Biomedical & Pharmacology Journal Vol. **6**(1), 111-124 (2013)

Synthesis, Characterization and Antidiabetic Study of Nd (III) Complex of 1-(p-(2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)Ethyl) Phenyl)Sulfonyl)-3-(trans-4-methyl Cyclohexyl) Urea Amaryl or Glimepiride, An Oral Antidiabetic Drug

SIBI JOSE[1] and S.A. IQBAL[2]

[1]Department of Chemistry, Sadhu Vaswani College, Bairagarh, Bhopal - 462 001, India.
[2]Department of Chemistry, Crescent College of Technology, Bhopal - 462 038, India.
*Corresponding author E-mail: iqbalospc@yahoo.com, kojose@yahoo.com

(Received: April 18, 2013; Accepted: June 06, 2013)

ABSTRACT

This paper deals with the synthesis and characterization of a glimepiride-neodymium complex, rare earth's metal. Glimepiride is an oral hypoglycemic agent. The conductometric titrations were conducted using monovariation method which shows the ligand metal ratio as 2:1. Molar conductance value indicate that complex is non-ionic in nature. Analytical results agree to the molecular formula $(C_{24}H_{34}N_4O_5S)_2$ $Nd.4H_2O$. rare earth's element Nd shows the high coordination sites. The structure of the complex was assigned as hexagonal in which ligand moleculas joining the central Nd(III) atom with nH_2O molecules. Infrared spectral studies have supported coordination of sulphonyl oxygen on one side and enolic oxygen attached from other side and four water molecule attached by four co-ordination sides with the neodymium ion. Mass spectral, thermal and magnetic studies supports structure of the complex proposed on the basis of analytical data X-ray diffraction studies.

Key words: Synthesis; Characterization; neodymium-Glimepiride complex, Mass, Thermal, X-Ray and Infrared spectroscopy.

INTRODUCTION

The study of chemistry and chemical reaction of co-ordination compound help in establishing structure activities relationship. It has been reported that in biological activity metal complex is more potent and less toxic as compared to the free ligand[1-6]. Inorganic chemistry and the use of metals in therapeutic drugs have become increasingly important over the last two of decades resulting in a variety of exciting and valuable drugs such as cis-platin for cancer.

Recently metals in medicine has been recognised internationally as an important area for research. In this account the role of rare earth's metal neodymium has been undertaken for study[7-10].

In recent years, much attention is given to the use of sulphonyl ureas because their high complexing nature with essential metals. Sulphonyl ureas are effective for non-insulin dependent diabetes mellitus[11-13].

These compounds are completely absorbed on oral administration. They are metabolized by liver and are excreted predominantly through urine. Complexation of sulphonyl ureas with rare earth's metals have been studied in detail by several workers[14-16]. A persual of available literatures shows that systemic study on complexation neodyamium with variours hypoglycemic drugs is relatively more important[17-19].

Here in the synthesis and characterization of neodymium trioxide complex with glimepiride have been described[20-22].

Ligand-metal ratio

For determining the ligand-metal ratio molar solutions were prepared of metal salt and ligand in 1:2 ratio and conductometric titrations were carried out by using monovariation method (Fig 2). The ligand-metal ratio (1:2) was also confirmed by the way of doing the jobs method[23] of continuous variation as modified by Turner and Anderson[24] using Δ conductance as index property. The indexed values were indicates 1:2 metal ligand ratio (Fig 3).

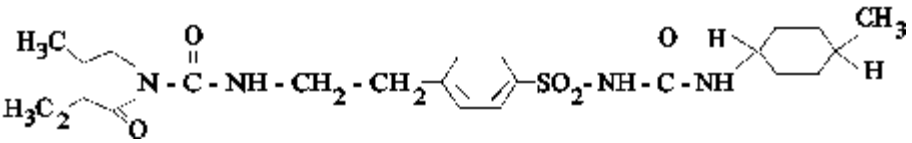


Fig. 1: Structure of Glimepiride

EXPERIMENTAL

Pure sample of G.P (trade name, amaryl) with m.f. ($C_{24}H_{34}N_4O_5S$) was received from Ipca laboratories Limited, Ratlam. Solvents and metal salts used were of the analytical grade. Melting point was determined by Parkin Elmer model melting point apparatus and are uncorrected, pH values determined on Lab. India pH analyser. IR spectra of ligand and complex were recorded with Perkin Elemer Spectrometer in the range of 4000-450 cm^{-1} (CDRI Lucknow). Mass spectral results of ligand and complex were obtained from CDRI Lucknow, India while X-ray diffraction studies were carried out by X-ray diffractometer model with 45kV rotating anode and Cukα (1w=1.54060A) radiation (Panjab University, India). Thermal analysis (DSC) was also varied the proposed structure of complex.

Synthesis

A weighed quantity of glimepiride (2mol.) was dissolved in minimum quatity of 80% DMF. The neodymium trioxide solution was prepared by dissolving it separately in the same solvent. A few drops of alkali NaOH solution was added to metal solution to increase the solubility. Metallic solution was added slowly with stirring into the solution of ligand at room temperature maintaining the pH between 6 to 8 by adding dilute NaOH solution and refluxed for 2-4 hours 25-27 at 80^0 ±5C. The solutions were left for crystallization at room temperature for 18-20 hours, shiny grey coloured crystals of complex were obtained which were filtered, washed, dried and then their melting points determined were recorded.

