



## Synthesis, Characterization and Spectral Study of Zinc Complex with Gliclazide( *N*-(hexahydrocyclopenta[*c*]pyrrol-2(1*H*)-ylcarbamoyl)-4- methylbenzenesulfonamide) and its Hypoglycemic Activity

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### ABSTRACT

The present paper describes Synthesis and characterization, spectral, x-Ray and hypoglycemic activity studies of zinc complex with gliclazide, an oral antidiabetic drug. The conductometric titration using monovariation method indicate that complexes are non-ionic and  $L_2M$  type. Analytical data agrees with the molecular formula  $(C_{15}H_{20}N_3O_3S)_2Zn$ . Structure of complex was assigned tetrahedral for zinc, supported by IR, X-Ray. Structure (I) is proposed for complex. Alloxan induced model was used to compare the hypoglycemic activity and found that zinc complex of gliclazide is more potent than parent drug.

**Key words:** Gliclazide, Sulphonylurea, Complex, IR, X-Ray and hypoglycemic activity.

### INTRODUCTION

Metal ion is required for many critical functions in humans. Scarcity of some metal ions can leads to disease<sup>1</sup>. Well – known example can leads to *pernicious anaemia* 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 Phatak,<sup>2</sup>; Bloomgarden<sup>3</sup>, ; Sanger and Thompson,<sup>4</sup>. 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 sulphonylureas because of their high complexing nature with essential metals. Sulphonylureas are effective for non-insulin dependent diabetes mellitus, [Sadilat and Phatak<sup>2</sup>, Bioomgarden,<sup>3</sup> Sanger and Thompson,<sup>4</sup>. These compounds are completely absorbed on oral administration. They are metabolized by liver and are excreted predominantly through urine.

Complexation of sulphonylureas with lighter transition metals has been studied in detail by Yoshinaga and Yamamoto,<sup>5-6</sup> Iqbal *et al.*<sup>7-10</sup> and other author synthesis the complexes of various metals with compound<sup>11-18</sup>. It is interesting to have an insight in to the synthesis of zinc complex with gliclazide and to diagnose various structural aspects of the isolated complex and compare its hypoglycemic activity to parent drug.

Here the synthesis and characterization of zinc with gliclazide have been described.

## EXPERIMENTAL

### Ligand-Metal Ratio

Pure Gliclazide m.p. 180°C (Lit. 179.5-180.5), 0.005 M, were diluted to 100 ml as required and titrated conductometrically against zinc chloride at 30±1°C. Results were plotted in the form of a graph which indicate ligand metal ratio as 2:1 (L<sub>2</sub>M).

Formation of 2:1 (L<sub>2</sub>M) ratio was further confirmed by Job's method<sup>19</sup>(Table-1 fig. 1a,b) of continuous variation as modified by Turner and Anderson<sup>20</sup>spectrophotometric studies were conducted using absorbance as index property, from these values the stability constant (log k) and free energy change (-F), were also calculated (Irving and Rossotti)<sup>21-22</sup>.

### Synthesis of Complexes

The chemicals used in this synthesis were all of analar grade Hi-media. A weighed quantity of Gliclazide (2 mol) (supplied by Zim laboratory Nagpur) was dissolved separately in minimum quantity of 90% ethanol. The Zinc chloride solution was prepared by dissolving (1 mol) 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 hours and on cooling the complexes separated out. Which were filtered off, washed well with ethanol and finally dried in vacuum and weighed.

## RESULTS AND DISCUSSION

### Physico-chemical Characteristics of "Gliclazide-zinc complex"

Molecular formula (C<sub>15</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>S)<sub>2</sub> Zn, Mol. wt: 443.92; Colour:white;6 Yield:53.16%; m.p86:1°C, -DF: 16.00; LogK: 10.75; Anal. Calcd (%) for (C<sub>15</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>S)<sub>2</sub> Zn : C,51.03; H,6.03; N,11.64; S,9.01; Zn,6.0; Found (%): C,50.83; H, 5.63; N,11.86; S,9.03; Zn,8.74.

### Infra-red Spectra Studies

The IR spectra (Fig 2) of ligand and isolated complex was recorded within the range 4000-400 cm<sup>-1</sup>. Assignments of the infrared spectral bands are based on literature IR spectrum shows important bands due to ν(M-O) 400-600 cm<sup>-1</sup>, ν(Ar-S) 700-800 cm<sup>-1</sup>, ν(-S-N) 1085±20 cm<sup>-1</sup>, ν(SO<sub>2</sub>-N) 1120±20 cm<sup>-1</sup>, ν(C-N) 1210±20 cm<sup>-1</sup>, ν(S=O) 1340±20 cm<sup>-1</sup>, ν(C=O) 1710 cm<sup>-1</sup>( present only in pure drug and variation in complex),1600±20 cm<sup>-1</sup>vs, (C-H Stretch)2800-2900cm<sup>-1</sup>, ν(Ar-H) 3150±20 cm<sup>-1</sup>, ν(NH-stretch) 3274±20 cm<sup>-1</sup>.The proposed structure (I) for the isolated complex is also supported by IR absorption, C.N.Rao<sup>23</sup>, Bellamy<sup>24</sup>,and Weissberger<sup>25</sup>.

