BIOCOORDINATION AND COMPUTATIONAL MODELING OF STREPTOMYCIN WITH CO (II), NI (II), IN (II) AND INORGANIC SN (II)

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ABSTRACT

The coordination compounds of streptomycin with Co(II), Ni(II), In(III) and Sn(II) have been synthesized and characterized by elemental analysis, vibrational spectra, electronic spectra, ^{1}HNMR spectra, magnetic susceptibility measurement ,thermal studies and X-ray powder diffraction studies. The complexes having crystallographic datas as a=21.9909(Å), b=13.12141(Å), c=11.88511(Å), V=3429.47(A^3), for complex 1; a=6.904155(Å), b=12.57103(Å), c=13.77902(Å), V=1090.3(A^3) for complex 2; a=6.709547(Å), b=9.231634(Å), c=8.6857(Å), V=347.1999(A^3) for complex 3 and a=16.10349(Å), b=10.4157(Å), =9.588465(Å), V=16084.26(A^3) for complex 4. The results of spectral techniques kinetic parameters were computed from the thermal data using Coat and Redfern method, which confirm first order kinetics. Molecular structures of the complexes have been optimized by MM2 calculations and supported octahedral, trigonal planar /tetrahedral geometry around Metal (II) ions and In (III) ions.

Keywords: Aminoglycosides, spectra, streptomycin, metal complexes, molecular modeling.

INTRODUCTION

Streptomycin having chemical name 1,-(4-(4-(3,5-dihydroxy-6-(hydroxymethyl)-4-(methylamino) tetrahydro -2H-pyran-2-yloxy)-5 -formyl-5-hydroxy -3-methyl tetra hydrofuran-2-yloxy)-2,5,6-trihydroxycyclohexane-1, 3-diyl)diguanidine is an aminoglycosidic antibiotic with three components : streptidine, streptose and N-methyl-L-glucosamine (Fig1). It is used to treat infections caused by Gram-negative bacteria and in the therapy of tuberclosis[1-4]. Serious toxicity is a major limitation of its usefulness, most notable is its ototoxicity, and causing deafness is severing cases. It has been proposed that the mechanism of action of this antibiotic could be related to an enhancement of the biological availability of magnesium and to a reduction of calcium [5-6] Some preliminary studies about the interaction in solution of some metal ions with streptomycin have been reported [7-9] but detailed chacterisation of the complexes are unavailable except for a neutral Cu(II) complex where Cu-O bonds with streptomycin are suggested[10-11]. Much of the structural effort has focused on aminoglycosides bound either to modular constructs of the ribosomal RNA decoding site in solution [10-12]. Furthermore, literature on streptomycin as a ligand reveals that there is clear disagreement about the metal – ligand binding sites in complexes of this ligand [13]. Therefore, in order to ascertain metal binding sites in streptomycin metal chelates, the synthesis and spectroscopic characterization of complexes of streptomycin with these transition/non-transition metal ions is attempted in this investigation. The structure of streptomycin is given in Fig.1.

Aminoglycosides kill bacteria primarily by inhibiting the translation step in microbial protein synthesis [2]. A major problem in therapy with aminoglycosides is their relatively high toxicity to the kidney and the inner ear. Nevertheless, streptomycin is currently the first choice antibiotic in developing countries and is still widely used in industrialized countries for the treatment of serious bacterial infections as tuberculosis. The adverse affects of aminoglycosides may result from complex formation with transition metal ions and the oxidative reactions the complexes subsequently promote.[3-5] co-administration of transition metal chelators and free radical scavengers, as well as over-expression of superoxide dismutase in model animals, suppress aminoglycoside-induced ototoxicity [6-9]. Systematic *in vitro* studies of iron interactions with streptomycin have led to a postulated mechanism of toxicity involving free radical formation by Fe(II)/Fe(III)-streptomycin complexe. Another body of evidence [10-12] has suggested that both pharmacological activity and toxicity of aminoglycoside antibiotics could be related to copper(II)-aminoglycosides complexes. Jezowska-Bojczuk et al. extensively investigated chelation of copper(II) ions by streptomycin-related aminoglycoside antibiotics using potentiometry and a variety of spectroscopic techniques.[10-13] Kanamicin B, tobramicin, geneticin, and amikacin strongly bind Cu(II) ions, forming monomeric complexes over a wide pH range. In naturally occurring aminoglycosides, the amino nitrogens and deprotonated alcoholic oxygens of the terminal aminosugar rings are involved in the coordination, forming five and six-membered chelate rings about central ions [14-16].

Amikacin, a semisynthetic derivative of kanamicin A, having the 1-amino group on the 2-deoxystreptamine moiety modified by acylation with 4-amino-2-hydroxybutyric acid, exhibits different binding modes by involving the amidated nitrogen in coordination [17]. Further, Cu(II)-amikacin complexes catalyze hydrogen peroxide disproportionate at pH 7.4 mediated by hydroxyl radicals and involving Cu(I)/Cu(II) and Cu(II)/Cu(III) redox pairs. [18] These complexes mediate oxidation of 2'-deoxyguanosine to 7,8-dihydro-8-oxo-2'-deoxyguanosine, double-stranded DNA cleavage, and both hydrolytic and oxidative t-RNA^{Phe} strand scission at a specific site in the

anticodon loop.[19] Under these circumstances, copper(II) ions are proposed to be involved in aminoglycoside toxicity. Therefore, in order to ascertain metal binding sites in streptomycin metal chelates, the synthesis and spectroscopic characterization antitumer activities of complexes of streptomycin (Figure 1) with this transition/main group metal ions provided in this investigation.