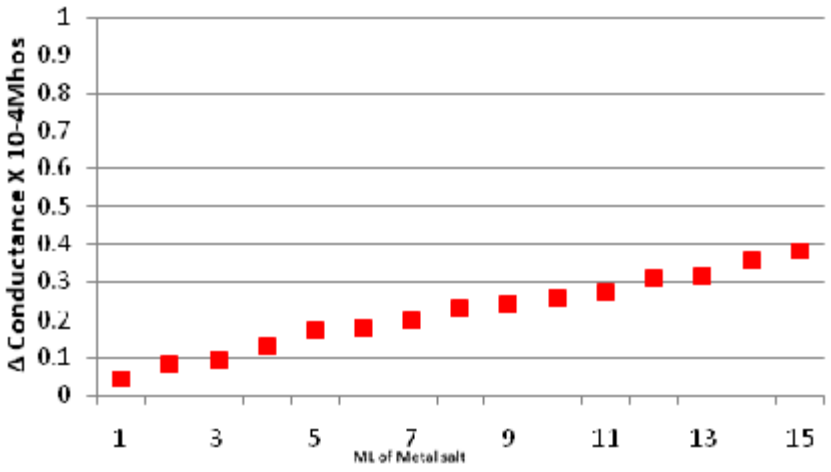


Fig. 2: Conductometric Titration graph of Neodymium-Glimepiride Complex

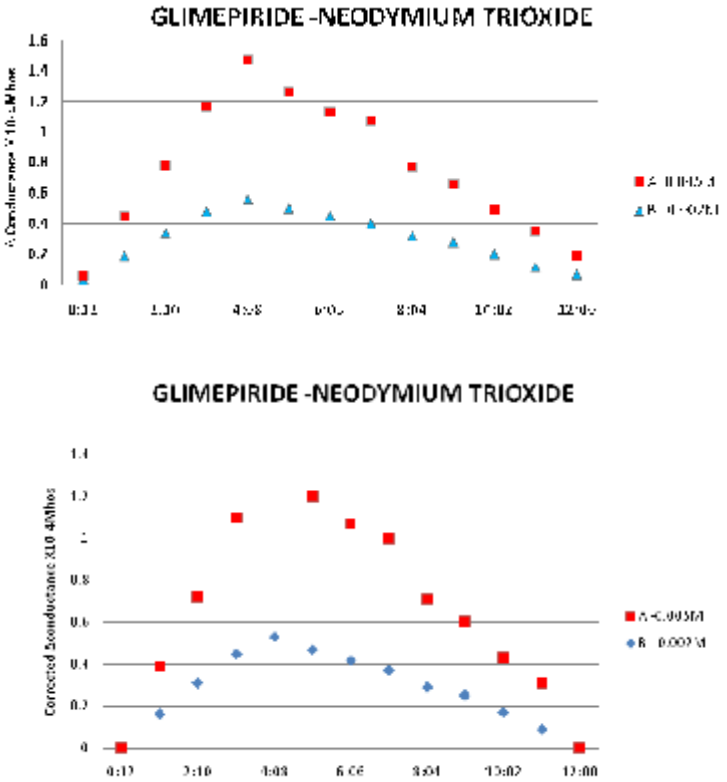


Fig. 3(b): Modified by Turner Anderson

Table 1: Synthesis and physico chemical characteristes of Glimepiride-neodymium Complex

Ligand/Complex	Ligend Metal Ratio	Colour	%yield	Stability constant Logk (L/mole)	Free energy change (-ΔF) Kcal/mole
Glimepiride (Ref)	-	White	-	-	-
Glimepiride-neodymium Complex	2:1	Shiny Greyish Crystal	60	11.93	-16.40

Table 2: Analytical data of complex

Drug/Complex	Elemental analysis found (calculated)						m.p.
	C	H	N	S	Metal	Water	°C
$C_{24}H_{34}N_4O_5S$	58.77 (58.80)	6.93 (6.95)	11.92 (11.94)	6.53 (6.57)	-	-	207
$(C_{24}H_{34}N_4O_5S)_2.Nd(OH_2)_4$	48.78 (49.61)	5.78 (9.64)	8.97 (9.64)	6.20 (6.13)	12.01 (12.40)	5.18 (3.10)	218

Analysis of complex

The resulting complex so sormed was characterized by its elemental analysis through SEM-EDAX method and IR, Mass, X-ray, and Thermal studies table. 3, 4, 5 and 6 and metal was estimated by EDTA method.

RESULTS AND DISCUSSION

Structure determination

IR Absorption Studies

The infrared spectrum of glimepiride and metal complex were recorded on Perkin Elemer Spectrometer RX1 (4000-450cm^{-1}). The major absorption bands for the infrared frequencies and the correspondign assignments are listed in Table 3.

The Glimepiride metal complex showed a prominent IR absorption band in the region 1706cm^{-1} and 1701cm^{-1} due to (C=O) carbonyl group[28-29]. The next IR band of structure significance of the ligand appears at 1215cm^{-1} which may be assigned to (S=O) which got shifted at 1216cm^{-1} in the complex. The NH group observed at 3681cm^{-1} in the ligand (glimepiride) shifted to 36080cm^{-1} in neodymium glimepiride comlex. The IR frequencies of (C=N) group was appeared at 2363cm^{-1} in the complex while absent in ligand. The linkage through amide-O and sulphone-O- atom was further supported by the appearance of a band in the far IR region at 670cm-1 in the complex that may be assignable to M-O frequency which was absent in ligand. The IR frequency region at 3403cm^{-1} was assigned to nH_2O molecule that may be not found in ligand frequency region. In pure ligand there is no absorption band detected for C-O and C=N due to enolisation are further supporting the structure for glimepiride neodymium complex.

