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Physico-Chemical and Various Spectral Investigations of Copper (II) Complex Of Glimepiride, An Oral Active and Highly Potent Antidiabetic Agent

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The synthesis and characterization of a copper complex with glimepiride (an oral antidiabetic drug) has been studied. The conductometric titration using monovariation method indicates that complex is non-ionic and of ML_2 type.Analytical data agrees with the molecular formula $(C_{24}H_{34}N_4O_5S)_2$ Cu. Structure of the complex was assigned as square planar in which ligand molecules lies horizontally joining the central copper atom. Infrared, Mass spectral and particle size analysis had confirm the co-ordination of sulphonyl oxygen on one side and enolic oxygen attached from other side with the metal ion. The structure for complex was proposed on the basis of analytical data and elemental analysis.

Key words: Synthesis; Characterization; Glimepiride metal ion complex, Infrared spectroscopy.

Glimepiride 1-(p-(2-(3-ethyle-4-methyl-2-oxo-3-pyroline-1-carboxamido)ethyl)phenyl sulfonyl)-3-(trans-4-methylcyclohexyl) Urea is a third generation hypoglycemic sulfonylurea, which is useful in the treatment of non-insulin dependent *diabetes mellitus* (NIDDM)[1-2]. Glimepiride is a white crystalline powder, relatively insoluble in water. It exhibits slow gastrointestinal absorption rate and inter individual variation of its bioavailability.[3]

The slow absorption rate of drug usually originates from either poor dissolution of drug from the formulation or poor permeability of drug across gastrointestinal membrane. For poorly water soluble and highly permeable drugs the rate of oral absorption is often controlled by the dissolution rate in the gastrontestinal tract[4]. Complexation of sulfonylurea with lighter transition metal has been studied in detail by Yoshinaga and Yamamotto (1966)[5], Qureshi and Iqbal (1985)[6]. A persual of available literature shows that systemic study on complexation of copper with sulphonyl ureas is relatively scanty.[7-10] The study of chemistry and chemical reaction of structure co-ordination compound helps in establishing structure activity relationship. It has been reported that in biological activity metal complex is more potent and less toxic as compared to the free ligand[11]. In view of the above and in continuation of our work, It is interesting to have an insight into the synthesis of copper complex with glimepiride and to diagnose various structural aspects of the isolated complex. Here the synthesis and characterization of cupric chloride with glimepiride has been described.

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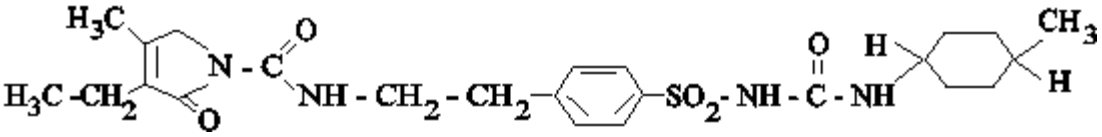


Fig. 1. Structure of glimepiride

Ligand-metal ratio

To confirm the ligand metal ratio, conductometric titrations using monovariation method were carried out at 27± 1^{0}C 0.005M solution of glimepiride drug was prepared in 80:20 mixture of DMF and water. Similarly solution of metal salt $CuCl_2.2H_2O$ was prepared in the same solvent of 0.01M concentration. 20mL of ligand was diluted to 200ml with the same solvent. The ligand was titrated against metal salt solution using monovariation method. Conductance was recorded after each addition, Graph is plotted between corrected conductance and volume of metal salt added (Fig II). From the equivalence point in the graph it has been concluded that the complex formation has taken place in the ratio of 2:1 (L:M). Stability constant and free energy changes were also calculated using Job's method[12] of continuous variation (Fig. 3).

