

X-Ray Diffraction and structural studies of Cu(II) complex with Gliclazide(*N*-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-ylcarbamoyl)-4-methylbenzenesulfonamide),and its hypoglycemic activity.

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Abstract

Synthesis and characterization by spectroscopic, X-ray and hypoglycemic activity study of copper complex with gliclazide (*N*-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-ylcarbamoyl)-4-methylbenzenesulfonamide) an oral antidiabetic drugs. The conductometric titration using monovariation method indicate that complex are non-ionic and L₂M (2:1) type. Analytical data agree with the molecular formula (C₁₅H₂₀N₃O₃S)₂Cu. of complex for gliclazide. The structure of complex was assigned square planner supported by IR, ¹H-NMR, X-Ray, mass studies and propose structure (I) for complex. Alloxan induced model have been used to compare hypoglycemic activity of gliclazide complex.

Keywords: *Gliclazide, Oral antidiabetic drugs, Complexes, IR, NMR. X-Ray, Hypoglycemic activity.*

1. Introduction

Metal ion are required for many critical function in humans .Scarcity of some metal ions can leads to disease¹. Well – known example can leads to pernicious anemia resulting from iron deficiency; growth retardation arising from insufficient dietary zinc, and heart disease in infants owing to copper deficiency. The ability to recognize, to understand at the molecular level and to the diseases caused by inadequate metal- ion function constitutes an important aspect of medicinal bioinorganic chemistry. Understanding the biochemistry and molecular biology of natural detoxification mechanisms and designing and applying ion-specific chelating agents to treat metal over-loads are the two components of a second major aspect of the new science that is evolving at the interface of bioinorganic chemistry and medicine.

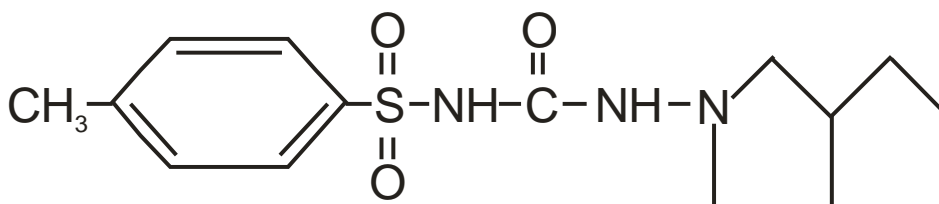
Diabetes is a deceptive disease and if not detected in early stage may cause even death. It is considered hereditary but actual genetic disorder is still a mystery. Several million people are suffering from this disease all over the world (Sadilot and Phatak², Bloomgarden³, Sanger and Thompson,⁴). Zinc- insulin was discovered as early as in 1921 and later it proved to be a very efficacious medicine in the treatment of diabetes mellitus. To avoid the daily pricks of hypodermic syringe, oral hypoglycemic agents were discovered which has revolutionized the treatment of diabetes .It is worthwhile to mention here that the majority of the essential metallic elements of biological importance are transition metals, whose ability to form coordination complexes and chelates is the characteristic aspects of their chemistry.

In recent years much attention is given to the use of sulphonyl-ureas because of their high complexing nature with essential metals. Sulphonyl-ureas are effective for non- insulin dependent diabetes mellitus, (Sadilot and Phatak², Bloomgarden,³ Sanger and Thompson,⁴). These compounds are completely absorbed on oral administration. They are metabolized by liver and are excreted predominantly through urine.

Complexation of sulphonyl-ureas with lighter transition metals has been studied in detail by Yoshinaga and Yamamoto⁵⁻⁶, Iqbal *at. et.*,^{7, 10, 11}. A perusal of available literature shows that systematic study of complexation of copper with gliclazide is relatively scanty. It is interesting to have an insight into the synthesis of copper complex with gliclazide and to diagnose various structural aspects of the isolated complex.