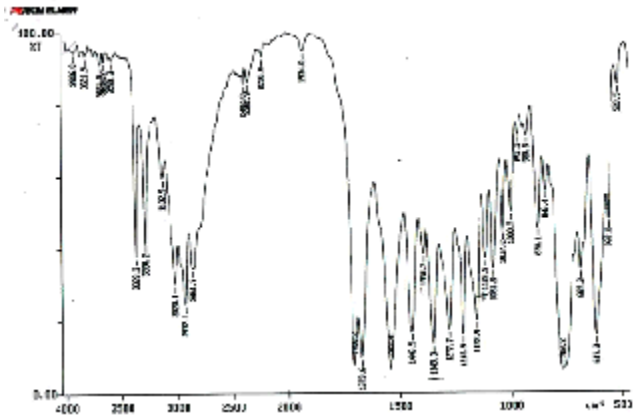


Fig. 4: IR Spectra of pure drug Glimepiride

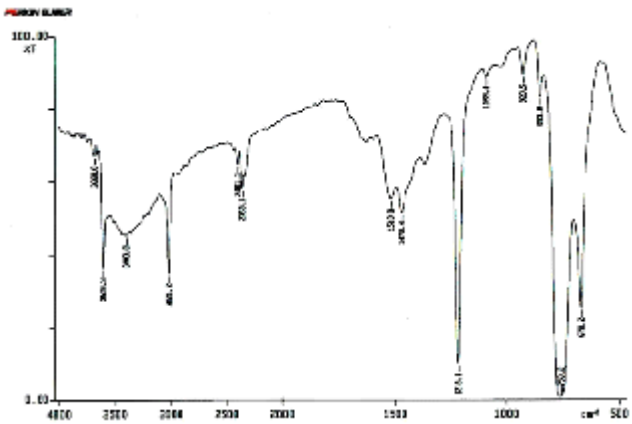


Fig. 5: IR Spectra of Glimepiride-Neodymium complex

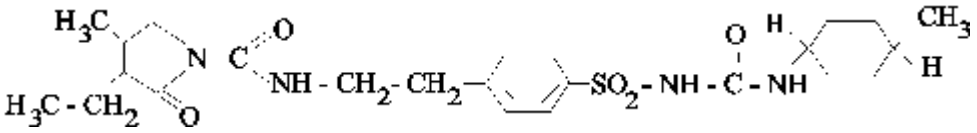
Table 3: IR Absorption data of the complex in cm^{-1}

Ligand/Complex	υ (NH)	υ (C=O)	υ (S=O)	υ (C-O)	υ (C=N)	υ (SO_2N	υ (M-O)	υ (H_2O)
$C_{24}H_{34}N_4O_5S$	3681	1706	1215	-	-	-	-	-
$(C_{24}H_{34}N_4O_5S)_2$.Nd.$4H_2O$	3680	1701	1216	1656	2363	3021	670	3403

Mass spectral studies

NMR data of the complex get summarized in Table-6 and their proposed structure are given in Fig-IV. the ^{1}HNMR spectra of the ligand was reported on a Bruker DRX-300 spectrometer (CDRI Lucknow) and isolated complex was reported on a Bruker Avance II 400 NMR spectrometer (Saif Panjab University, Chandigarh). DMSO was used as a solvent. The other features of NMR spectrum were the aromatic proton resonances located and the presence of unresolved multiplet is suggestive of excessive deshielding of aromatic protons[26-30]. The NMR signal of enolic OH group is observed in the ligand while absent in the complex indicates the involvement of enolic OH group in complexation. Moreover the enolization of N1 hydrogen is not possible because it is simultaneously attracted from the groups SO_2 from one side and C=O on the other side[31].

Mass Spectra of Pure Ligand Glimepiride



Molecular formula :- $C_{24}H_{34}N_4O_5S$
Molecular mass : - 490 g/mol

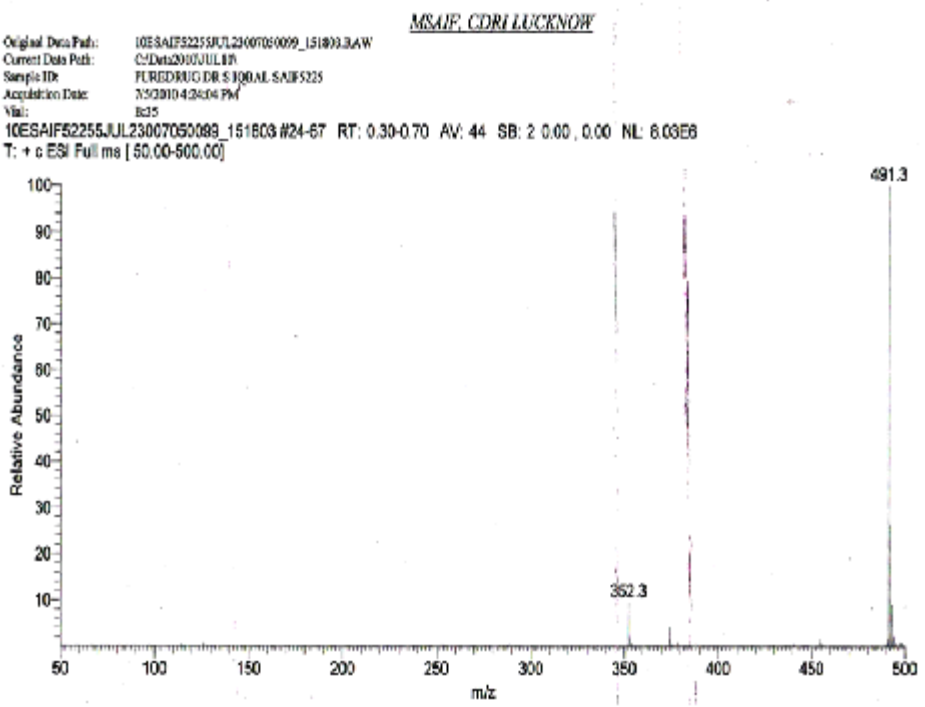
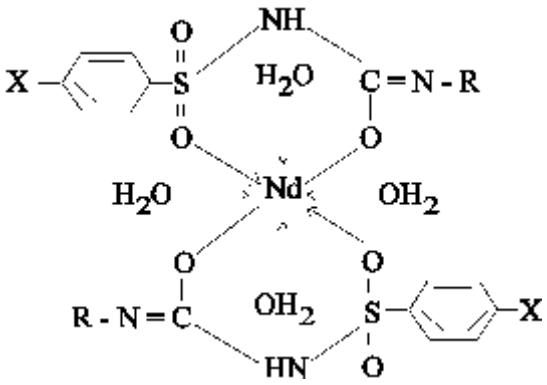


Fig. 6: m/z 491 due to $(C_{24}H_{34}N_4O_5S)^+$ parent ion peak or (m) and m/z 352 due to $(C_{17}H_{16}N_3O_4S)$