Conductometric titration monovariation method

Table 1. Glimepiride with cupric chloride (job's method)

Glimepride – 0.005 M	$CuCl_2.2H_2O$ – 0.005 M				
Solvent – 90% Ethanol	Temperature 31±1°C				
Mole metal ligand ratio	Conductance X10^{-4}Mhos			Conductance X10^{-4}Mhos	Corrected X10^{-4}Mhos
	M:S C_1	S:L C_2	M:L C_3	$C_1+C_2-C_3$	Δconductance
0:12	0.94	1.62	2.52	0.04	0.00
1:11	1.57	1.55	2.96	0.16	0.12
2:10	2.20	1.48	3.34	0.34	0.30
3:9	2.80	1.46	3.73	0.53	0.49
4:8	3.45	1.36	4.16	0.65	0.61
5:7	3.95	1.15	4.66	0.44	0.40
6:6	4.47	1.06	5.07	0.46	0.42
7:5	4.58	0.89	5.29	0.28	0.24
8:4	5.14	0.75	5.68	0.21	0.18
9:3	5.13	0.71	5.69	0.15	0.11
10:2	5.62	0.42	5.19	0.13	0.09
11:1	6.21	0.32	6.45	0.08	0.04
12:0	6.69	0.22	6.87	0.04	0.00

EXPERIMENTAL

All chemicals used were of analytical grade. Pure sample of Glimepiride (Molecular fornula $C_{24}H_{34}N_4O_5S$ and mol.wt 490.62) was obtained from Ipca laboratories Ltd, Ratlam in powdered form m.p 207^{0}C.

Metal salt $CuCl_2.2H_2O$ was of merck chemical. The solvent used were distilled water and DMF. Metal-ligand ratio was calculated by using systronic digital conductivity meter. Melting point was determined by Parkin Elmer melting point apparatus and are uncorrected and pH values determined on LabIndia pH analyser.

IR spectra of ligands and complexes were recorded with perkin Elmer spectrometer in the

Table 2. Glimepiride with cupric chloride (job's method)

Glimepride – 0.005 M $CuCl_2.2H_2O$ – 0.002 M
Solvent – 90% Ethanol Temperature 31±1°C

Mole metal ligand ratio	Conductance X10^{-4}Mhos			Conductance X10^{-4}Mhos	Corrected X10^{-4}Mhos
	M:S C_1	S:L C_2	M:L C_3	$C_1+C_2-C_3$	Δconductance
0:12	0.93	1.66	2.51	0.08	0.00
1:11	1.58	1.54	2.93	0.19	0.08
2:10	2.21	1.44	3.32	0.33	0.27
3:9	2.84	1.36	3.70	0.50	0.42
4:8	3.47	1.25	4.18	0.54	0.48
5:7	3.96	1.14	4.64	0.46	0.38
6:6	4.48	1.06	5.12	0.42	0.34
7:5	4.61	0.92	5.14	0.39	0.31
8:4	5.02	0.79	5.44	0.37	0.29
9:3	5.14	0.60	5.45	0.29	0.21
10:2	5.62	0.46	5.89	0.19	0.09
11:1	6.21	0.34	6.42	0.13	0.07
12:0	6.69	0.21	6.84	0.06	0.00

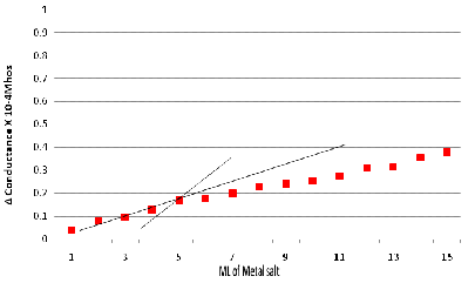


Fig. 2. Conductometric titration monovariation method glimepiride with cupric chloride

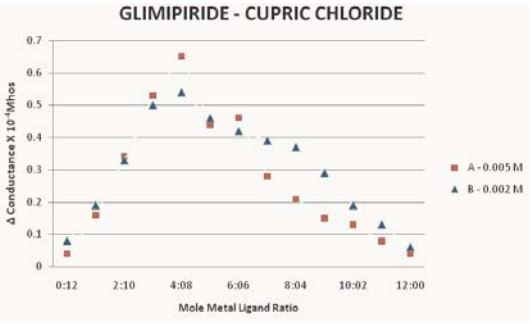


Fig. 3. Job's Method

range of 4000-450 cm^{-1} (CDRI Lucknow, India). Mass spectral results of ligand and complex were obtained from CDRI Lucknow while Electronic spectral studies were carried out on a Lambda 25 instrument model at 480 nm/min scan speed from Sadhu Vaswani College, Bhopal.

