleaves the guanido group of

International Journal of Universal Science and Technology
ISSN: **ISSN: 2454-7263** Copyright © Universal Print
Volume No. 03, Issue No. 01, Page No. 47-51 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Studies on Metal Complexes of Some……

arginine to form NO. Nitric oxide serves important functions. Nitric oxide is a vasodilator and smooth muscle relaxant. It regulates blood flow and blood pressure. It inhibits platelet aggregation and adhesion. It is neurotransmitter and helps macrophages in the bactericidal action.

The formamido group (– C – NH_2) can be transferred to glycine to form guanidoacetic acid (glycocyamine) which can be methylated to form creatine. It is the immediate precursor in the formation of urea by the liver. Arginine, by the action of agrinase, is converted to ornithine and urea. The ornithine, on transamination, is converted to glutamic acid semialdehyde, which can be oxidized to glutamate. Thus, it is glycogenic. Ornithine, in conjunction with methionine, serves as a precursor for the synthesis of polyamine spermidine and spermine. These polyamines are growth factors and are required for cell proliferation. Since, they carry a high positive charge, readily associate themselves with polyanions like DNA and RNA and help in stabilizing those structures and may also stimulate their synthesis. They also act as inhibitors of certain enzyme synthesis, particularly the kinases. In pharmacological doses, they act as hypothermic & sedatives. Spermidine and spermine are oxidized to putrescine and other products by the enzyme, 'polyamine oxidase' which is present in the liver peroxisomes. Large amounts of putrescine and spermidine are excreted in urine[86] in a acetylated form.

Glutamic acid is acidic non-essential glycogenic amino acid with one amino group and two carboxylic group. 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, on deamination it form oxaloacetate and α - ketoglutarate and form glycogen. Its wide range contribution in urea formation, purine, and pyrimidine rings synthesis. Glutamic acid on decarboxylation gives rise to gamma aminobutyric acid. It controls the neuronal activity. Glutamic acid is one of the constituent of glutathione which is important in the activity of sulphadryl enzyme system.

Glutamine is acidic non-essential glycogenic amino acid. It is a constituent of folic acid. Basically it is used in higher animal for conjugation, detoxification of phenyl acetic acid.

Alanine is a non-essential, glycogenic amino acid. It was first isolated in 1888 from silk fibrin where it occurs in abundance along with glycine and serine. It is the parent substance of all the amino acids except glycine. The various amino acids may be derived from alanine by replacement of one or two H atoms of the methyl group present on α-carbon atom. Alanine is the least hydrophobic of the 8 non-polar aminoacids because of its small methyl side chain. Deamination or transamination produces pyruvic acid, which can be readily converted to glucose or oxidised in citric acid cycle. β-alanine is a constituent of pantothenic acid.

Glucose is released from liver by glycogenolysis and gluconeogenesis during muscular contraction. Glucose is utilized by muscle by glycolysis, producing pyruvate. While part of this pyruvate is converted to lactate, the rest is aminated to form alanine. Both are returned to the liver and can participate in gluconeogenesis, to form fresh glucose. Formation of alanine from pyruvate in muscle also helps in removing some of the NH_3 formed in that tissue during amino acid metabolism. The cycle of transport of glucose from liver to muscle and of alanine from muscle to liver is known as glucose – alanine cycle.

Glycine is the neutral, aliphatic, optically inactive non-essential, glycogenic amino acid. 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. Haeme is a tetra pyrol ring system with transition metal iron. The nitrogen from each pyrol is denied from Glycine. It can form serine, creatine and purine. It is essential constituent of glutathione and also take part in detoxication mechanism. There are several abnormalities in glycine metabolism such as primary hyperoxaluria, due to diversion of more glycine to oxalate formation glycinuria, urine contain large amounts of oxalates as well as less reabsorption of glycine in the kidney.

Survey of literature reveals that no work has been reported on complex tendencies of amino acids with transition metal ion copper (II) in ethanol-water solution. Therefore in order to understand the complex formation tendencies of amino acids with copper (II) in 80 % (v/v)

International Journal of Universal Science and Technology
ISSN: **ISSN: 2454-7263** Copyright © Universal Print
Volume No. 03, Issue No. 01, Page No. 47-51 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Studies on Metal Complexes of Some……

ethanol-water medium at 27°C at a fixed ionic strength 0.1M NaClO4

2. Materials and method

Amino acids in pure form were obtained from recognized chemical supplier and used as received. Ethanol was purified as described in literature. Double distilled water was used for the preparation of ethanol-water mixture and stock solution of metal and amino acids.

All chemicals used were AnalaR grade. $NaClO_4$ (O.lM) and NaOH solution was prepared in carbon dioxide 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 copper (II) to prevent hydrolysis and standardized by using standard EDTA solution[2].