### X-ray diffraction studies

X-ray diffraction studies also confirm the complex and formation due to new bonds<sup>26-33</sup>. The number of peaks in gliclazide are 22 while (GLZ)<sub>2</sub>Zn are 11 (Fig-3). Thus indicating the something new formation i.e 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

Table 1: Gliclazide with zinc

S. No.	Metal:Ligand ratio	Absorbance		Corrected Absorbance	
		0.002M	0.005M	0.002M	0.005M
1	0:12	0.012	0.019	0.00	0.00
2	1:11	0.045	0.067	0.033	0.048
3	2:10	0.065	0.127	0.052	0.107
4	3:9	0.120	0.152	0.106	0.131
5	4:8	0.146	0.195	0.132	0.174
6	5:7	0.135	0.172	0.120	0.151
7	6:6	0.126	0.166	0.111	0.143
8	7:5	0.101	0.125	0.084	0.101
9	8:4	0.092	0.105	0.074	0.081
10	9:3	0.080	0.096	0.062	0.071
11	10:2	0.068	0.083	0.047	0.058
12	11:1	0.045	0.055	0.027	0.029
13	12:0	0.025	0.028	0.00	0.00

Table 2: Hypoglycemic activity of gliclazide and its complexes at 3<sup>th</sup> day(mg/dl)

Group-I	Group-II	Group-III	Group-IV
90	108	175	171
95	157	180	170
105	160	168	165
106	122	170	118
109	170	195	175
85	165	190	170

Table 2: Hypoglycemic activity of gliclazide and its complexes at 7<sup>th</sup> day(mg/dl)

Group-I	Group-II	Group-III	Group-IV
107	112	135	110
89	180	130	115
97	188	140	120
110	132	135	100
102	197	126	116
98	197	125	108

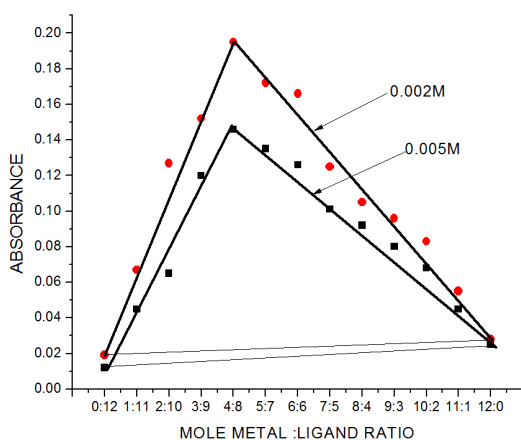


Fig.1(a)

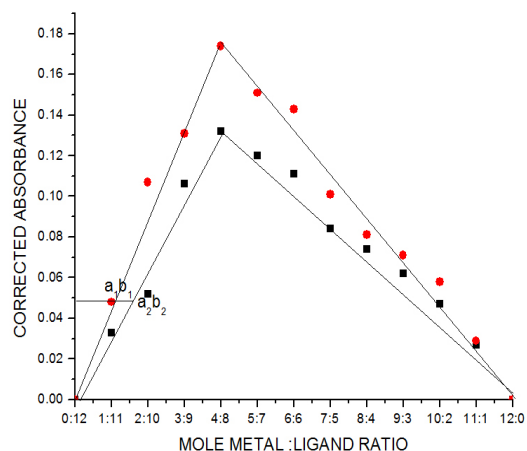


Fig.1(b)

is not in good agreement with available information and thus confirms the formation of totally new complexes. 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, from cell data and crystal lattice parameters of Zn(II) indicates complex attributed to orthorhombic crystal system. The particle size is 7.28 nm which is calculated from x-ray line broadening using the Scherrer formula  $D_{hkl} = \kappa\lambda/\beta_{hkl} \cos\theta$ , where D is

the particle diameter in ångstroms,  $\kappa$  is a coefficient and is equal to 0.89 here,  $\lambda$  is the half-maximum line width, and  $\theta$  is the wavelength  $\frac{d_{true} - d_{obs} \times 100}{d_{true}}$  is 6.84 calculated by formula and volume of the unit cell is 14065 is calculated by  $\text{Volume}(\text{Å}^3) = abc \sin\gamma$  and  $a, b, c$  are lattice parameters. Density =  $\frac{\text{Weight}}{\text{Volume}}$  is found 0.050 g/cm<sup>3</sup>. Space group is Pmmm and  $\alpha=90^\circ$ ,  $\beta=90^\circ$ ,  $\gamma=90^\circ$ , crystal system- orthorhombic. Cullity<sup>34</sup>(1978), Duval<sup>35</sup> Bragg and Bragg<sup>36</sup>, Guinie<sup>37</sup>(1952), Henry et al<sup>38</sup>, (1951), Kidwai<sup>39</sup>(2011).