Mass Spectra of Glimepiride-Neodymium complex



Molecular formula :- $(C_{24}H_{34}N_4O_5S)_2Nd.4H_2O$

Molecular mass : - 1190 g/mol

The mass spectrum of the pure ligand (Fig VI) shows a molecular ion peak m+ at m/z 491 due to $(C_{24}H_{34}N_4O_5S)^{\bullet+}$ Parent ion peak[30] 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 mass132 and the mass spectrum of $[Nd(C_{24}H_{34}N_4O_5S)_2.(H_2O)_4]$ shows a moleclar ion peak m.+ at m/z 1417 which corresponds to molecular weight of complex and also the excess co-ordination nature of the metal ion with supporting ligand[31-32]. The peaks of appreciable intensity have been observed at m/z values 149, 237, 283, 315, 413, 485, 513, 591, 606,

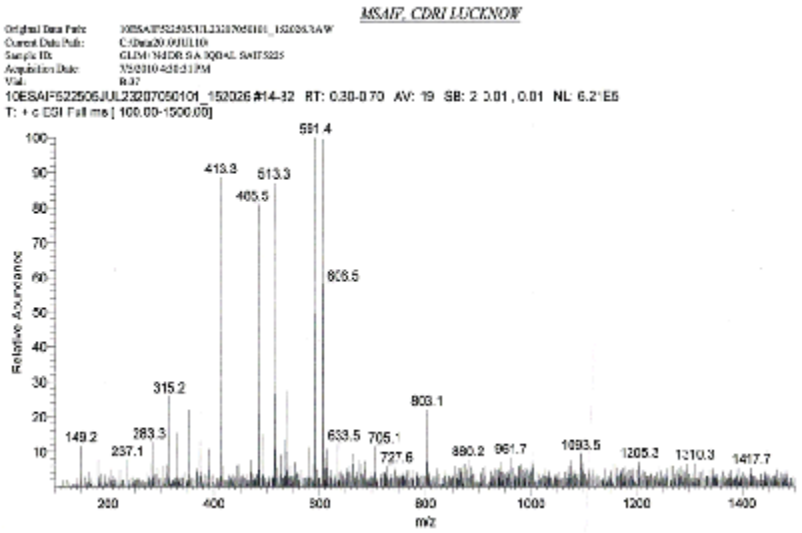


Fig. 7: m/z 1205 due to $[M(L_2)(H_2O)_4]^{\bullet+}$ or $[Nd(C_{50}H_{66}N_8O_9S_2).(H_2O)_4]^{+}$ molecular peak ion or parent ion; m/z 1093 due to $[Nd(C_{47}H_{50}N_8O_{10}S_2)]^{+}$; m/z 961 due to $(C_{47}H_{61}N_8O_{10}S_2)^{+}$ fragment ion; m/z 803 due to $(C_{42}H_{57}N_7O_7S)^{+}$ radical ion; m/z 591 due to $(C_{32}H_{39}N_4O_5S)$ base peak ion; m/z 513 due to $(C_{26}H_{33}N_4O_5S)^{+}$ fragment ion; m/z 485 due to $(C_{24}H_{30}N_4O_5S)$ fragment ion; m/z 315 due to $(C_{14}H_{25}N_3O_3S)^{+}$ fragment ion and m/z 149 is due to $(C_{62}H_{15}NOS)^{+}$ fragment ion

Table 4: Mass Spectral Intensities of Metal Complex of Glimepiride

S.No.	Ligand/Metal Complex	Ms (ESI) m/z Values	Assignment
1.	Pure ligand Glimepiride	491 (m^{+}) $(C_{24}H_{34}N_4O_5S)^{+}$	molecular ion peak or parention
		m/z 352 $(C_{17}H_{16}N_3O_4S)^{+}$	fragmention or majorproduction
2.	Glimepiride-Neodymium Complex	m/z 1417 (m^{+})	molecular ion peak
		m/z 1205 $[Nd(C_{24}H_{34}N_4O_5S)_2(H_2O)_4]^{+}$	molecular weight of complex
		m/z 1093 $Nd(C_{24}H_{34}N_4O_5S)^{+}$	due to loss of co-ordinatedwater
		m/z 591 $(C_{32}H_{39}N_4O_5S)^{+}$	base peak

633, 705, 727, 803, 880, 961, 1093, 1205, 1310, and 1417. The peak observed at m/z 1205 corresponds to exact molecular weight of complex $[Nd(C_{24}H_{34}N_4O_5S)_2(H_2O)_4]$.

X-ray diffraction studies

X-ray diffractometer model 00011023505 with 45kV rotating anode X-ray generator was used for scanning the ligand and respective complex. Radiation used was Cu (1w=1.54060A). The samples were scanned in the range 25°C. Powder data was indexed using computer software (Panjab University). X-ray diffraction studies also confirm the complexation and formation of new bands.