The experimental procedure, by the potentiometric titration technique, involves the titration of carbonate free solution of

1)Free $HClO_4$

2)Free $HClO_4$ + Ligand Amino acids

3)Free $HClO_4$ + Ligand Amino acids + Copper Metal Ion

Against standard solution of sodium hydroxide and amino acid. The ionic strength of the solutions was maintained constant i.e. 0.1 M by adding appropriate amount of 1M sodium per chlorate solution. The titration were carried out at 27°C in an inert atmosphere by bubbling oxygen free nitrogen gas through an assembly containing the electrode to expel out $C0_2$. pH meter reading in 80%(v/v) ethanol-water were corrected by method of Vanuitert and Hass.[3]The formation constant of binary complexes were determined by computational programmed to minimize the standard derivation.

3. Results and Discussion

Binary metal complexes

The proton ligand constant and metal ligand stability constant of amino acids with copper (II) determined in 80 %(v/v) ethanol-water mixture at 27°C and ionic strength μ= 0.1 M $NaCI0_4$ are given in Table I

The experimental data obtained from pH metric titration in 80% (v/v) Ethanol-water medium of a representative system is presented in Table, was used to calculate pK and logK values of metal complexes

The acid and acid + ligand curve of representative system coincide up to pH 3.8 and the latter deviates from the former after pH 10. The ligand curve deviates from the acid curve in the region of low pH to right side indicates the deprotonation of ligand). The proton-ligand stability constant needs to be determined very accurately since pL values are dependent on them. The accuracy of n_A is determined by the accuracy with which V_2-V_1 can be determined (the volume of alkali added can be read on the microburette accurately upto the second place of decimal and with slight uncertainty to the third place). The addition of alkali was made in minute drops of ≈ 0.01 to 0.02 ml. The burette reading was noted immediately after the drop of alkali was added. The difference V_2-V_1 can be estimated from the graph accurately up to second place of decimal and with limited accuracy to the third place. The values of V_2 and V_1 used in the calculations of nA are given in Table 2.4 for a representative system. The values of nA have been plotted against pH of the solution to get the formation curve for the proton ligand complex. The pK values i.e. proton-ligand stability constant were calculated by following methods.

Point wise calculation method.

The $\bar{n}_A$ values in the region 1.2 to 1.8 and 0.2 to 0.8 were used to obtain pK_1, and pK_2 by using equation. The average of these values was taken as the correct values of pK_1 and pK_2. These values were further corrected by straight-line plot. This method was used to obtain correct pK values for all amino acids.

Half integral method

The graph plotted between n_A against pH is known as formation curve. Approximate values of proton-ligand stability constants can be determined by this method. The values of

International Journal of Universal Science and Technology
ISSN: **ISSN: 2454-7263** Copyright © Universal Print
Volume No. 03, Issue No. 01, Page No. 47-51 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Studies on Metal Complexes of Some……

pH for which n_A = 1.5 and n_A=0.5 corresponds to pK_1 and pK_2 respectively. The values of pK obtained by half-integral method and point wise calculation method are found to be in good agreement.

Determination of metal-ligand stability constants.

The following conditions should hold for the system, for conversion of any Calvin-Bjerrum titration data into stability constant.

1) Formation of complex, under the experimental condition.
2) Absence of metal ion hydrolysis, polynuclear, hydrogen and hydroxyl bearing complexes and anion complexes of metal ion.
3) Absence of complexes of very high and very low stability.

The deviation along the volume axis of Metal+ligand titration curve from the ligand titration curve is an indication for chelation in solution. The metal solution used in present investigation is $1.0x10^{-3}$ M. Therefore the possibility of formation of polynuclear complexes is not expected. All the metals are used in perchlorate form.

Calculation of n and pl

The values of metal-ligand formation number, n were obtained by equation. To calculate n, volume of NaOH required obtaining the same pH i.e. V_2 & V_3 can be read directly from the ligand titration curve and ligand + metal ion titration curve respectively. The n were calculated in the pH range where there is no possibility of hydrolysis of metal ion. The values for V_2, V_3, n and pL for representative system are presented. The values of pL were calculated using the equation. For the calculation of metal-ligand stability constant, following method are used.

Point wise calculation method.

The metal ligand stability constants $logK_1$ and $logK_2$ were calculated by using this method. The average of these values was taken as the correct value of $logK_1$. The values of n selected where in the range 0.2 to 0.8.Point wise calculations for $logK_1$ values are shown The formation constant in the case of 1:2 complexes were calculated using the linear equation given in chapter first, for different values of n and pL. The average of these values was taken as the correct values of $logK_2$. The values of n selected were in the range of 1.2 to 1.8.