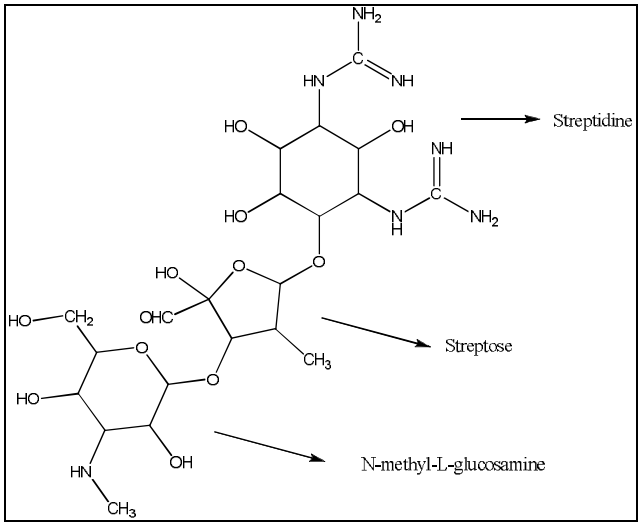

Figure 1: 1,-(4-(4-(3,5-dihydroxy-6-(hydroxymethyl)-4-(methylamino) tetrahydro-2H-pyran-2-yloxy)-5-formyl-5-hydroxy-3- methyltetrahydrofuran- 2-yloxy)-2,5,6-tri hydroxy cyclohexane -1, 3 -diyl) diguanidine (Streptomycin)

MATERIALS AND METHODS

All the chemicals used in this study were of analytical grade which obtained from Merck. Streptomycin was purchased from Merck. Solvents used were of analytical grade and were purified by standard procedures. The stoichiometric analyses(C, H and N) of the complexes were performed using Elementar vario EL III (Germany) model. Metal contents were estimated on an AA-640-13 Shimadzu flame atomic absorption spectrophotometer in solution prepared by decomposing the respective complex in hot concentrated HNO_3. Their IR spectra were recorded on Perkins–Elmer FTIR spectrophotometer in KBr and polyethylene pellets. The electronic spectra were recorded in water on Beckman DU-64 spectrophotometer with quartz cells of 1 cm path length. ^1H NMR spectra were recorded in DMSO-d_6 solvent on a Bruker Advance 400 instrument. Rigaku model 8150 thermoanalyser (Thermaflex) was used for simultaneous recording of TG-DTA curves at a heating rate of $10^o min^{-1}$.

For TG, the instrument was calibrated using calcium oxalate while for DTA, calibration was done using indium metal, both of which were supplied along with the instrument. A flat bed type aluminium crucible was used with α- alumina (99% pure) as the reference material for DTA. The activation energy and Arrhenius constant of the degradation process was obtained by Coats and Redfern method. The XRD powder pattern were recorded on a vertical type Philips 1130/00 X- ray diffractometer, operated at 40kVand 50Ma generator using the Cukα line at 1.54056 A° as the radiation sources. Sample was scanned between 5° to 70° (2θ) at 25°C. The crystallographic data was analyzed by using the CRYSFIRE –2000 powder indexing software package and the space group was found by the CHECK CELL program. Debye – Scherer relation with the help of 100% peak width determined the particle size. The experimental density was determined by Archimedes method.

Molecular modeling

3D molecular modeling of the proposed structure of the complexes was performed using CsChem3D Ulta -11 program package. The correct stereochemistry was assured through the manipulation and modification of the molecular coordinates to obtain reasonable low energy molecular geometries. The optimized structures of the complexes were performed by MM2 programme contained CS chem. Office programme. The potential energy of the molecule was the sum of the following terms: $E = E_{str} + E_{ang} + E_{tor} + E_{vdw} + E_{oop} + E_{ele}$.Where all E's represent the energy values corresponding to the given types of interaction. The subscripts str, ang, tor, vdw, oop and ele denote bond stretching, angle bonding, torsion deformation, vander waals interactions, out of plain bending and electronic interaction, respectively.

Antibacterial sensitivity assay

Antibacterial sensitivity assay was performed using Streptomycin (SM) and its metal complexes (Co-SM, Ni-SM, In-SM and Sn-SM) on *Agrobacterium sp* BN-2A. Various concentrations (0, 25, 50, 100, 150 and 200 µg/mL) were made and filter sterilized with 45mm sterile filter paper. Antibiotic discs were prepared with Watt's man filter paper to cut by paper punch machine. All paper discs were autoclaved and soaked with filter sterilized antibiotic solutions of different concentrations separately in sterile condition then excess water of solution was dried in oven.

Now, antibiotic discs of different concentrations were ready to use. 100 µL aliquot of overnight nutrient broth grown culture (*Agrobacterium sp* BN-2A) was spread over Nutrient agar (NA) solid Petri plate and antibiotic discs were kept gently on the surface. The Petri plates were incubated in BOD incubator at 37^0C for growth. Inhibition zone was visualized around antibiotics disc after overnight growth. The diameter of the zone of inhibition and antibiotic discs were recorded. Bacterial inhibition index (BII) was calculated using formula written below:

$$\text{Bacterial Inhibition Index (BII)} = \frac{\text{Diameter of inhibition zone} - \text{Diameter of antibiotic disc}}{\text{Diameter of antibiotic disc}}$$

The experiment was performed in triplicate and repeated three times. Average of all readings and standard deviations were calculated. Statistical calculations (t-test) were done and P-value was recorded. P value ($p \leq 0.005$) showed the significant data.

Synthesis of complexes

To a methanolic solution of different metal chlorides (0.5 mmol) in a separate flask was added a aqueous methanolic solution of the streptomycin (0.5 mmol). The solution was stirred for 6h, after which the volume was reduced on a

warm water bath. The product obtained was washed with a small amount of methanol and air – dried. The above product was redissolved in excess warm methanol, and clear solution was left undisturbed for weeks to give beautiful crystals of the complexes.

RESULTS AND DISCUSSION

Satisfactory results of elemental analysis (Table 1) and spectral studies revealed that the complexes were of good purity. Various attempts to obtain the single crystals have so far been unsuccessful. X-ray diffraction studies indicate crystalline nature of the metal complexes. The complexes were soluble in polar solvents.