Synthesis

Complex was synthesized by mixing the solution (80% DMF) metal salt solutions with that of ligand in 1:2 molar ratios; respectively at room temperature maintaining the pH between (6.5-8) by the addition of dilute NaOH solution. On refluxing the mixture content for 3hrs at 80^{0}C and on cooling the Harbour blue crystals of glimepiride-copper complex were obtained.[13-17] The complex was washed with 80% DMF or alcohol and weighed (yield-70%).

Table 3. Synthesis and Physicochemical characteristics of Glimepiride-copper complex

Ligand/ Complex	Ligand Metal Ratio	Colour	% Yield	Stability Constant LogK (L/mole)	Free Energy Change (-ΔF) KCal/mole
Glimepiride	-	White		-	-
Glimepiride-copper Complex	2:1	Harbourblue	70%	12.40	-17.05

Table 4. Analytical data of Complex

Ligand Complex	Elemental analysis Found (Calcd)					
	C	H	N	S	Metal	M.P°C
$C_{24}H_{34}N_4O_5S$	58.77 (58.50)	6.93 (6.95)	11.92 (11.94)	6.53 (6.57)	-	207
$(C_{24}H_{34}N_4O_5S)_2.Cu$	55.07 (55.17)	6.48 (6.51)	9.90 (10.72)	6.01 (6.13)	5.98 (6.03)	218

RESULTS AND DISCUSSION

The synthesized complex is blue and stable, being soluble in DMSO, acetone and insoluble in water, ethanol etc. Analytical data (table 4) and conductometric studies suggest 2:1 (L:M) ratio. Measured conductance values of these complex are too low to account for their electrolytic behaviour.

Structure Determination

IR absorption studies

The IR spectrum[18-21] of the ligand and the isolated complex were scanned in the range 4000-450 cm^{-1} and the probable assignments are given in (table 5). The proposed structure for the isolated complex is also supported by IR absorption bands and characterized by the absorption of carbonyl (C=O) and sulphonyl urea group at 1700 cm^{-1} and 1216 cm^{-1} respectively. The NH group observed at 3681 cm^{-1} in the ligand (glimepiride) was shifted to 3758 cm^{-1} in copper glimepiride complex. The next IR band of structural significance of the ligand appears at 1017 cm^{-1} which may be assigned to u (C-O), which was absent in pure ligand and the considerable frequency of υ (C=N) was obtained at 1540 cm^{-1} in metal complex while absent in pure ligand were indicates that these specific IR absorptions are appeared due to complexation. The linkage through amide-O and sulphone –O- atom was further supported by the appearance of a band in the far IR region at 671 cm^{-1} in the complex that may be assignable to M-O frequency (Fig IV a&b).