Here the synthesis and characterization of copper with gliclazide have been described

Structure of Gliclazide



2. Experiment

2. 1.Ligand-Metal Ratio

- Pure gliclazide m.p. 180°C (Lit. 179.5-180.5), 0.005 M, pure were diluted to 100ml. and titrated conductometrically against cupric chloride at 30±1°C. results were plotted in the form of graph which indicate ligand metal ratio as 2:1 (L₂M).
- Formation of 2:1 (L₂M) ratio was further confirmed by Job's method⁸ of continuous variation as modified by Turner and Anderson⁹(Table-1) spectrophotometric studies were conducted using absorbance as index property, from these values the stability constant (log k) and free energy change (-ΔF), were also calculated Tables 1, and fig. 1 given for gliclazide -copper complex.

Table-1 GLICLAZIDE WITH CUPRIC CHLORIDE

Gliclazide-0.002M, 0.005M
 90% Ethanol
 pH: 6.4

Cupric chloride-0.002M,0.005M
 Temperature: 31⁰C±1

Solvent:
 Wavelength: 610 nm

S.No.	Metal:Ligand ratio	Absorbance		Corrected Absorbance	
		0.002M	0.005M	0.002M	0.005M
1	0:12	0.006	0.01	0.00	0.00
2	1:11	0.065	0.095	0.059	0.085
3	2:10	0.112	0.135	0.106	0.125
4	3:9	0.167	0.19	0.161	0.175
5	4:8	0.205	0.260	0.198	0.241
6	5:7	0.185	0.240	0.178	0.220
7	6:6	0.145	0.198	0.138	0.185
8	7:5	0.098	0.123	0.090	0.109
9	8:4	0.090	0.112	0.082	0.099
10	9:3	0.052	0.075	0.044	0.065
11	10:2	0.033	0.043	0.025	0.029
12	11:1	0.021	0.025	0.020	0.019
13	12:0	0.009	0.014	0.00	0.00

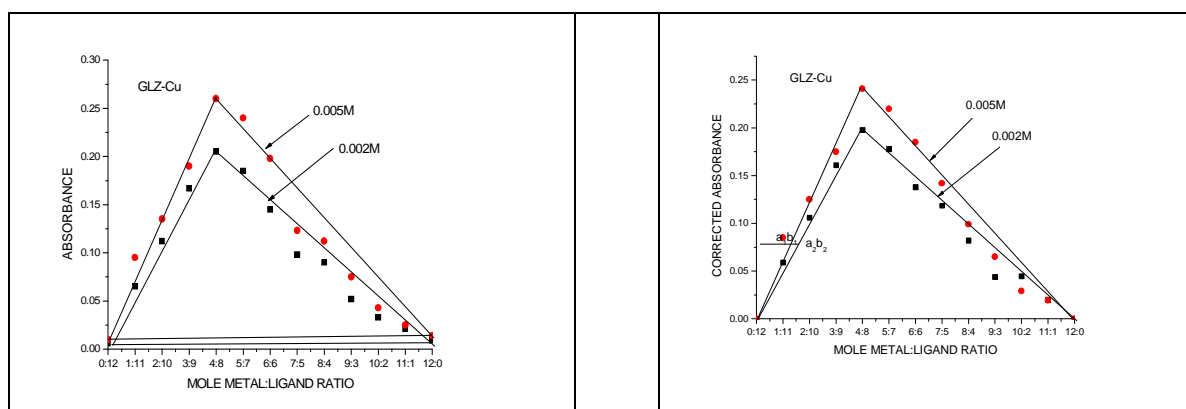


Fig.1 Job's Method of continuous variations modified by Turner and Anderson

2.2 Synthesis of Complex

The chemicals used in this synthesis were all of AnalaR grade and Hi-media. A weighed quantity of Gliclazide (2 mole) (supplied by Zim laboratory, Nagpur) was dissolved separately in minimum quantity of 90% ethanol. The cupric chloride solution was prepared by dissolving (1 mole) separately in the same solvent. Ligand solution was added slowly with stirring into the metallic salt solution at room temperature, maintain the pH between 6.0 to 6.5 by adding dilute NaOH solution. On refluxing the mixture for 3 hour and on cooling the complex separated out. Which was filtered, washed well with ethanol and finally dried in vacuum and weighed.