Table 1: Color, reaction yield and elemental analysis of complexes

Complex	Empirical formula	Color	Yield (%)	Analysis: found (calculated) (%)				M.Pt. (^{O}C)
				C	H	N	M	
[Co(L) Complex (1)	$C_{21}H_{37}N_7O_{12}Co$	Pink	75	39.65 (39.50)	5.15 (5.84)	15.65 (15.36)	9.15 (9.23)	65
[Ni(L) Complex(2)	$C_{21}H_{37}N_7O_{12}Ni$	greenish	80	37.13 (39.52)	6.11 (5.84)	14.41 (15.36)	9.60 (9.20)	66.5
[In(L)H_2O Complex(3)	$C_{21}H_{39}N_7O_{13}$ In	White	84	35.61 (35.41)	5.85 (5.85)	15.46 (15.36)	9.42 (9.20)	85.5
[Sn(L) Complex(3)	$C_{21}H_{37}N_7O_{12}Sn$	brown	79	36.61 (36.12)	5.31 (5.35)	13.91 (14.04)	17.17 (17.00)	88.2

L: Ligand streptomycin

Table 2: IR spectral data (cm^{-1}) of the metal complexes

Frequency	ν_{N-H}	OH	OH	NH_2	NH_2	M – O
$C_{21}H_{37}N_7O_{12}Co(1)$	3417(s,b)	1637(m)	1528(s)	1232(m)	690(s)	4150(m)
$C_{21}H_{37}N_7O_{12}Ni(2)$	3425(s,b)	1639(m)	1525(s)	1225(m)	685(s)	426(s)
$C_{21}H_{39}N_7O_{13}$ In(3)	3437(s,b)	1644(m)	1503(m)	1217(w)	686(s)	467(m)
$C_{21}H_{37}N_7O_{12}Sn(4)$	3425(s,b)	1629(m)	1469(s)	1226(w)	696(s)	425(s)

Vibrational spectra

Streptomycin molecule exhibits absorptions 1055, 1760, 2938cm^{-1}. These bands are very metal complexes indicating non – involvement of the oxygen atoms of hydroxyl group in coordination with the metal ions[19].The stretching frequencies of streptomycin hydroxyl and give bands at 3368 and 3434 cm^{-1} with a shoulder at about 3550 cm^{-1}. These bands appear in the complexes as strong band absorption in the region 3420 - 3445 cm^{-1}. These bands appear for the new complex at the same wave number, ruling out the participation of hydroxyl oxygen in the coordination. These results confirm that complexation occurred and suggest that the oxygen of the hydroxyl group is involved in the coordination sphere [16]. The vibrational bands due to rocking & wagging modes of water and metal – oxygen stretching modes are observed in the 800 – 350 cm^{-1} region for all the complexes may be attributed to coordinated water[17-20]. This can be confirmed with the help of thermo grams. A new band in the 615 – 300 cm^{-1} regions in the spectra of the complexes is assignable to ν (M - O).

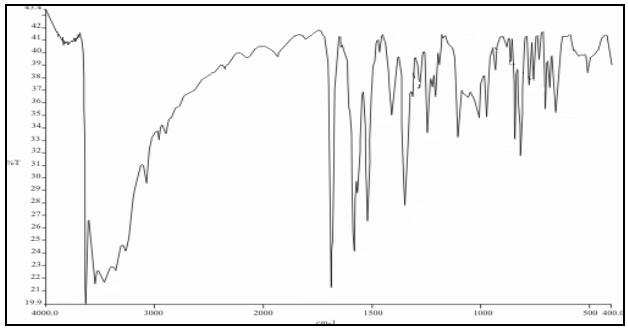

Figure 1b: FT-IR spectra of complex 3

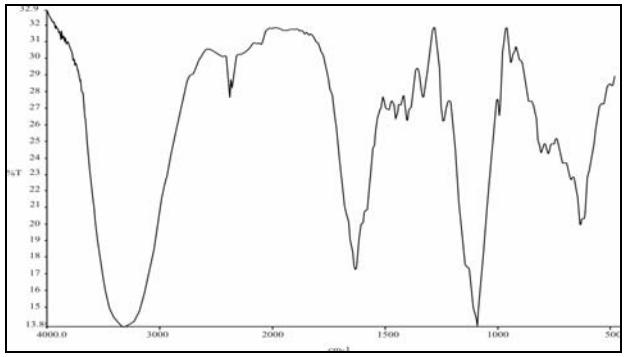

Figure 2a: FT-IR spectra of complex1

^{1}HNMR

The ^{1}HNMR spectra of the streptomycin complexes of Co(II), Ni(II), In(II) and Sn(II) in a DMSO-d_6 solvent show well-resolved signals inj Figure 4. ^{1}HNMR spectrum of complex 1. The N-H protons of amine, which would have undergone very rapid exchange with the solvent, appear as quite broad ragged doublet around 3.58(ppm) and 3.65 (ppm) coordinated with metals(II) which disappeared in the metal complex spectra. In complex 3, peaks range 1.21-1.71 ppms are from coordinated water. The various assignments of ^{1}HNMR of the complexes are summarized in table 3. Chemical shift are in ppm from TMS & multiplicity in parentheses (bd, broad; d, doublet; m, multiplet).

Table 3: ^{1}HNMR assignments of ligand and its complex 1 and complex3

Assignment	Ligand	Complex1	Complex3
NH	2.0(d)	8.17bd)	7.55
C_1H,C_2H	3.27	3.26(m)	3.11
C_2H,C_6H	1.93,2.13,1.91	1.95,2.05,1.94(d)	3.012
C_1OH	3.8(s)	6.54	3.05
C_3OH	4.85(bd)	5.04	…….
C_5OH	3.58	…….	……
C_2H	3.65	…….	……..
C_3H	3.36	4.45	2.765,2.66
CH(H)	0.96	3.76	2.591