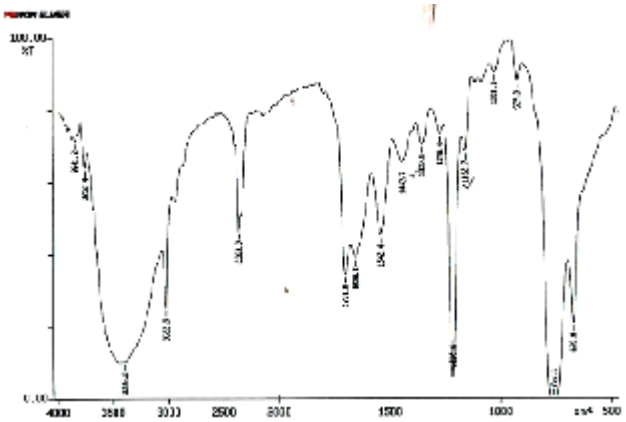


Fig. 4(a). IR Spectra of Pure Drug Glimepiride

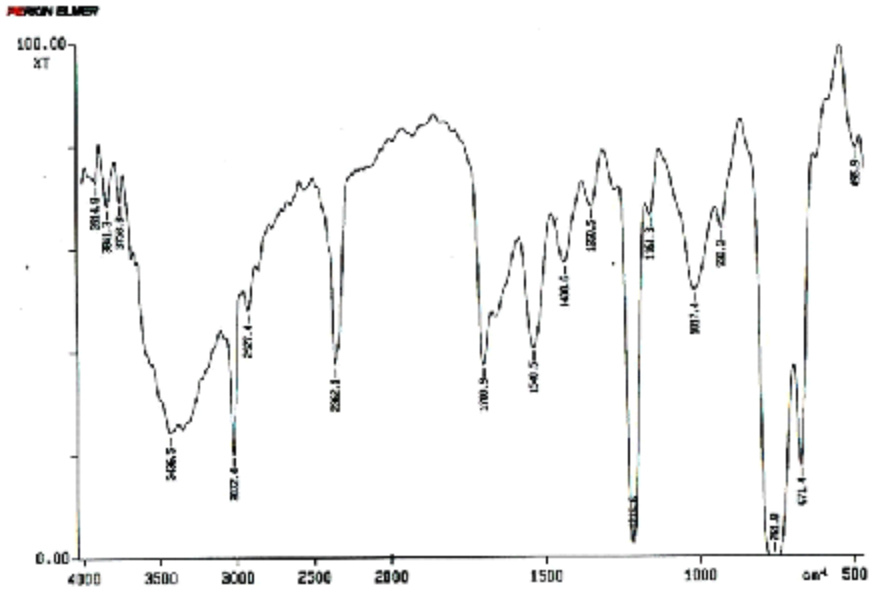
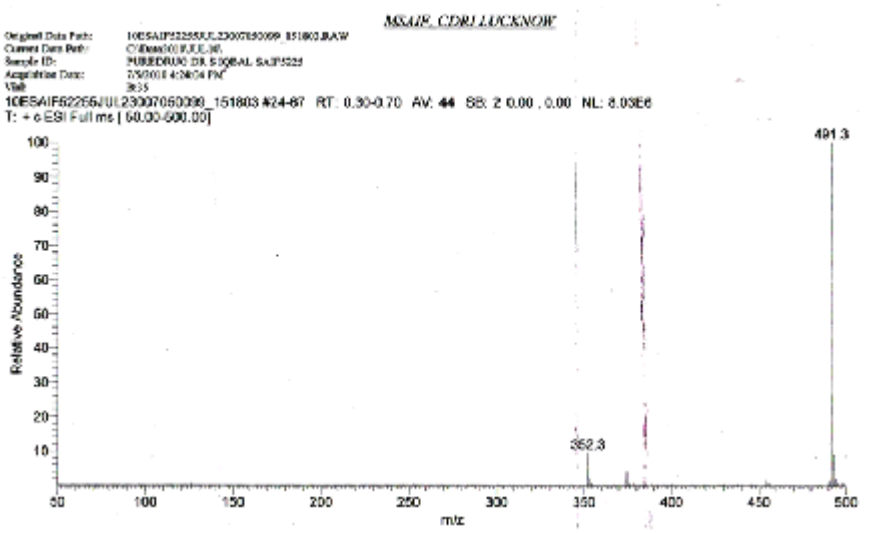


Fig. 4(b). IR Spectra of Glimepiride-Copper complex

Table 5. IR Absorption data of the complex in cm^{-1}

Ligand/Complex	v(NH)	v(C=O)	v(S=O)	v(C-O)	v(C=N)	v(SO_2N)	v(M-O)
$C_{24}H_{34}N_4O_5S$	3681	1706	1215	-	-	3020	-
$(C_{24}H_{34}N_4O_5S)_2$.Cu	3758	1700	1216	1017	1540	3022	671