### Hypoglycemic Studies

Pharmacotherapy includes the treatment

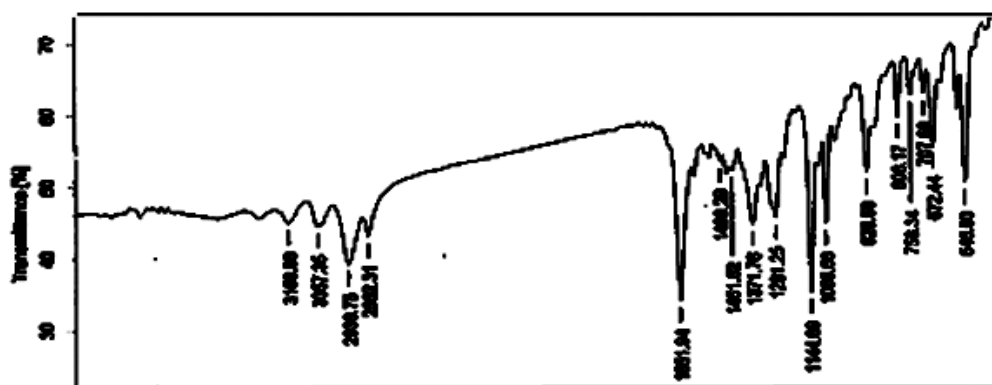


Fig. 2: Ir spectra of glyclazide-zinc complex

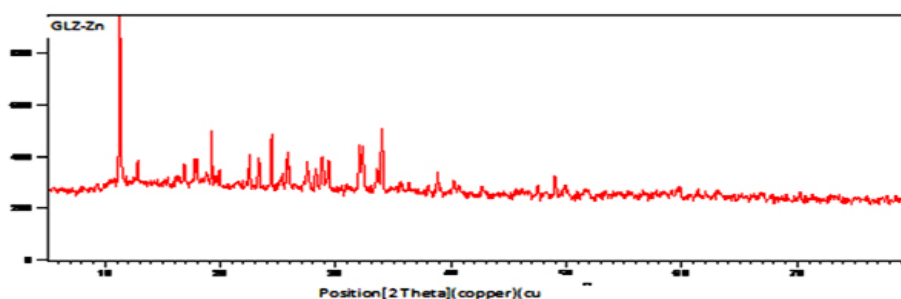
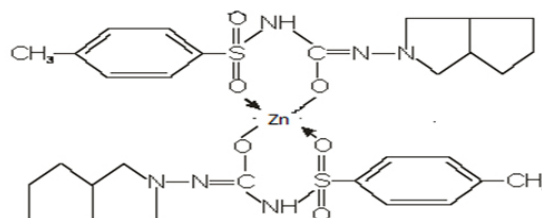


Fig.3: X-ray diffractogram of glyclazide-zinc complex



Structure (I)

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. 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 of pure drug and its complexes with zinc 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 Laboratory, 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<sup>41-43</sup>

The diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of Alloxan monohydrate (120 mg/kg b.w.). 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 5 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, p.o.) for 4 days and alloxan 120g/kg, i.p. on 1<sup>st</sup> day.

### Group IV

Rats received gliclazide-zinc complex, (2mg/kg, p.o.) once daily for 4 days and Alloxan 120g/kg, i.p. on 1<sup>st</sup> day.

### Sample collection

At the end of study (7<sup>th</sup> day), blood samples were collected through tail vein and blood glucose levels were estimated using an electronic glucometer and result is given in table 2, 3.

## DISCUSSION

For supporting the proposed structure of gliclazide-zinc 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 agrees to the molecular formula  $(\text{C}_{15}\text{H}_{20}\text{N}_3\text{O}_3\text{S})_2\text{Zn}$ , ( $L_2M$ ).

For determining the proposed structure on the basis of stoichiometry and analysis of the complexes. Advance spectroscopic methods like IR was 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. A detailed study of X-Ray

also supports the complexes 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 nitrogen and to enolization is strongly supported.

Zinc complex of gliclazide was evaluated for their hypoglycemic effect on experimental rats using alloxan induced diabetes model. The effect of zinc complex of gliclazide on blood glucose level at 3<sup>rd</sup> and 7<sup>th</sup> day of alloxan induced diabetes in rats shown in table-3, 4 respectively. So from table we can conclude that zinc complex of gliclazide have more hypoglycemic activity as compared to parent

drug. These interesting observations on metal-complex of oral sulphonylureas used as antidiabetic agents for lowering blood sugar concentration may likely substantiate the use of these complexes after extensive clinical studies.

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