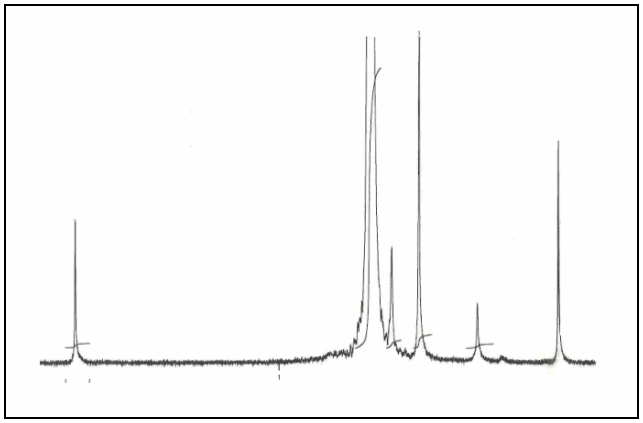

Figure 3a: ^{1}H NMR Spectra of Co-SM complex

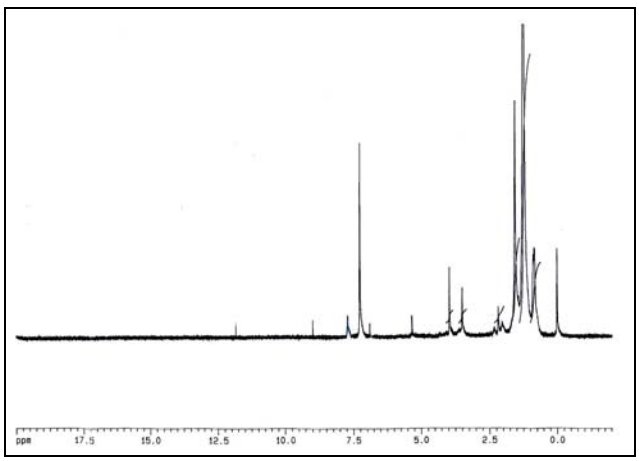

Figure 3b: ^{1}H NMR Spectra of Ni-SM complex

Electronic spectra

The electronic spectra of the ligands and its metal complexes have been studied in the range 190---800nm. The shoulder band observed at 275nm in water solvent in legand may be assigned to n→n* transition within the OH group of hydroxyl moiety in the free ligand. This band disappeared in all complexes, revealing the involvement of OH in chelate formation. The band observed at 298 nm in SM (Streptomycin) may be assigned to π→π* transition of the oxygen of hydroxyl group of ligand.In cobalt complex, the absorption band in the visible region 509-570nm and 475-420nm assignable to 2B2g→ 4Eg(P) and 2B2g → 4A2g(P) transition, suggested square planar where as indium complex3 376 nm may be assigned to π→π*.

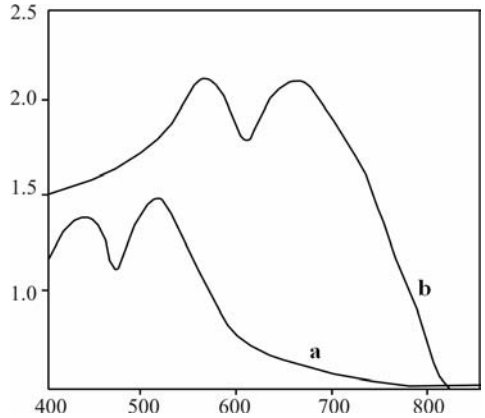

Figure 4: Electronic spectra of the (a) Cobalt –S M complex (b) Nickel – SM complex

Kinetics of thermal decomposition

Recently, there has been increasing interest in determining the rate- dependent parameters of solid-state non-isothermal decomposition reactions by analysis of TG curves [21, 22]. Thermogravimetric (TG) and differential thermo gravimetric (DTA) analyses were carried out for different metal–streptomycin complexes in ambient conditions. The thermogravimetric analysis revealed that the complexes of Zn & Cd loses mass between 65°C and 140°C, corresponding to nearly 15 % of the total mass, followed by considerable decomposition up to 600°C, which corresponds to the decomposition of the ligand molecule leaving metal oxide (NiO & InO, respectively) as residue. The complexes of In & Sn decomposes nearly 9% of the total mass up to temperature 170°C, followed by considerable decomposition of the ligand molecule up to 650°C, leaving metal oxide (CoO and SnO respectively) as residue. On the basis of thermal decomposition, the kinetic analysis parameters such as activation energy (E*), enthalpy of activation (ΔH*), entropy of activation (ΔS*), free energy change of decomposition (ΔG*) were evaluated graphically by employing the Coats – Redfern relation [23]

$$\text{Log } [-\text{Log } (1- \alpha) /T^2] = \log [AR/ \theta E^*(1-2RT/E^*)]-E^*/2.303RT$$

Where α is the mass loss up to the temperature T, R is the gas constant, E*is the activation energy in J mole^{-1}, θ is the linear heating rate and the term (1-2RT/E*) ≅ 1.A straight line plot of left hand side of the equation (1)

against 1/T gives the value of E* while its intercept corresponds to A (Arrhenius constant). The Coats and Redfern linearization plots, confirms the first order kinetics for the decomposition process [25]. The calculated values of thermodynamic activation parameters for the decomposition steps of the metal complexes are reported in Table 4. According to the kinetic data obtained from the TG curves, the activation energy relates the thermal stability of the metal complexes. Among metal complexes, activation energy increases as complex 3 ~ complex 2 < complex 4 < complex 1, same trends happens with thermal stability of metal complexes. All the complexes have negative entropy, which indicates that the complexes are formed spontaneously. The negative value of entropy also indicates a more ordered activated state that may be possible through the chemisorptions of oxygen and other decomposition products. The negative values of the entropies of activation are compensated by the values of the enthalpies of activation, leading to almost the same values for the free energy of activation.

Table 4: Thermodynamic activation parameters of the metal complexes

Complex	Order/n	Steps	E*/Jmol^{-1}	A/sec^{-1}	ΔS*/JK^{-1}mol^{-1}	ΔH*/Jmol^{-1}	ΔG*/ kJmol^{-1}	k×10^{2}s^{-1}
$C_{21}H_{37}N_7O_{12}Co$	1	I II	56.66 68.804	1.125×10^5 1.256×10^5	-91.49 -109.175	112.745 96.114	61.228 89.18	1.62 1.01
$C_{21}H_{37}N_7O_{12}Ni$	1	I II	58.066 7.178	6.27×10^5 1.16×10^5	-80.136 -109.603	74.10 125.89	55.29 93.104	3.25 1.718
$C_{21}H_{39}N_7O_{13}$ In	1	I II	56.35 61.08	1.4501×10^5 1.171×10^4	-66.345 -74.96	118.76 54.691	44.135 74.96	1.41 1.142
$C_{21}H_{37}N_5O_9Sn$	1	I II	54.59 67.88	2.28×10^6 1.53×10^6	-56.01 -80.731	70.98 28.16	36.566 80.547	1.01 0.611