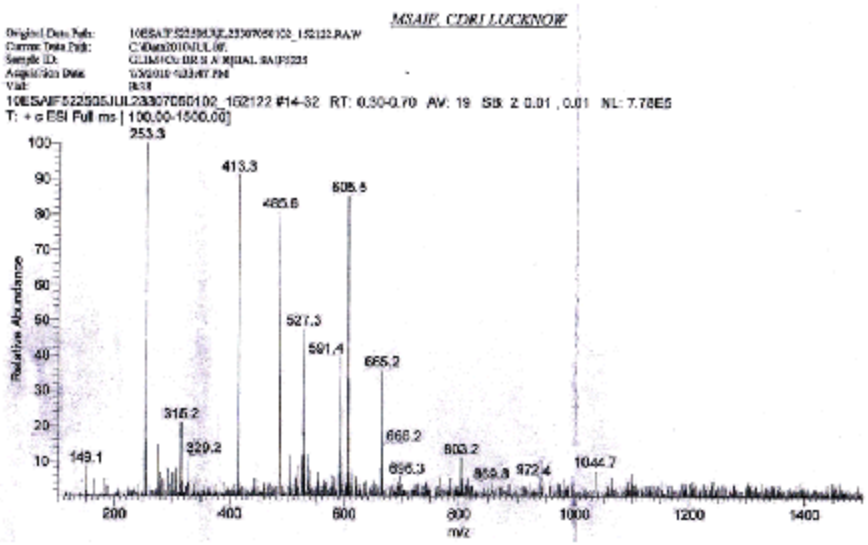
(ii) Mass spectral analysis



m/z 491 due to ($C_{24}H_{34}N_4O_5S$) parent ion peak or (m) and m/z 352 due to ($C_{17}H_{16}N_3O_4S$)

Fig. 5(a). Mass spectra of pure ligand glimepiride

Mass Spectra of Glimepiride-Copper Complex



m/z 1044 due to $[M(L_2)]^{\bullet+}$ molecular ion or parent ion; m/z 972 due to (L_2) ; m/z 803 due to $(C_{42}H_{57}N_7O_7S)$ fragment ion; m/z 605 due to $(C_{35}H_{47}N_3O_4S)$ fragment ion; m/z 413 due to $(C_{24}H_{33}N_2O_2S)$ fragment ion and m/z 253 due to $(C_{12}H_{17}N_2O_2S)$ base peak ion with 100% relative abundance.

Fig. 6(b). Mass spectra of complex (glimepiride-copper complex)

The mass spectrum of the pure ligand shows a molecular ion peak $m_{\bullet}^{+}$ at m/z 491 due to $(C_{24}H_{34}N_4O_5S)$ parent ion peak[22] which is in accordance with the proposed formula of the ligand. The other peak of appreciable intensity has been observed at m/z value 352 correspond to species $(C_{17}H_{16}N_3O_4S)$ due to loss of $(C_7H_{18}NO)$ fragment radical cation having a molecular mass 132. While the mass spectrum of $[Cu(C_{24}H_{34}N_4O_5S)_2]$ shows a molecular ion peak m at 1044 which corresponds to molecular weight of complex supported for the monomeric structure[23-24]. Beside this peak the complex showed the fragment ion peak at m/z 972 due to (L_2) radical cation. The peaks of appreciable intensity have been observed at m/z values 149, 253, 315, 329, 413, 485, 527, 591, 605, 665, 666, 696, 803, 869, 972 and 1044 which indicate the fragmentation pattern. The peak observed at m/z 972 due to (L_2) . The complex showed the fragment ion peak at m/z 803 due to species $(C_{42}H_{57}N_7O_7S)$. The peaks of appreciable intensity has been observed at m/z value 253 due to $(C_{12}H_{17}N_2O_2S)$ base peak.

Some important mass spectral intensities of metal complex of Glimepiride are summarised in table.

Table 6. Mass spectral Intensities of Metal Complex of Glimepiride

S.No.	Ligand/Metal Complexes	Ms (ESI) m/z values	Assignment
1.	Pure ligand Glimepiride	491(m)$(C_{24}H_{34}N_4O_5S)$	Molecular ion peak or parent ion.
		m/z 352 $(C_{17}H_{16}N_3O_4S)$	Fragment ion or major product ion.
2.	Glimepiride-Copper Complex	m/z 1044 (m)	
		$[Cu(C_{24}H_{24}N_4O_5S)_2]$	Molecular ion peak.
		m/z 972 (L2)	Due to ligand molecular weight
		m/z 253 $(C_{12}H_{17}N_2O_2S)$	Base peak

Electronic spectral studies of the complex

Electronic spectra offer great help in determining the structure of metal complexes. Electronic spectra of transition metal ions and complexes are obtained in the visible and ultraviolet region.[25-26]

In the present study the above spectra for the complex were obtained from Sadhu Vaswani College, Bhopal and the analysis were carried out on a Lambda 25 instrument model at 480 nm/min scan speed.

