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Thermal, X-ray diffraction, spectral and antimicrobial activity of bivalent metal (Zn, Cd, Hg, Pb and Sn) chelates of 2-hydroxy-1, 4-naphthoquinone -1, oxime

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Abstract: *The new complexes M-Lm {M= Zn, Cd, Hg, Sn and Pb: Lm = 2-hydroxy -1, 4- naphthoquinone -1, oxime} have been prepared. They were characterized by XRD, IR (FAR and MID), Electronic spectra and investigated by SEM with EDAX analysis. The antimicrobial activity of these metal complexes was tested against Escherichia coli, Bacillus subtilis, Klebsiella pneumonie, Staphylococcus aureus and Candida albicans by disc diffusion method. The results were encouraging and compared with standard Cisplatin complex which was known as chemotherapy agent for various cancer treatments.*

Keywords: *Lawsone monoxime, Metal chelates, IR, NMR, SEM, Antimicrobial activity, Electronic spectra*

I. Introduction:

The effect of monoxime of 2-hydroxy -1, 4- naphthoquinone on muscular work was tested on the rectus abdominis of Rana temporaria in Ringer solution at pH 7.2 (1). Ligand field stabilization energies were presented for metallic domplexes of (copper, nickel, cobalt, zinc, lead, cadmium, manganese and magnesium mono oximates) by R. K. Sharma et. al. (2). The antimicrobial activity of the Ho (III) complexes of C3 substituted 2-hydroxy -1, 4- naphthoquinone-1, oxime by disc diffusion method against *S. aureus, Klebsiella, pneumonie, Salmonella typhimurium, Proteus morganii, Providencia typhimurium and e. coli*. They have reported a variable the antimicrobial activity against all tested microorganisms and S aureus have showed good sensitivity against all metal complexes (3). X-ray diffraction, spectral and antimicrobial activity of bivalent metal (Zn, Cd, Hg, Pb and Sn) chelates of 2-hydroxy 1-4 naphthoquinone -1, oxime have been reported and they carried out antimicrobial activity against *Escherichia coli, Bacillus subtilis, Klebsiella pneumonie, Staphylococcus aureus and Candida albicans* . The results were encouraging. (4). Sonawane (5) studied antifungal screening viz. C*andida albicans, Saccharomyeas arevisiae and Aspergillus niger* with lawsone monoxime. P.T. Kulkarni (6) reported antimicrobial activities of lawsone (Lw) and lawsone monoxime (Lm) as well as zinc lawsonate (Zn-Lw) with dichlone as standard. The spectral data reveal that Lawsone chelate through the O atoms and lawsone monoximes chelate through N and O with Co(III). An octahedral geometry is suggested for the chelates. Preliminary antimicrobial activity studies showed that the activity of the ligands diminishes on chelation (7). This work reports the synthesis of metal chelates, their characterization employing X-ray diffraction, Far and Mid infra red spectra, Electronic spectra, Thermal analysis TG/DTA curves, SEM, EDAX and antimicrobial activity against micro organisms.

Computational details

The calculations of the title compound were carried out with Gaussian 09 mechanics program. Geometry optimization was calculated using RHF / 6-311 G* level of theory. The wave number value computed by HF method and 6-311 G* level contain known systematic errors due to negligence of electron correlation (8). We therefore have used the scaling factor as 0.90 for HF/6-311G* (d p) set.

II. Materials and Methods

Synthesis of 2-hydoxy-1, 4-napthoquinone-1, oxime was carried as per the reported method (7) which was recrystalized using methanol used as it is, supplied by AR grade Thomas Baker chemicals. A stock solution of chlorides of Zn (II), Cd (II), Hg (II) Pb (II) and Sn (II) were prepared by using AR grade chemicals. Deionised water was used during synthesis.

2.1 Preparation of metal chelates.

The chelates were prepared by mixing metal salt solution and ligand in 1:2 for all metals. The mixture was constantly stirred for one hour with magnetic stirrer. The pH of the mixture was maintained between pH 5.0-6.0 by adding ammonia solution to it. Mixture was warmed on water bath for about 15 minutes. On cooling it was filtered and found to be colored.