The elemental analysis of the isolated complex was carried out using Coleman Analyzer at the Departmental Microanalytical Laboratory CDRI Lucknow. The IR spectra of the ligand as well as of the complex was recorded on Perkin Elemer Spectrometer(I.I.T Bombay) and ^1H NMR spectra of the ligand and isolated complex was recorded on a Bruker DRX-300 spectrometer and d_6 -DMSO was used as a solvent. ^1H -NMR spectra recorded in CDRI Lucknow, India, and X-Ray diffractogram from Punjab University, Chandigarh, India. From stoichiometry and analytical data, the composition of the complex comes out to be $(\text{C}_{15}\text{H}_{21}\text{N}_3\text{O}_3\text{S})_2\text{Cu}$, which favours 2:1 (L_2M) ratio. The tentative structure (I) assigned to complex on the basis of analytical data and IR, NMR and X-ray studies.

3. Result

3.1 Table-2 Physico-chemical characteristics of gliclazide-copper complex

S. No.	Complex	Color	Yield (%)	m.p.°C	$-\Delta F$	Log K	Molar conductance $\Omega^{-1}\text{cm}^{-1}\text{mole}^{-1}$
1.	$(\text{C}_{15}\text{H}_{21}\text{N}_3\text{O}_3\text{S})_2\text{Cu}$	Maple green	58.79	169	16.65	12.022	30.1
S.No.	Formula of complex	Molecular weight (g/mol)	Analytical data calculated (theoretical)				
			C	H	N	S	Metal (%)
1.	$(\text{C}_{15}\text{H}_{21}\text{N}_3\text{O}_3\text{S})_2\text{Cu}$	708	50.19 (50.50)	6.00 (6.01)	10.47 (11.47)	9.00 (8.47)	7.99 (7.75)

3.2 X-Ray Diffraction Studies.

X-ray diffraction studies also confirm the complex and formation due to new bonds ¹⁰⁻²³. The number of peaks in gliclazide are 22 while $(\text{GLZ})_2\text{Cu}$ are 10

(Fig.2) . Thus indicating that complex formed is a well kit one. All the reflections present are new ones and the patterns are fairly strong. On comparing the pattern obtained with available literature. It is evident that its pattern is not in good agreement with available information and thus confirms the formation of totally new complex. The X-ray pattern have been indexed by using computer software(FPSUIT 2.0V) and applying interactive trial and error method keeping in mind the characteristics of the various symmetry system, till a good fit was obtained between the observed and the calculated $\text{Sin}^2\theta$ value. The unit cell parameters were calculated from the indexed data and given below, from cell data and crystal lattice parameters of $(\text{GLZ})_2\text{Cu}$, indicates complex attributed to orthorhombic crystal system Table 3. $a(\text{\AA}) = 21.762$, $b(\text{\AA}) = 23.4271$, $c(\text{\AA}) = 27.274$, $\alpha=90^\circ$, $\beta=90^\circ$, $\gamma =90^\circ$, Volume $(abc)\text{\AA}^3= 14062.94$, $D_{\text{cal}}=5.39107\text{g/cm}^3$, $D_{\text{obs}}=5.40791\text{g/cm}^3$, Crystal system= orthorhombic, Porosity(%) = 4.411, Density = 0.05473g/cm^3 , Particle size = 15.0 microns, Space group = Pmmm