The observed electronic spectral bands of the glimepiride ligand and metal complex (copper-glimepiride) are given in table (7) as follows :-

Table 7. Electronic absorption spectral data and meff (Effective magnetic moment) values of copper-glimepiride complex

S. No.	Complex	Electronic Absorption (cm^{-1})	Band Assignment	Magnetic moment (μeff) value B.M.	Geometry
1.	Glimepiride -copper complex	25490 20533	LMCT (Ligand to Metal Charge Transfer)^{2}Big g^2A_1g	1.77	Square planar

The electronic spectra of the Cu(II) complex of glimepiride shows an absorption bands at 25490 $cm^{-1}$and 20533 cm^{-1} attributed to the LMCT (ligand to metal charge transfer) and ^{2}Big g^2A_1g transition which is compatible with this complex having an square planar structure around the Cu(II) metal ion.[27-30]

Antidiabetic activity of glimepiride copper complex

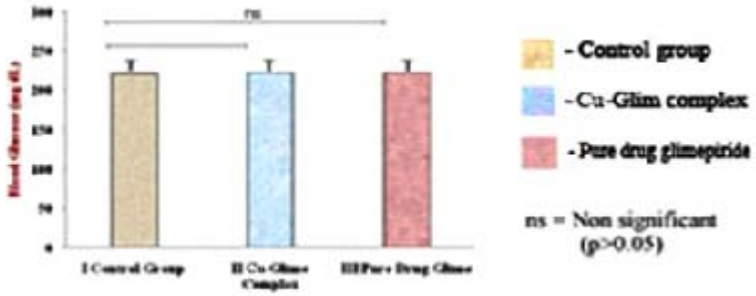
This isolated glimepiride-metal complex were found to be more potent as compared th the parent drug. Hence as compare to standard synthetic drug the glimepiride-copper complex[31] was having more hypoglycemic activity. The hypoglycemic effect of glimepiride as well as metal complex were investigated on the blood sugar levels of male wistar rats by alloxan induced antidiabetic test (PBRI, Lab Bhopal, India). Analysis of data presented in (table 8) reveals that the drug caused a marked decrease in blood sugar level. On comparing the hypoglycemic effect of copper comple with parent drug it was revealed that in case of Cu-glimepiride treated male wistar rats blood sugar falls to 90.8±1.9235 mg/dl while in glimepiride treated rats blood sugar falls to .961.5811 mg/dl. These results clearly indicate a better hypoglycemic activity of Cu-glimepiride complex over its parent drug.

Table 8. Antidiabletic Activity Analysis by Allozan Induced Antidiabetic Test

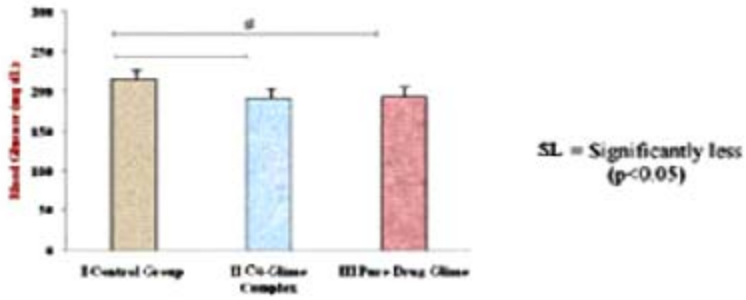
Group	Treatment Group	Dose	Blood Glucose level (mg/dL)			
		mg/kg	Initial	1 h	3 h	6 h
I	Control group + Glucose (2g) + Vehicle	2 g	222.6±1.1401	216.2±1.3038	217.8±1.7888	223±1.5811
II	Copper complex of Glimepiride (2mg) + Glucose + Vehicle	2 mg	223 1.5811	191.2 1.9235	116.6 2.0736	90.8 1.9235
III	Pure drug Glimepiride (2mg) + Glucose +Vehicle	2 mg	223.4±1.8165	193.6±2.0736	115.2±1.9235	96 ±1.5811

P>0.05 when compared to vehicle treated control group but P<0.05 & ** when compared to vehicle control group after 30 min. (*) & after 90 min (**).