2.2 Instrumental Analysis.

The IR spectra were recorded on a JASCO FTIR in the region of 4000 to 350 cm^{-1} model in a KBr matrix. Electronic spectra were recorded on JASCO 530 UV-VIS spectrometer, in solid state in KBr matrix and in methanol solution. DTA/TGA curves were recorded on Shimatzu 60H model using 10 C^o / min. heating rate, 800 C^o maximum temperature in air. X-ray diffraction patterns were obtained by using Brucker D8 advanced diffractometer. Elemental analysis was carried out with a Perkin Elmer 2400 series for C, H, O, & N. Scanning electron microscopy was carried out on Vega 2SB model and EDAX on OXFORD INCA PENTA with TECAN VEGA 2SB.

2.3 Bioassay

Test organisms: The antimicrobial activity ligands, metal salt and synthesized metal chelates were examined against bacteria and fungi *Escherichia coli* (NCIM 2065), *Bacillus subtilis* (NICN 2063), *Klebsiella pneumonie* (NCIM 5082), *Staphylococcus aureus* (NCIM 2079) *and Candida albicans* (NICM 3471)] strains collected from NCL Pune, India.

2.4 Maintenance and Culture:

The culture of bacteria and fungi were maintained on Nutrient agar (Himedia Laboratories Pvt. Ltd. Ref. M 002-500G 99% Purity and sub cultured accordingly. These plates were incubated at 35^{o}C for 24 hours in incubator.

2.5 Inoculums preparation:

One loopful growth of bacteria and fungi were transferred in to the 100µL of the organism suspension. Finally 100µL of ligand, metal salt and metal chelates were placed in to each well.

III. Result and discussion

The chelates of lawsone monoxime are stable at room temperature, insoluble in water and protic solvents while soluble in aprotic solvents such as DMF and DMSO.