Table 5: Crystallographic data for complexes

Compounds	Complex 1	Complex 2	Complex 3	Complex 4
Formula	$C_{21}H_{37}N_7O_{12}Co$	$C_{21}H_{37}N_7O_{12}Ni$	$C_{21}H_{39}N_7O_{13}$ In	$C_{21}H_{37}N_7O12Sn$
FW	637.33	637.69	711.82	697.71
Temp (K)	293	293	293	293
Wavelength	1.54056	1.54056	1.54056	1.54056
Crystal System	Orthorhombic	Triclinic	Monoclinic	Orthorhombic
Space group	P mmm	P 1	P 2/m	P mmm
Unit cell dimension				
a(Å)	21.9909	6.904155	6.709547	16.103449
b(Å)	13.12141	12.57103	9.231634	10.4157
c(Å)	11.885	13.77902	8.6857	9.5884
α^o	90.00	84.3499	90.00	90.00
β^o	90.00	97.198	96.346856390	90.00
γ^o	90.00	68.49413	90.00	90.00
Volume (A^3)	3429.47	1090.30	347.1999	1608.426
θ range (0)	21.696-75.106	13.811-61.987	10-65	12-67
Limiting indices	$0 \le h \le 4$ $0 \le k \le 6$ $0 \le l \le 3$	$-6 \le h \le 4$ $0 \le k \le 7$ $0 \le l \le 4$	$-3 \le h \le 1$ $-4 \le k \le 4$ $0 \le l \le 4$	$-7 \le h \le 5$ $0 \le k \le 8$ $0 \le l \le 5$
Particle size(nm)	11.922	80.82	55.99	10.92
Intensity (%)	7.2–100	5.9–100	4.5-100	3.4-100
R indices	0.0000156	0.0000615	0.0000754	0.0000362
Density	1.07405	1.7437	1.034	1.151
Z	2	2	1	1

TOF–MS spectra

Mass spectrometry has been successfully used to investigate molecular species $[MH]^+$ in solution [26]. The molecular ion peaks of the ligands and complexes have been used to confirm the proposed formula (Table 4). The pattern of the mass spectrum gives an impression of the successive degradation of the target compound with the series of peaks corresponding to the various fragments. Their intensity gives an idea of stability of fragments. The ligand starts degradation and finally forms $C_7H_{15}NO_5]$=193, (100 % m/z values. In the TOF–mass spectra of metal complexes initial fragmentation pattern is again similar (loss of two water molecules), a mononuclear nature for these complexes $[M(L)]^+$ can be deduced. The last two fragments appears in nearly all the complexes at positions (m/z values) 163(100% complex 1, 100 % complex 2, 100 % complex 3 and 57 % complex (4) and 263/264/267 (10 % complex I, 50% complex 2 and 100 % complex 4) corresponds to $[C_8H_{18}N_6O_4]^+$ and $[C_{21}H_{41}N_7O_{12}]^+$ respectively, which could be the result of degradation & demetallation of the complexes (Figure 5a-b. Scanned TOF-MS spectrum of complexes 3 and 4 with specific fragments).