Half integral method

To get the formation curve, the values of n, were plotted against pL. The formation curves for each metal ligand system were drawn separately. The number of complexes formed in the reaction and the values of stability constants can be deducted from the formation curve. From the formation curve the values of $logK_1$ and $logK_2$ were calculated by the known value of pL at n = 0.5 and 1.5 respectively.

Method of least squares

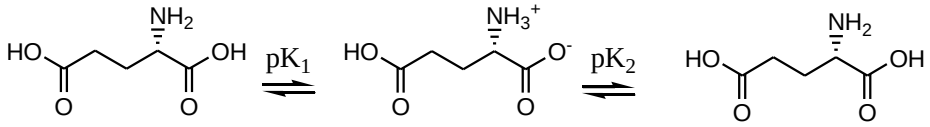
The metal ligand system having a ratio of $logK_1/K_2$ less than 1.8 was subjected to the method of least squares to achieve greater accuracy.

The method of least squares was carried out to determined $logK_1$ and $logK_2$ values.

It is convenient to plot n/(n-1)L against (2-n)/(n-1) to obtain the slope K_1K_2 and the intercept – K_1 of the straight line. The n values between 0.8 to 1.2 were however, not taken for calculation as in this range the values n/(n-1) and (n-2)/(n-1) become very large in the center of curve and very sensitive to even slight experimental errors in n points in this small region.

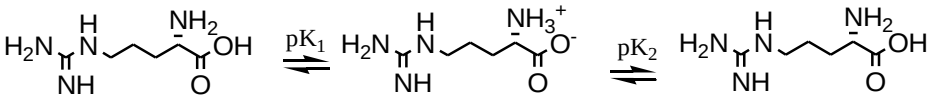
In the present investigation all values of $logK_1$ and $logK_2$ were calculated by all the methods.

Proton ligand dissociation constant of amino acid is shown below.[4-7]



(Dissociation of glutamic acid)

International Journal of Universal Science and Technology
ISSN: **ISSN: 2454-7263** Copyright © Universal Print
Volume No. 03, Issue No. 01, Page No. 47-51 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Studies on Metal Complexes of Some……



(Dissociation of arginine)

The proton ligand constant and metal ligand stability constant of dapsone and amino acids with copper (II) determined in 80 % (v/v) ethanol-water mixture at 27^0C and ionic strength μ=0.1M $NaClO_4$ are given in Table. [8-16]

AMINO ACIDS	PK_1	pK_2	$logK_1$	$logK_2$
Glycine	2.7700	9.7400	9.6900	8.9800
Glutamic acid	3.1360	5.8987	10.9800	8.6400
Glutamine	3.0100	9.2800	9.5400	7.8900
Arginine	4.2659	12.200	-	-
Alanine	3.7000	10.180	-	-

4. Conclusion

Proton ligand stability constant and metal ligand stability constant of non essential amino acid with Cu(II) were determined at 27^0C and μ=0.1 m $NaClO_4$ in 80% (v/v) ethanol- water medium and presented in Table pK values of the ligands generally increase as the dielectric constant of the medium decreases thereby enhancing logK values. Similar trends are observed in the pK and logK values of amino acids and their complexes with Cu (II).

5. References

1. A textbook of biochemistry by A.V.S.S. Rama Rao, UBSPD Publications.
2. Vogel A.I., " A Text Book of Practical Organic Chemistry", Pergamon Green and Co. Ltd., London (1956)
3. Van-Vitart L.G. and Hass C.G., J. Am. Chern. Soc., (1953) 75, 451
4. Biochemistry of Leningers.
5. Khade B.C., Deore P.M. and Arbad B.R., Acta Sciencia Indica (2007) vol XXXIIIC, No.1, 105-108.
6. Bhosale V.N. Thesis submitted to Dr. B. A. M. university Aurangabad (1993)
7. Khade B.C., Deore P.M. and Arbad B.R., Acta Sciencia Indica (2007) vol XXXIIIC, No.1, 105-108.
8. Shoukry M.M., Mohamed M., Shehata M.R. and Mohmoud A.M., Mikrochim.
9. Acta. (1998) 129,107
10. Biochemistry of Leningers.
11. Shoukry M.M., Khairy M.E. and Khalid KG. Transition Met. Chern. (1997) 22,465
12. Gupta R., Vyas P.C., Arora M. And Bapna R., Trans SAEST (1997) 32, 21
13. Mahamoud A.A.A., Farghely O.A., Ghandour M.A. & Said El. Montsch. Chem, (2000) 131, 1031.
14. Lozano M.J. & Borras J.J. Inorg. Biochem., (1987), 31, 197
15. Garg B.S. & Dwived Poonam, J. Indian Chem. SOC., Vol. 83, (2006) 229-232.