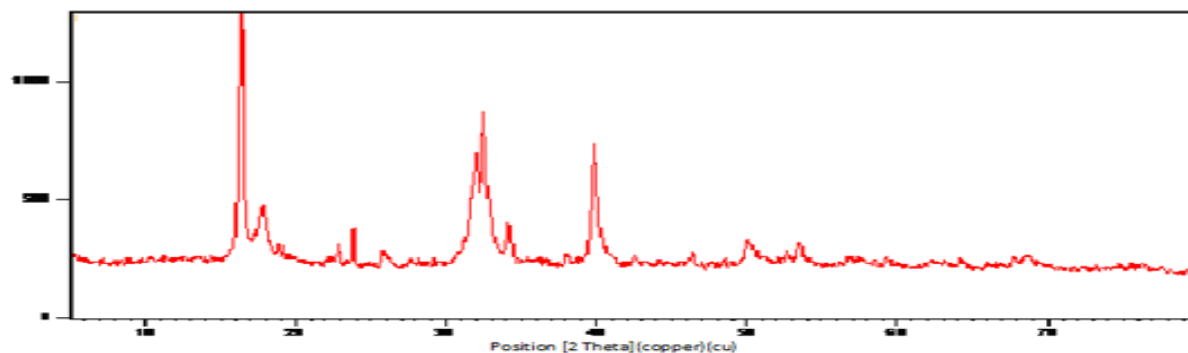


Fig.2 X-ray diffractogram of gliclazide-copper complex

Table-3 Cell data and crystal parameter of gliclazide-copper complex

2θ	I/I_0	$D_{(\text{Obs})}$	$D_{(\text{Cal})}$	h	k	l
16.3917	100	5.40791	5.39107	0	4	2
17.8081	20.64	4.98085	4.99204	0	2	5
22.8482	8.89	3.89225	3.88698	0	1	7
23.8088	15.71	3.73734	3.73578	5	2	3
31.7966	32.84	2.81435	2.81304	2	8	1
32.4666	62.17	2.75779	2.75569	7	3	3
34.2064	16.25	2.62140	2.62045	5	5	6
39.8484	48.48	2.26229	2.26061	0	7	9
50.0436	8.51	1.82276	1.82107	4	11	6
53.5242	8.71	1.71068	1.71068	4	13	0

3.3 Infra-red Spectra Studies

The IR spectra of ligand and isolated complex (Fig.3) was recorded within the range $4000-400\text{ cm}^{-1}$. Assignments (Table-4) of the infrared spectral bands are based on literature. IR

spectrum shows important bands due to $\nu(\text{M-O})$ $500\text{-}600\text{ cm}^{-1}$, $\nu(\text{Ar-S})$ $700\text{-}800\text{ cm}^{-1}$, $\nu(\text{-S-N})$ $1085\pm 20\text{ cm}^{-1}$, $\nu(\text{SO}_2\text{-N})$ $1120\pm 20\text{ cm}^{-1}$, $\nu(\text{C-N})$ $1210\pm 20\text{ cm}^{-1}$, $\nu(\text{S=O})$ $1340\pm 20\text{ cm}^{-1}$, $\nu(\text{C=O})$ 1710 cm^{-1} , $\nu(\text{NH-stretch})$ $3274\pm 20\text{ cm}^{-1}$. The proposed structure for the isolated complex is also supported by IR absorption²³⁻²⁸

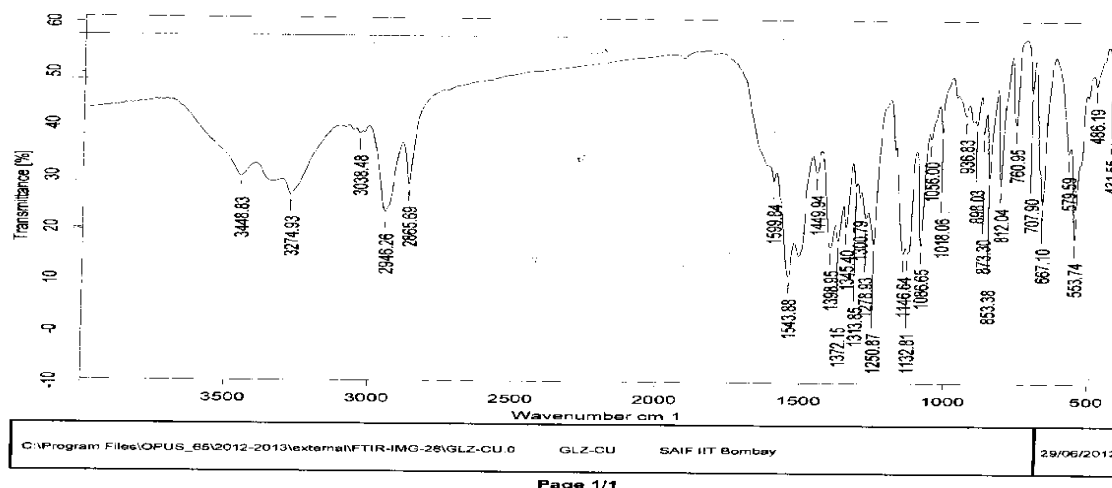


Fig.3 gliclazide –copper complex

Table -4 Specific IR assignment of gliclazide (ligand) and gliclazide complex with Cu(II)