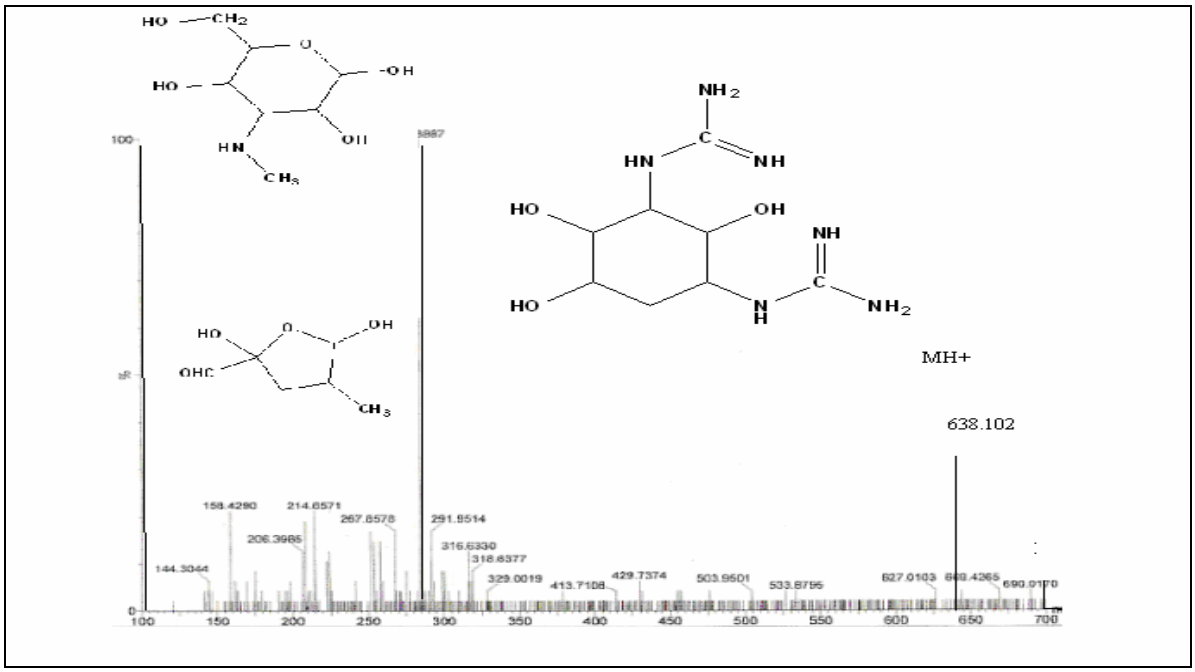

Figure 5a: TOF –Mass spectra of Co-SM complex

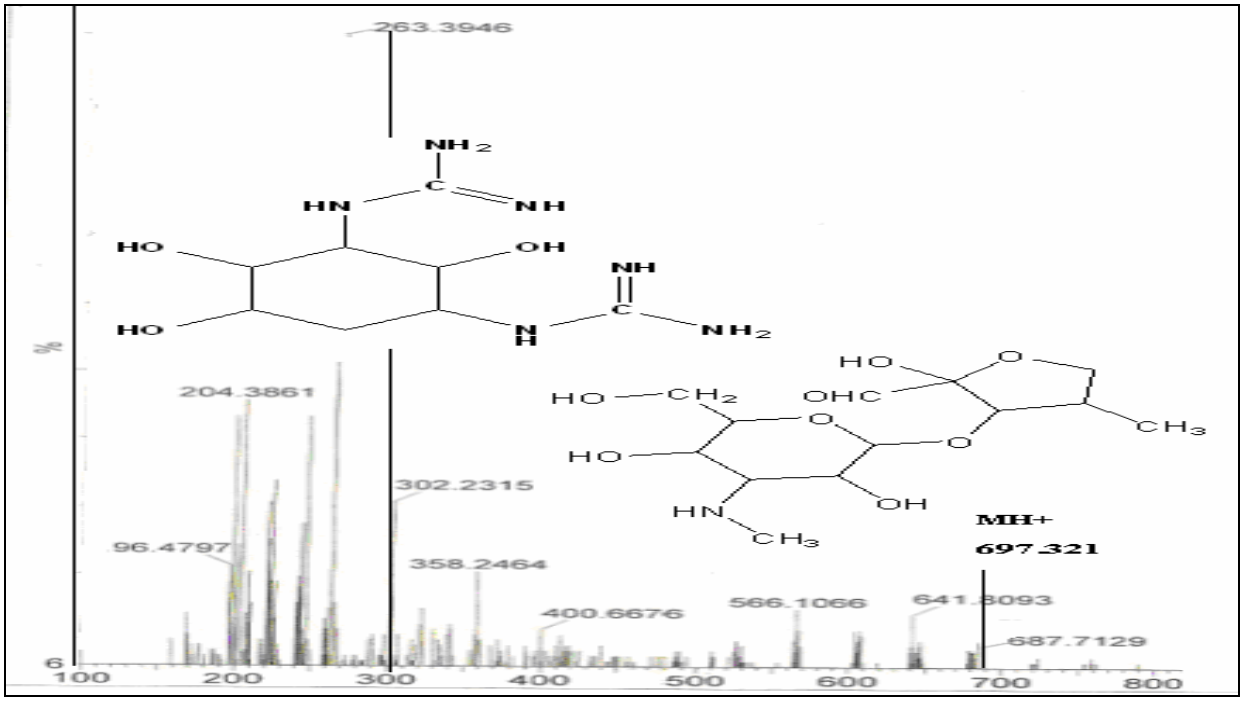

Figure 5b: TOF-Mass of Sn-SM complex

X-ray powder diffraction studies

In absence of single crystal, x-ray powder data is especially useful to deduce accurate cell parameters. The diffraction pattern reveals the crystalline nature of the complex. The indexing procedure were performed using (CCP4, UK) Crysfire programme [24] giving different crystal system with varying space group. The merit of fitness and particle size of the metal (II) complexes has been calculated (Figure 6a-6d) spectra of complexes. The cell dimensions of the complexes are shown in table 5.

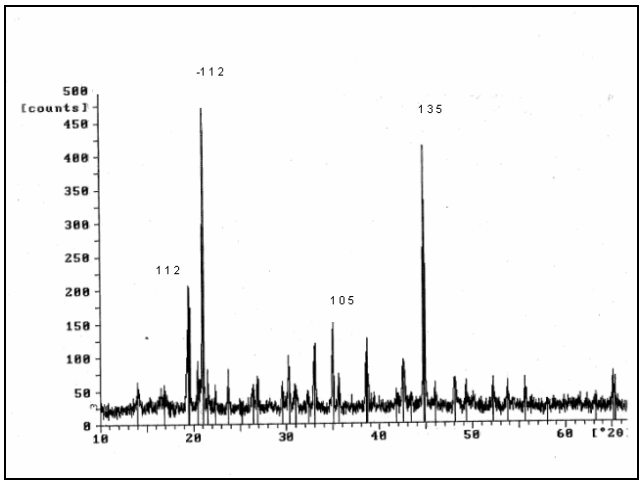

Figure 6a: XRPD spectra of Co-SM complex

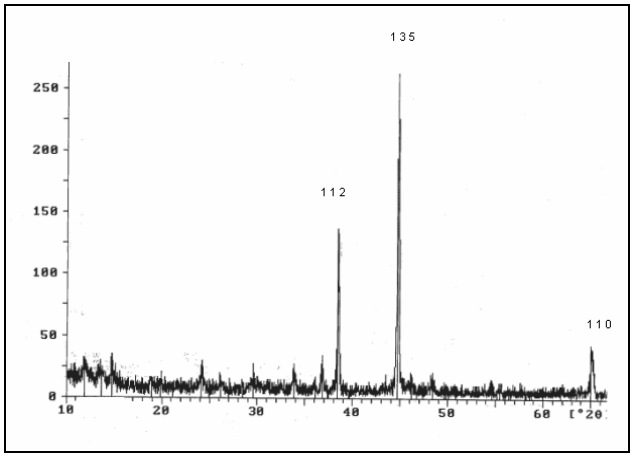

Figure 6b: XRPD spectra of In-SM complex

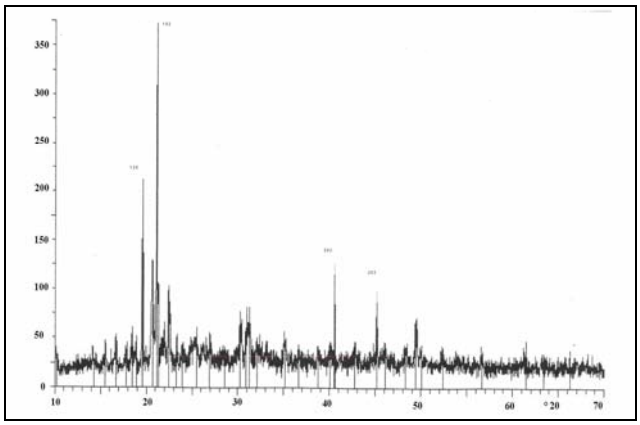

Figure 6c: XRPD spectra of Ni-SM complex

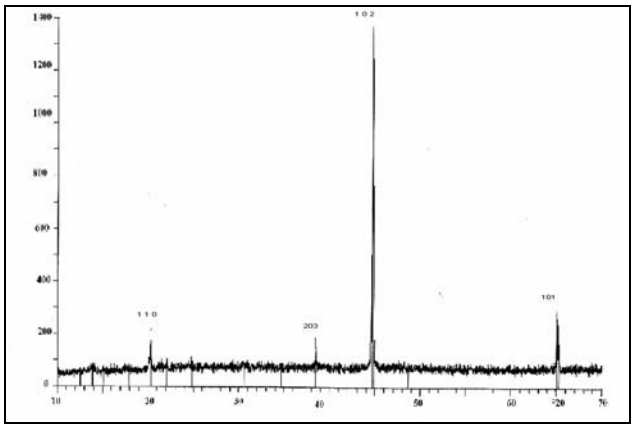

Figure 6d: XRPD spectra of Sn-SM complex

Molecular structures & analysis of bonding modes

In order to ascertain the structural preferences and coordination behavior of streptomycin to metal ions, molecular mechanics calculations on the $[ML]^{n+}$ species were undertaken. The optimized molecular structure of complex 1,2,3 and 4 is given in fig. 7-12. Energy minimization was repeated several times to find the global minimum [25-27]. The energy minimization values for the optimized structure for the complex 1, 2, 3 and complex 4 are 35.12, 25.31, 38.24 and 41.34 kcal/mol respectively. The selected bond length and bond angles of all the complexes are represented in Table 5. The optimised structure of complex 1 is octahedral, complex 2 is trigonal planar, complex 3 is trigonal planar and complex 4 is tetrahedral geometry respectively [25-30].