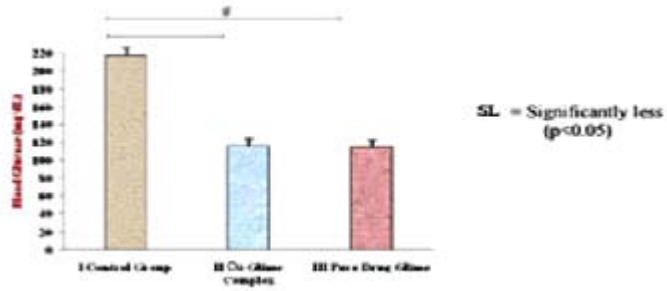
Blood-sugar level (Zero hour)



Blood-sugar level after 1 hr.



Blood-sugar level after 3 hr.



Blood-sugar level after 6 hr.

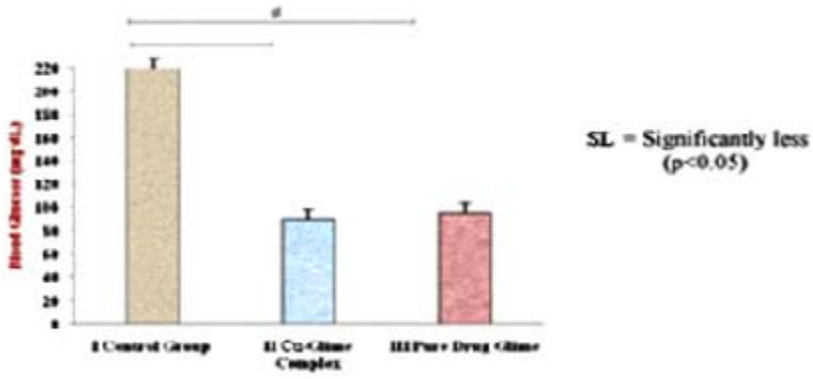


Fig. 6. Graphical Representation of Comparative Antidiabetic Activity Analysis of Complex of Copper-Glimepiride by Alloxan Induced Antidiabetic model

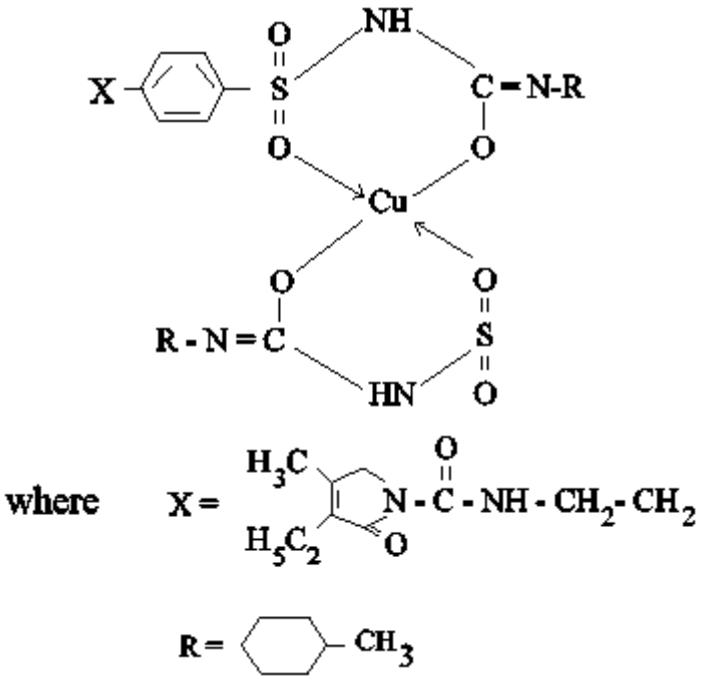


Fig. 7. Structure of Copper-Glimepiride complex. (Square planar geometry)

CONCLUSION

From the monovariation studies conducted by conductivity method and further confirms by the Job's method of continuous variation suggest the ligand-metal ratio as L_2M. Analytical data agrees to the molecular formula $(C_{24}H_{34}N_4O_5S)_2Cu$. The structure of the complex proposed on the basis of analytical data and stoichiometry was further supported by IR, Mass and Electronic spectral studies which suggest the linking of sulphonyl and enolic oxygen to the metal atom. The disappearance of enolic hydrogen in complex as indicated by IR values. The results of Mass spectra and electronic spectra are well in agreement with the mol.wt of the complex. Hypoglycemic activities of the complex shows more blood sugar lowering effect as compared to parent ligand. Calculating the toxicity in the complexes and on many trials on monkey's and men the complex may be introduced as medicine in future.

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