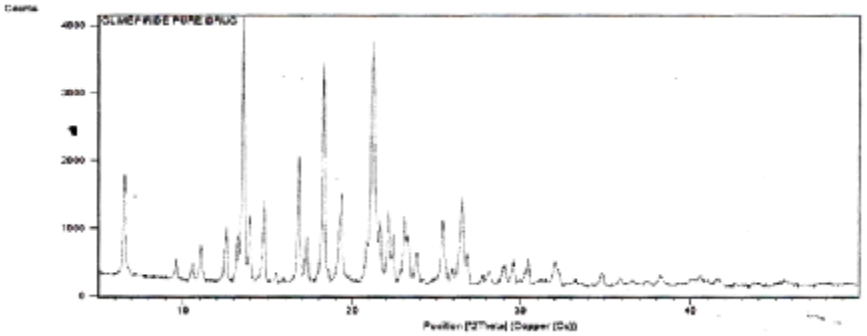


Fig. 8: XRD data of pure ligand glimepiride

Dataset Name	GLIMEPIRIDE PURE DRUG
File name	C:\X'Pert Data\feb2011\GLIMEPIRIDE PURE DRUG.xrdml
Comment	Configuration=Flat Sample Stage, Owner=jagtar, Creation date=6/11/2007 3:57:00 PM
	Goniometer=PW3050/60 (Theta/Theta); Minimum step size 2Theta:0.001; Minimum step size Omega:0.001
	Sample stage=PW3071/xx Bracket
	Diffractometer system=XPERT-PRO
	Measurement program=PU, Owner=jagtar, Creation date=4/15/2008 1:52:59 PM
Measurement Date / Time	2/28/2011 10:10:23 AM
Operator	Panjab University
Raw Data Origin	XRD measurement (*.XRDML)
Scan Axis	Gonio
Start Position [°2Th.]	5.0084
End Position [°2Th.]	49.9904
Step Size [°2Th.]	0.0170
Scan Step Time [s]	25.1954
Scan Type	Continuous
PSD Mode	Scanning
PSD Length [°2Th.]	2.12
Offset [°2Th.]	0.0000
Divergence Slit Type	Fixed
Divergence Slit Size [°]	0.9570
Specimen Length [mm]	10.00
Measurement Temperature [°C]	25.00
Anode Material	Cu
K-Alpha1 [Å]	1.54060
K-Alpha2 [Å]	1.54443
K-Beta [Å]	1.39225
K-A2 / K-A1 Ratio	0.50000
Generator Settings	40 mA, 45 kV
Diffractometer Type	0000000011023505
Diffractometer Number	0
Goniometer Radius [mm]	240.00
Dist. Focus-Diverg. Slit [mm]	91.00
Incident Beam Monochromator	No
Spinning	No

Table 5: Particle Size Analysis of Glimepiride-neodyamium complex

Drug/Complex	Particle size (in microns) mm	Appearance
Pure drug (Glimepiride)	10	heavy compact crystals
Glimepiride neodymium complex	2	minute particles

Table 6: Hypoglycemic activity of glimepiride and its Nd-Complex

Group	Treatment (mg/kg body weight)	Observation Tables Glucose Tolerance Test Blood Glucose (mg/dL)		
		Fasting	30 min.	90 min.
I	Control group + Glucose (2g) + Vehicle	73.8 ± 2.588	142.8 ± 2.863	120.6 ± 2.408
II	Neodyamium complex of Glimepiride (2mg) + Glucose + Vehicle	72.6 ± 2.0736	103.8 ± 2.3874*	84.8 ± 3.4928**
III	Pure drug Glimepiride (2mg) + Glucose + Vehicle	74.4 ± 2.8809	100.6 ± 2.7018*	90.2 ± 2.5884**

The numbers of peaks in the complex is 17 which indicated that the complex formed is a well knit one[33-35]. Moreover the complexe lack of periodicity in the diffractrogram of the resultant complex indicates its amorphous nature[36].

Particle size analysis

Paricle size and Elemental Analysis of Glimepiride Pure by SEM-EDAX Method

Surface morphology of pure ligand (Glimepiride was studied by SEM method and the compositional analysis of pure drug and the complex were investgated by EDAX (Energy dispersive X-ray spectroscopy)

Thus the particle size analysis shown in Fig 10 and 11 have revealed that the original