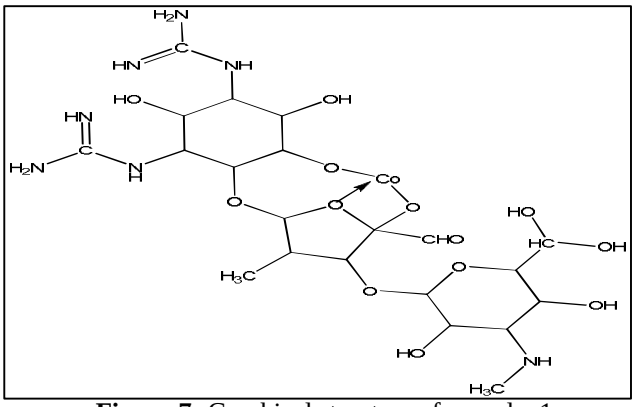

Figure 7: Graphical structure of complex1

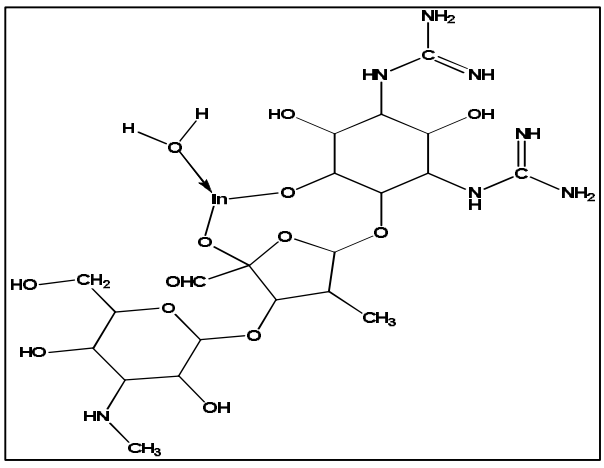

Figure 8: Ggraphical structure of complex3

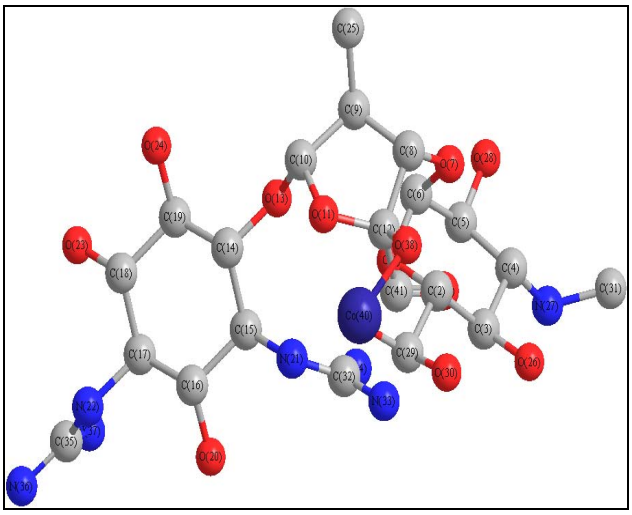

Figure 9: Optimised structure of Co-SM complex (without H-Atoms)

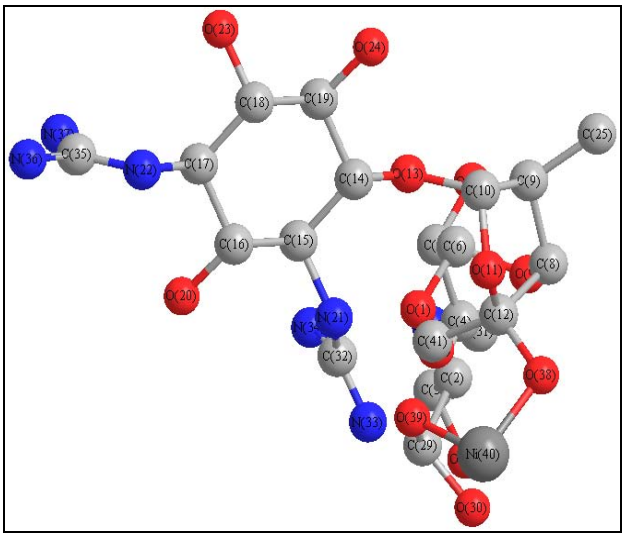

Figure 10: Optimised structure of Ni-SM complex (without H-Atoms)

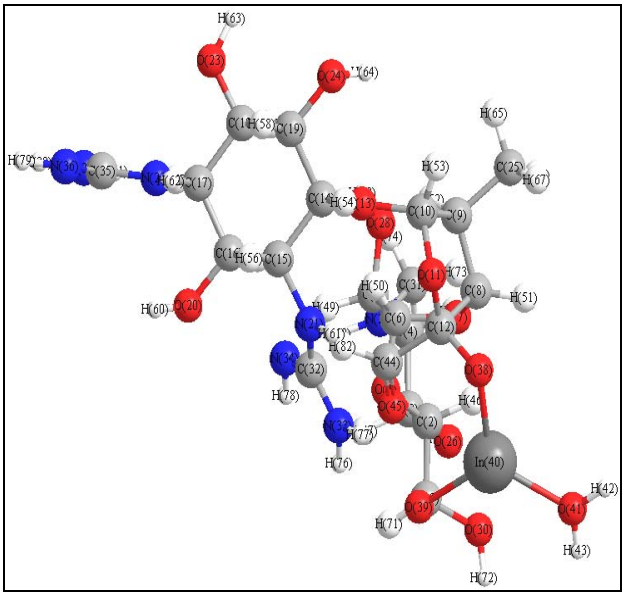

Figure 11: Optimised structure of In-SM complex. (without H-Atoms)