Pure drug (Gliclazide)	Gliclazide-Cu complex
632 cm^{-1} s, 668 cm^{-1} vs, 1087 cm^{-1} vs 1240 cm^{-1} vs,1348 cm^{-1} vs,1710 cm^{-1} vs, 2867 cm^{-1} , 2950 cm^{-1} vs, 3274 cm^{-1} vs Vs=very strong, s=strong, m=medium W=weak	547 cm^{-1} s ,753 cm^{-1} vs,1122 cm^{-1} s, 1216 cm^{-1} vs,1622 cm^{-1} vs, 1337 cm^{-1} s, 1524 cm^{-1} s, 2947 cm^{-1} m, 3021 cm^{-1} s, 3674 cm^{-1} m, Vs=very strong, s=strong, m=medium, W=weak

3.4 ¹H-NMR Studies

¹H-NMR spectral (Fig.4) data are given in (Table-5). It was observed that the singlet due to the imide (NH) proton around (δ 8.033) in the spectrum of the ligand disappeared in the spectra of (NH) group in the complex molecule due to formation of M-O band. This also confirms the deprotonation of imide NH group through enolization (the appearance of $>\text{C}=\text{N}$ stretching band observed in IR spectra). Other features of NMR spectrum were the aromatic presence of unresolved multiplet suggestive protons²⁹⁻³⁸.

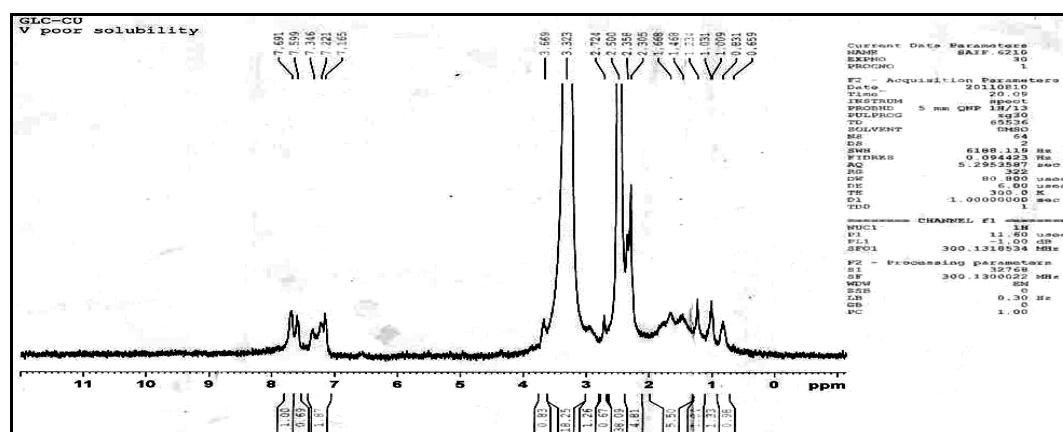


Fig-4 ¹H NMR spectra of gliclazide-copper complex

Table-5 NMR-Assignments of gliclazide-copper complex

(C ₁₅ H ₂₁ N ₃ O ₃ S) ₂ pure drug Gliclazide	(C ₁₅ H ₂₀ N ₃ O ₃ S) ₂ Cu
δ8.041 (s,1H,NHCO, J=0.334H _z), δ7.817 (d,benzene J=1H _z), δ7.395 (d,benzene, J=1H _z), δ6.28 (s,SO ₂ NH), δ3.320 (NH-CO, J=0.929H _z), δ2.901 (s,CH ₃ group attached to benzene, J=2.160 H _z), δ1.388 (s,CH ₃ group, J=2.955H _z , s=singlet, d=doublet, t=triplet, q=quartrate, m=multiple	δ7.691 (d,1H,NH-CO, J=1H _z), δ7.346 (d,benzene, J=0.69H _z), δ6.328 (s,SO ₂ -NH, J=1H _z), δ3.668 (s,NH-CO-Cu, J=0.83H _z), δ2.724 (s,CH ₃ -group attached to benzene, J=1.26H _z), δ1.608 (q,CH ₃ group,J=5.50H _z), δ1.009 (CH ₃ group,J=2.03H _z