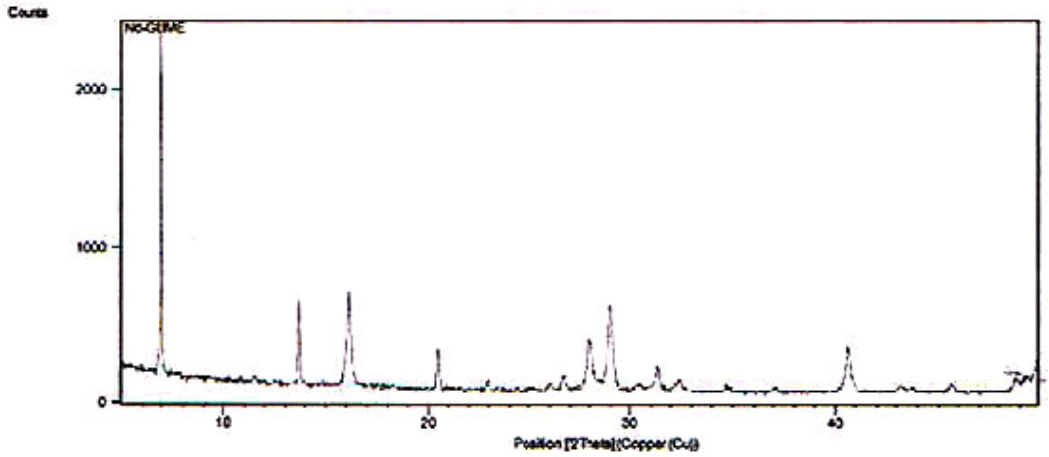


Fig. 9: XRD data of glimepiride-neodymium complex

Table 6: XRD data table of pure ligand, glimepiride

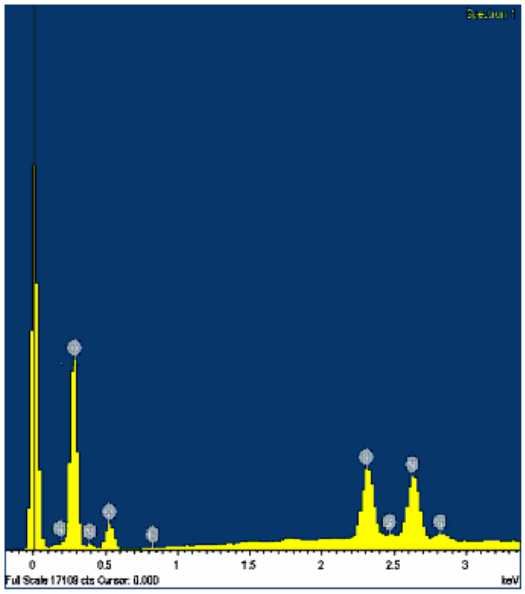
Pos. [°2Th.]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Area [cts*°2Th.]
6.5769	0.1171	13.43963	36.96	165.77
9.6248	0.0836	9.18947	7.42	23.77
10.5561	0.1171	8.38075	5.61	25.15
11.0783	0.0836	7.98682	11.03	35.33
12.5609	0.0836	7.04728	19.86	63.64
13.2334	0.0836	6.69059	17.45	55.90
13.5966	0.1171	6.51272	100.00	448.51
13.9677	0.1004	6.34050	23.61	90.77
14.8031	0.1171	5.98450	30.37	136.22
15.5129	0.1004	5.71222	3.31	12.73
16.8615	0.1004	5.25829	47.20	181.44
17.3321	0.1171	5.11656	16.19	72.60
18.3051	0.1338	4.84673	83.27	426.83
19.3576	0.0836	4.58550	33.15	106.21
20.8063	0.0836	4.26938	13.41	42.95
21.2003	0.1506	4.19093	90.48	521.73
21.6577	0.0836	4.10344	20.80	66.65
22.1546	0.1171	4.01252	27.82	124.76
22.4490	0.1004	3.96056	17.81	68.47
23.1159	0.1338	3.84778	25.39	130.14
23.3171	0.1004	3.81504	16.36	62.91
23.8470	0.1506	3.73144	11.45	66.05
25.3875	0.1004	3.50840	23.47	90.24
25.9445	0.1004	3.43433	5.43	20.89
26.5144	0.1171	3.36180	29.99	134.53
26.7990	0.1004	3.32674	11.34	43.60
27.7402	0.1004	3.21597	2.84	10.91
28.1590	0.1673	3.16909	4.71	30.17
29.0505	0.2342	3.07383	7.10	63.71
29.5916	0.1171	3.01884	9.27	41.57
30.4222	0.0669	2.93829	9.83	25.19
32.0273	0.1338	2.79460	8.49	43.52
33.2354	0.1004	2.69573	1.85	7.10
34.8346	0.1673	2.57555	4.23	27.13
35.9001	0.1673	2.50152	2.54	16.26
36.6025	0.2007	2.45511	1.81	13.93
37.4142	0.3346	2.40369	1.56	20.01
38.2693	0.3011	2.35193	3.86	44.56
40.5891	0.2007	2.22271	3.54	27.24
41.6287	0.2676	2.16957	2.33	23.86
43.8666	0.4015	2.06394	0.88	13.53
45.6466	0.6528	1.98587	1.39	46.95

Table 8:

Pos. [°2Th.]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Area [cts*°2Th.]
6.9600	0.0669	12.70077	100.00	150.15
11.5366	0.2676	7.67059	1.62	9.73
13.6982	0.1004	6.46462	22.40	50.45
16.1523	0.1673	5.48753	26.25	98.54
20.4570	0.0669	4.34149	9.88	14.84
22.9701	0.1338	3.87188	2.18	6.54
26.6635	0.1673	3.34333	3.88	14.55
27.9515	0.2342	3.19214	13.92	73.14
28.9580	0.0836	3.08344	22.57	42.36
31.3108	0.1673	2.85690	6.69	25.11
32.4197	0.2007	2.76167	3.35	15.11
34.6908	0.2676	2.58590	1.77	10.66
37.0691	0.2676	2.42527	1.43	8.62
40.6105	0.2342	2.22158	12.10	63.60
43.1738	0.2007	2.09544	2.01	9.06
45.7124	0.2676	1.98480	2.54	15.27
48.8201	0.3264	1.86394	3.96	39.25

Particle size analysis

Paricle size and Elemental Analysis of Glimepiride Pure by SEM-EDAX Method



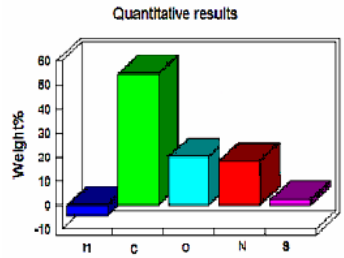
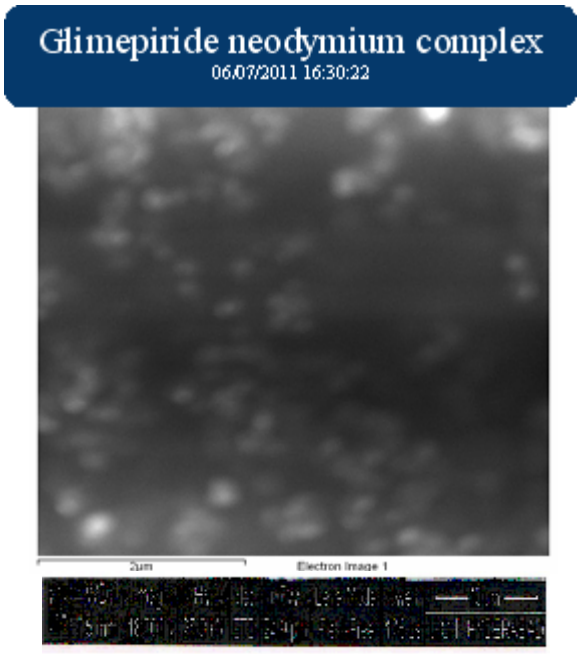
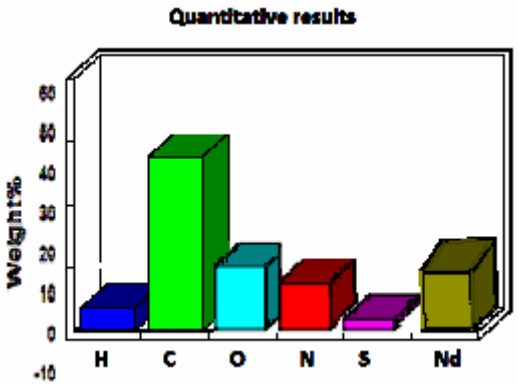


Fig. 10:

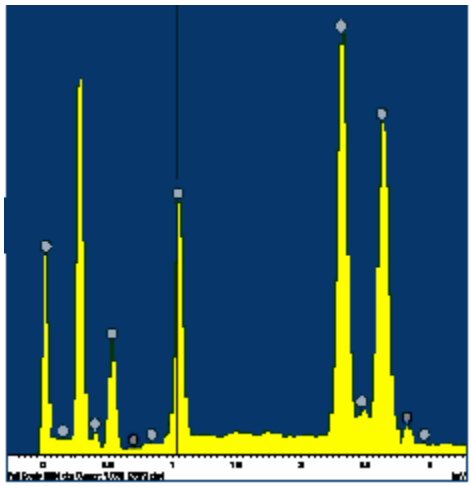
Element	
C	
H	6.93
O	16.32
S	6.53
N	11.92
Totals	100.00

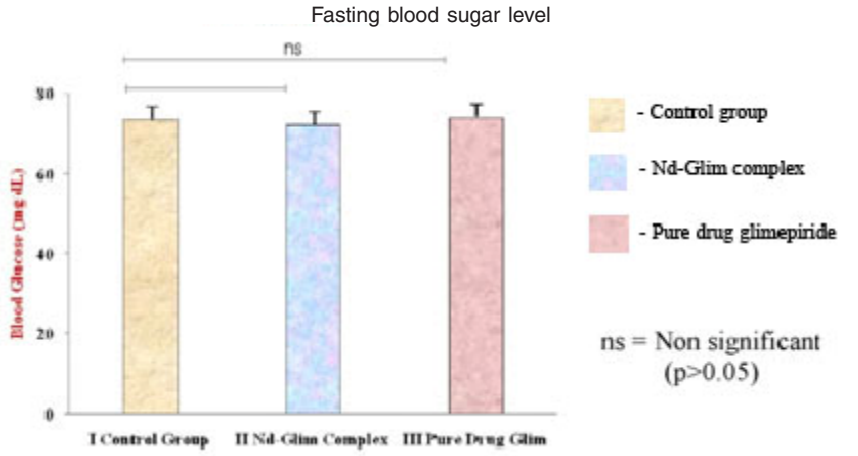
Paricle size and Elemental Analysis of Glimepiride-Nd Complex by SEM-EDAX Method



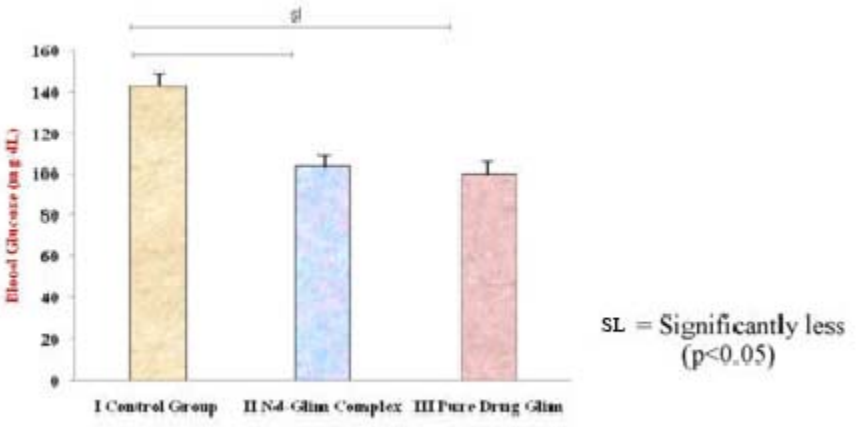


Element	Atomic %
C	49.61
H	5.85
O	13.78
S	9.64
N	6.13
Nd	12.4
Totals	100.00





(Blood sugar level) After 30 minutes



(Blood sugar level) After 90 minutes

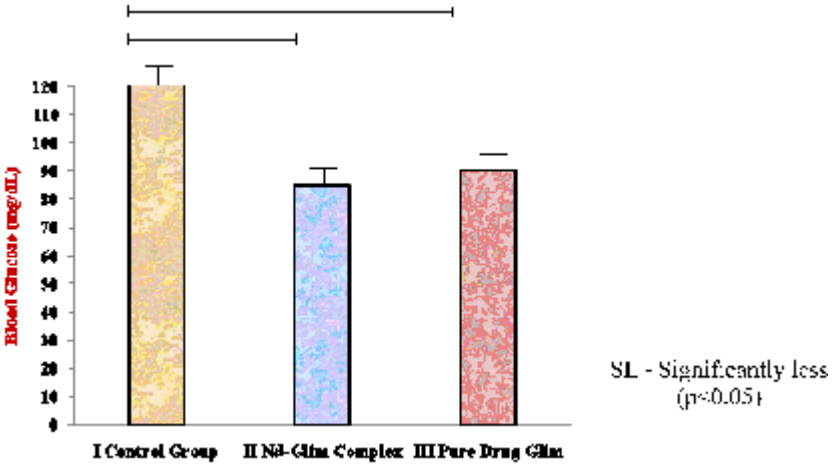


Fig. I: Oral Glucose Tolerance

morphology of pure components disappeared, which supported that the metal complex is more potent and being absorbed more readily than its parent drug[37-38].

Antidiabetic activity

The isolated glimepiride-metal complex were found to be more potent as compared to the parent drug. Hence as compare to standard synthetic drug the glimepiride-neodymium[39-41] complex is 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 Oral glucose tolerance test[42]. (PBRI, lab. Bhopal). Analysis of data presented in table (6) reveals that the drug caused a marked decrease in blood sugar level. On comparing the hypoglycemic effect of neodymium complex with parent drug it was revealed that incase of Nd- glimepiride treated male wistar rats blood sugar falls to 64.8+ 3.4928 ng/dl while in glimepiride treated rats blood sugar level falls to 90.2 +2.5884 mg/dl. These results clearly indicate a better hypoglycemic activity of Nd-glimepiride complex over its parent drug.[43-46]

REFERENCES

Ammar, H.O., Salama, H.A., Ghorab, M., and Mahmoud, A.A., *Asian J. Pharm. Sci.,* **2**(2): 44-55 (2007).