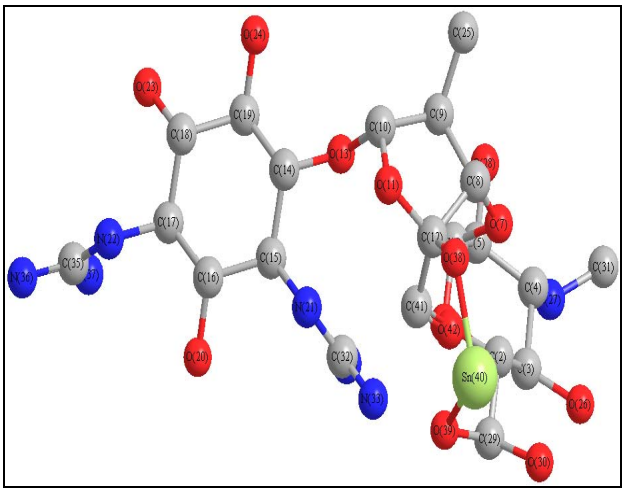

Figure 12: Optimised structure of Sn-SM complex (without H-Atoms)

Antibacterial activities

The antibacterial sensitivity assay shows that there is reduction of inhibitory potentials of antibiotic Streptomycin by the formation of complex with metal. The bacterial strain sensitive to Streptomycin easily grow in the presence of 200 µg/mL of Co-SM, Ni-SM and Sn-SM where as the In-ST was not effective up to 100 µg/mL and less effective on the concentration of 150 µg/mL and more[33-35]. It can be seen in the table 6 and Fig.13 that only 25 µg/mL of Streptomycin showed inhibitory effect on *Agrobacterium sp* BN-2A but the same strain is resistant to all concentrations of Co- SM and Ni-SM. The Bacterial Inhibition Index (BII) of Streptomycin (ST) was highest (4.2 ± 0.245) at the concentration of 200 µg/mL and minimum inhibitory concentration (MIC) was 25 µg/mL where BII was 0.8 ± 0.047. (Table-6)

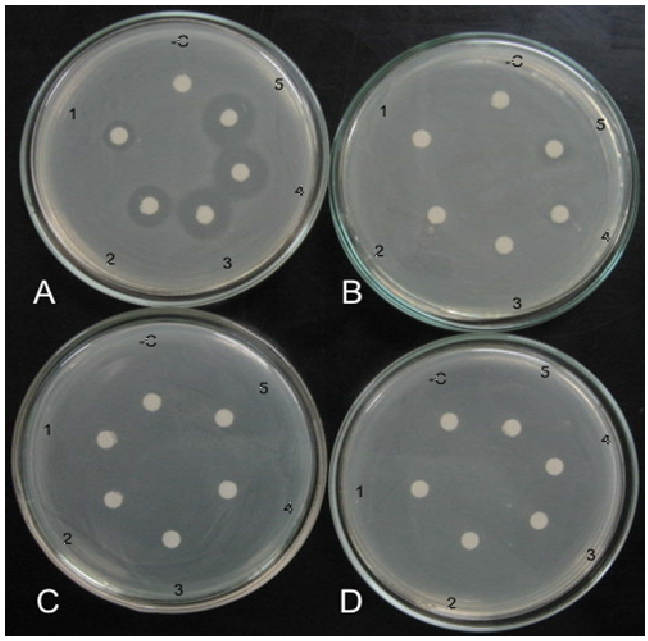

Figure 13: Antibacterial sensitivity assay of metal added antibiotics Streptomycin on *Agrobacterium sp* BN-2A. A, Streptomycin (SM); B, Cobalt-Streptomycin complex (Co-SM); C, Ni-Streptomycin complex (Ni-SM); D, Indiuml-Streptomycin complex (Ni-SM). (-C, 0.0 µg/mL; 1, 25 µg/mL; 2, 50 µg/mL; 3, 100 µg/mL; 4, 150 µg/mL; 5, 200 µg/mL concentration in SM and all metal complexes of SM).

Table 6: Antibacterial sensitivity assay of Streptomycin (SM) and its metal complexes (Co-SM, Ni-SM, Ni-SM and Sn-SM) on *Agrobacterium sp* BN-2A

Concentration (µg/mL)	BII of antibacterial Streptomycin-metal complexes[#]				
	SM	Co-SM	Ni-SM	In -SM	Sn-SM
Control (-)	0 ± 0 (0)	0 ±0.024	0 ± 0 (0)	0 ± 0.01 (0)	0 ± 0.321(0)
25	0.8 ± 0.047 (0.2)	0 ± 0.4	0 ± 0.247	0 ± 0.02 (0)	0 ± 0.245 (0)
50	1.2±0.082 (0.002)	2 ± 0.17	0 ± 0.321	0 ± 0 .0214(0)	0 ± 0.324(0)
100	1.6±0.163 (0.003)	1.5 ± 0.321	0±0.23145 (0.021)	0 ± 0.0214 (0)	0 ± 0.245 (0)
150	2.2±0.245 (0.004)	2.32±0.654	0.6± 0.082 (0.332)	0 ± 0.214 (0)	0 ± 00.214 (0)
200	4.2±0.245 (0.001)	3.2 ± 1.254	1.0± 0.082 (0.169)	0 ± 0 .214(0)	0 ± 0.3547 (0)

Values shown is the average ± standard deviation of three readings performed three times.
[#]value in the parenthesis indicates p-value of the data based on t-test ($p \leq 0.005$).

A number of studies have indicated the antimicrobial activities of many heavy metals due to their effects on iron uptake by bacteria (Bland et al., 2004). Iron is a co-factor for many essential enzymes (Domenico and Reich, 1996). Potent antimicrobial activity of complexes would result out of a combination of higher transport of the complex (1) through the cell membrane and iron limitation into the cells of the bacteria and less effective of complex 3.

CONCLUSION

Transport of organic ligands into bacterial cells can be facilitated by the formation of metal complexes. Hence, metal complexes of streptomycin were synthesized. They were characterized by UV, IR, TGA/DTA, XRPD and structure was optimized Chem Office Ultra-11 programme and elemental analysis. The complex was found to possess metal to ligand ratio of 1:4. It has been observed that complexation between metal ions and streptomycin takes place above pH 7. The Solubility of the complexes was found to be more than that of streptomycin. Agar diffusion method was used for antibacterial activity. The complexes were found to possess better activity (lesser MIC value) than that of streptomycin as well as metal chloride and streptomycin physical admixture. It was concluded that metal complexes can be a better alternative to streptomycin as an antibacterial agent.

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