3.5 Mass Spectral Study

The mass spectra of Cu(II) complex of the gliclazide, peaks attributable to the related molecular ions m/z 713.1[M-2H]²⁺, m/z 1068[M-2H]²⁺,324[M-H]²⁺ respectively³⁸⁻⁴⁸. The observed free ligand Gliclazide peaks for Cu(II), m/z 324.1 [L+H]⁺. The mass spectra of the complex is given in Fig. 5 and assignment are m/z 713 due to [Cu(C₁₅H₁₉N₃O₃S)₂]⁺ or (ML₂)⁺. Molecular ion peak (m⁺); m/z 366 due to (C₁₈H₁₉N₆O₃)⁺ fragment ion. m/z 324 due to [C₁₅H₂₁N₃O₃S]⁺. Base peak ion 100% relative abundance.

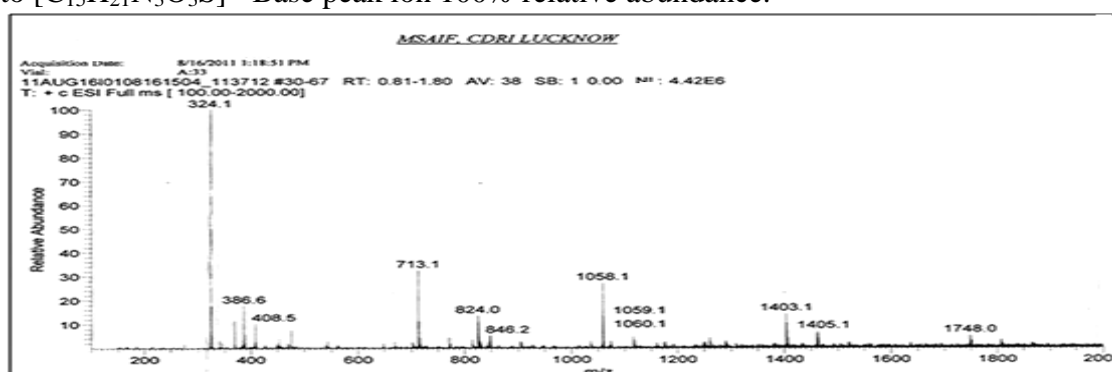


Fig.5 Mass spectra of gliclazide-copper complex

3.6 Hypoglycemic Studies

Pharmacology is mainly concerned with the responses of living organisms to chemical stimuli. One may further divide the subject from a medical view point, into pharmacodynamics and pharmacotherapy, the former is concerned with the response of living organism to chemical stimuli in the absence of disease, while the later with the response of

organism to such stimuli in a pathogenic state. This is the phase of pharmacology (i.e. pharmacotherapy) which is of special interest to the physician.

Pharmacotherapy includes the treatment of the sick with drugs and therefore is of prime importance in practice of medicine. It is fundamental to the health-economy of the people. A compound or a complex which is to be recommended as a drug of utility must be capable of easy absorption and excretion. It is also essential that neither the absence itself nor the metabolic products thereof should exercise toxicity or any adverse side effect to the patient.

1. **Animal Study-** Where necessary such tests should be carried out on animals as rats, rabbits and dogs. When a substance has given satisfactory results for the aforesaid animals then only it may be tried on monkeys and men.
2. In present study we analyze the hypoglycemic activity on albino rats:-

Animal care and handling

The anti-diabetic activity was carried out on Wistar albino rats of 4 months of both sexes, weighing between 140 to 200 gm. They were provided from Sapience Bio-analytical Research Lab, Bhopal, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature $25\pm 2^{\circ}\text{C}$ relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1413/PO/a/11/CPCSEA).