2. Massimo, M.B., *Clin Ther.,* **25**: 799-816 (2003).
3. Kouichi **et al.,** *Diab. Res. Clin. Pract.,* **68**: 250-57 (2005).
4. Geinsen, K., *Drug Res.,* **38**: 1120-30 (1988).
5. Baliar, S., Biswal, S., Sahoo, J., and Murthy, P.N., *Inter. J. Pharm. Sci and Nanotech.*, **2**: 1129 (2009).
6. Iqbal, S.A., and Qureshi, R., *Asian .J. Expt. Sci.,***1**: 68-70 (1985).
7. Theivarasu,C., and Sivaprakash.K., ., *Orient. J. Chem.,* **28**(3): 1379-87 (2012)
8. Iqbal, S.A., Jose, S., and Zaafarany, I., *Orient. J. Chem.,* **28**(1): 613-18 (2012).
9. Bellamy, L.J., *"The infrared spectra of complex molecules".* Methven & Co. Ltd. London (1954).
10. Nakamoto, K., *"Infra-red spectra of inorganic and co-ordination compound.* John Wiley & Sons, Newyork (1963).
11. Dyer, J., *"Application of Absorption Spectroscopy of Organic Compounds" Prentice Hall of India Pvt. Ltd.* Fourth printing., 36 (1978).
12. Rao, C.N.R., *"Chemical Application of Infrared spectroscopy".* Academic Press, Newyork 258 (1970).
13. Chandran, A., Varghese, H.T., Yohannan, C., and Rajendran, G., *Orient. J. Chem.,* **27**(2): 611-17 (2011).
14. Tawkir, M., Iqbal, S.A. Krishan, B., and Zaafarany, I., *Orient. J. Chem.,* **27**(2): 603-09 (2011).
15. Oliveira, G.G.G., Ferraz, H.G., and Matos, J.S.R., *J. Therm. Anal. Colorimetry.,* **29**: 267-70 (2005).
16. Khan, M.R., and Sahdev., *Orient. J. Chem.,* **27**(2): 649-53 (2011).
17. Santhi, S., and Namboori, C.G.R., *Orient. J. Chem.,* **27**(3)., 1203-08 (2011).
18. Verma, M., Llala, N.P., Rawat, R., and Phase, D.M., *Orient. J. Chem.,* **27**(4): 1775-78 (2011).
19. Cullity, B.D. "Elements of X-ray Diffraction"., Second Edn, Wesley Publishing Company INC., 40 (1978).
20. Zaitoon, B.A., Yousef, R., and Musleh, S.M., *Orient. J. Chem.,* **27**(4): 1357-74 (2011).
21. Seshadri, N., Suresh, C., Seshaiah, K., and Suresh, M., *Orient. J. Chem.,* **27**(4): 1685-90 (2011).
22. Geinier, A., *"X-ray diffraction in Crystals, Imperfect Crystals and Amorphous Bodies".*, San Francisco (1963).
23. Job, P., *Ann. Clin.,* **113**: 10 (1928).
24. Turner, S.E., and Anderson, R.C., *J. Am. Chem. Soc.,* **912**: 71 (1949).
25. Pena, K., Garcia-cuello, V.S., Giraldo, L., and Moreno-Pirajan, J.C., *Orient. J. Chem.,* **27**(4): 1293-1301 (2011).
26. Mehta, A., Vasanti, S., and Tyagi, R., *Current Trends in Biotech and Pharm.,* **3**(1): 76-84 (2009).
27. Pahovnik, D., Revan, S., Grdadolink, J., and

Zagar, E., *J. Pharm. Sci.,* **100**(11): 4700-09 (2011).
28. Ashraf M., Siddiqui M.A. and Bhatty Talanta M.K., **15**: 559 (1989).
29. Rai, B.K., Vidyarthi, S.N., Singh, R- and Ojha A., *Orient. J. Chem.*, **28**(3): 1403-09 (2012).
30. Bragg W.L. and Bragg W.H., The Crystalline State, A General Survey, London, Vol. I (1993)
31. Jenkins R. and Devries J.L., Worked Examples in X-ray Analysis, New York, Springer Verlag (1970).
32. Henry N.F.M., Lipson H. and Wooster W.A., The interpretation of X-ray Diffraction Photographs, Macmillan, London (1951).
33. Siewers R.E., Nuclear Magnetic Resonance Shift Reagents, Academic, New York (1973).
34. Sharma, K.Y. and Prasad, M., *Orient .J. chem.*, **28**(3): 1419-24 (2012).
35. Iqbal. S.A., Jose, S. and Zaafarany, I., *Orient.J. Chem.*, **28**(1): 613-18 (2012).
36. Nag., A.K., *Orient. J. Chem.*, **28**(1): 514-45 (2012).
37. Soleymani, R., Salehi, Y.M.., Yousofzad, T., and Cheshmeh Ali, M.K., *Orient. J. Chem.,* **28**(2): 627-38 (2012).
38. Obeidat, S.M., and Tayyem, B. AL., *Orient. J. Chem.,* **28**(2): 795-801 (2012).
39. Rehman, F., and Mairaj, S., *Orient. J. Chem.,* **28**(2): 881-85 (2012).
40. Mary, Y.S., Panicker, C.Y. and Varghese, H.T., *Orient. J. Chem.,* **28**(2): 937-41 (2012).
41. Mdhavadiya, V.A., *Orient. J. Chem.,* **28**(2): 921-25 (2012).
42. Saxena, A., *Orient. J. Chem.,* **28**(2): 1067-1070 (2012).
43. Vardhan, V., and Sharma., T.R., *Orient. J. Chem.,* **28**(2): 1077-80 (2012).
44. Kakadiya, J., Shah, M., and Shah, N., *Inter. J. Applied Bio. And Pharm. Tech.,* **1**(2): 276 (2010).
45. Jha, V.K., *J. Chem.,* **23**(7): 3285-86 (2011).
46. Sumana, G., and Suryawanshi, S.A., *Indian. J. Experi Biology.,* **39**: 748-58 (2001).
47. Rasheed, K., Tariq, M.I., Munir, C., Hussain, I., and Siddiqui, H.L., *Chemical & Pharmaceutical Bulletin.,* **56**(2): 168 (2008).