Chemicals

Alloxan monohydrate was purchased from Central Drug House (P) LTD. Gliclazide was provided as gift sample by Zim laboratories, Nagpur. All other chemicals used for this study were of analytical grade.

Induction of diabetes by alloxan^{49, 50}

The diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of Alloxan monohydrate (120 mg/kg body weight) Hyperglycemia was confirmed on the third day of Alloxan-injection. Rats with moderate diabetes having hyperglycemia (with blood glucose above 150 mg/dl) were taken for the experiment.

Experimental Design

In the experiment, a total of 24 rats were used. The rats were divided into 4 groups, comprising of 6 animals in each group as follows:

Group I: Normal control rats received 1ml/100gm of 0.5% CMC (carboxy methyl cellulose) using an intragastric tube for 4 days.

Group II: Negative control rats received Alloxan 120g/kg, i.p. for inducing diabetes.

Group III: Rats received gliclazide (2mg/kg body weight per orally) for 4 days and Alloxan 120g/kg, i.p. on 1st day.

Group IV Rats received gliclazide-copper complex, (2mg/kg, per orally.) once daily for 4 days and Alloxan 120g/kg, i.p. on 1st day

Sample collection

At the end of study, Blood samples were collected through tail vein and blood glucose levels were estimated using an electronic glucometer (Glucochek) and result is given in table 6 & 7.

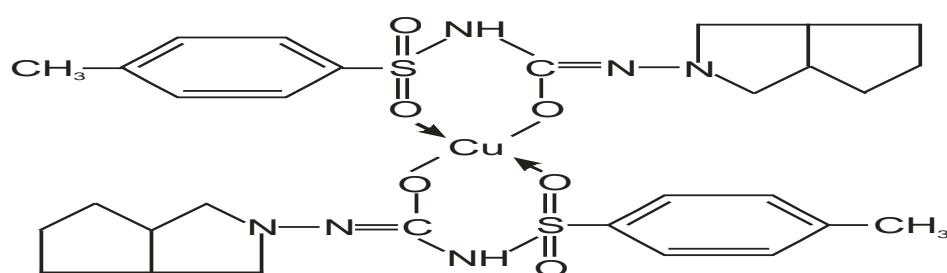
Group-I	Group-II	Group-III	Group-IV
90	108	175	170
95	157	180	175
105	160	168	195
106	122	170	187
109	170	195	167
85	165	190	155

Group-I	Group-II	Group-III	Group-IV
107	112	135	115
89	180	130	120
97	188	140	125
110	132	135	98
102	197	126	120
98	197	125	112

Discussion

For supporting the proposed structure of copper-gliclazide complex, initially Job's method of continuous variation as modified by Turner and Anderson was conducted which indicate 2:1 ligand:metal ratio of the complex, moreover stability constant and free energy change was also calculated. Analytical data (Table.2) agrees to the molecular formula $(C_{15}H_{20}N_3O_3S)_2Cu \cdot (L_2M)$.

For determining the proposed structure on the basis of stoichiometry and analysis of the complex. Advance spectroscopic methods like IR, 1H NMR, Mass were conducted which suggest the coordination of metal atom with enolic oxygen of the carbonyl group on one side and oxygen of the sulphonyl group from the other side. These observation were further supporting from the IR (Table.4) and NMR (Table.5) values of metal-oxygen and disappearance of M-H linkages in NMR. A detailed study of X-Ray (Table-3) also supports the complex formation and the various values like particle size, porosity, volume of unit cell, density as well as crystal system was evaluated and discussed. Moreover looking to the higher electronegativity of oxygen as compared to N^2 and to enolization is strongly supported. Copper complex of gliclazide was evaluated for their hypoglycemic effect on experimental rats using alloxan induced diabetes model. The effect of copper complex of gliclazide on blood glucose level at 3rd and 7th day in alloxan induced diabetes in rats shown in table-6, 7 respectively. So from table we can conclude that copper complex of gliclazide have more hypoglycemic activity as compared to parent drug. These interesting observations on metal-complex of oral sulphonyl-urea used as anti-diabetic agents for lowering blood sugar concentration may likely substantiate the use of these complex after extensive clinical studies.



Structure (I) for gliclazide-copper complex

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