3.1 Thermal analysis of Lawsone monoxime (Lm) and its chelates

TG / DTA curves of Lm and its chelates are shown in Fig. 1

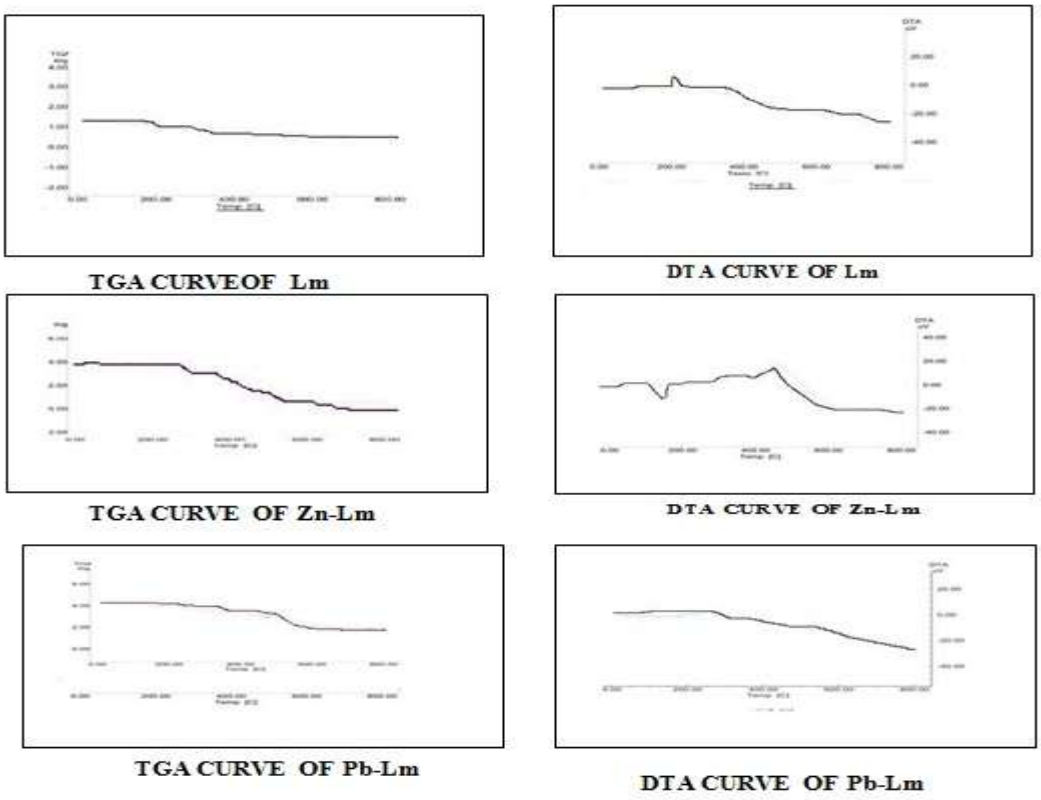


Fig.1 TG /DTA Curves of Lm (Lawsone monoxime) and its metal chelates

The TG curve of Lm shows only one step weight loss in the temperature range 127.01 ^{O}C to 275 ^{O}C up to 21.53 %. Then the weight loss is continuous up to 800 ^{O}C and finally it shows 31.85 % weight loss which corresponds to loss of NO2 moiety. The probable mechanism can be given as

$C_{10}H_7NO_3 \longrightarrow C_{10}H_7O_2 + NO$ Step I

$C_{10}H_7O_2 \longrightarrow 7C$ Step II

The TG curve of Zn-Lm shows two step weight loss in which first step is starting at 110.71 ^{O}C and ends at 304.13 ^{O}C with weight loss up to 16.49 % and second step starts at 335.22 ^{O}C & ends at 626.75 ^{O}C with weight loss up to 63.33 %. The first step is due to loss of 4 water molecules and the second step for the decomposition of Lm. Finally it gives ZnO and some carboneous matter.

$Zn(C_{10}H_5NO_3)_2.4H_2O$ --------- $Zn(C_{10}H_5NO_3)_2$ ---------- ZnO + C

The TG curve of Pb-Lm shows two step weight loss in which first step is starting at 37.63 ^{O}C and ends at 284.07 ^{O}C with weight loss 14.87 % and the second step starts at 473.03 ^{O}C which ends at 654.13 ^{O}C with weight loss 23.036 %. The first step is due to partial decomposition of Lm and in the second step it decomposes completely leaving residue as PbO and some carbon ash. The probable mechanism can be given as

$Pb(C_{10}H_7NO_3)_2$--------- Pb ($C_{10}O$) ---------- PbO + C

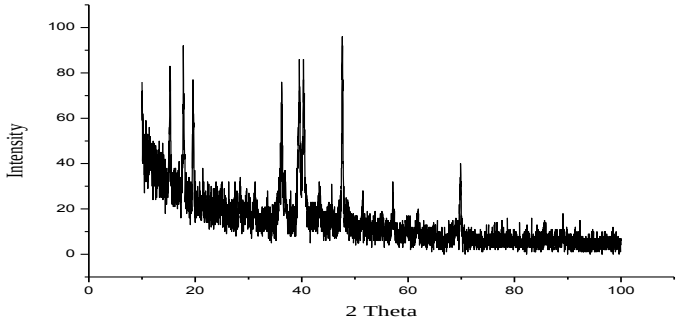
DTA of Lm shows only one exotherm in the temperature range of 192.23 ^{O}C to 226. 80^{O}C with peak temperature at 203.89 ^{O}C. This can attributed to the partial decomposition of Lm which loses NO moiety only.

DTA of Zn-Lm shows only one Endotherm which starts at 212.41 ^{O}C and ends at 160.3 ^{O}C. This is due to dehydration process in which it loses 4 molecules of water.

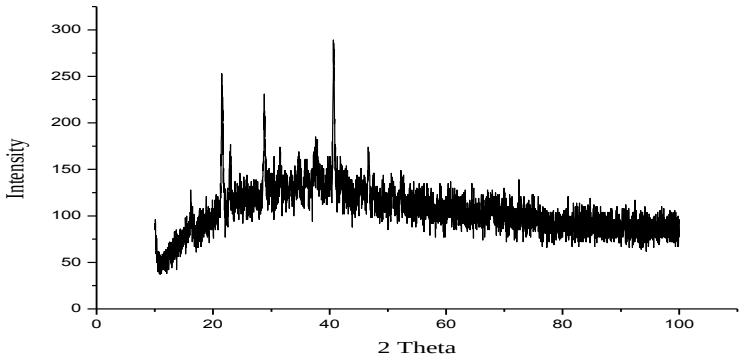
DTA of Pb-Lm shows a broad exothermic peak which starts at 192.44 ^{O}C and ends at 290.1 ^{O}C. Then it does not any observable peak.

3.2 XRD Studies of Lawsone monoxime (Lm) and Its chelates of Zinc and Lead

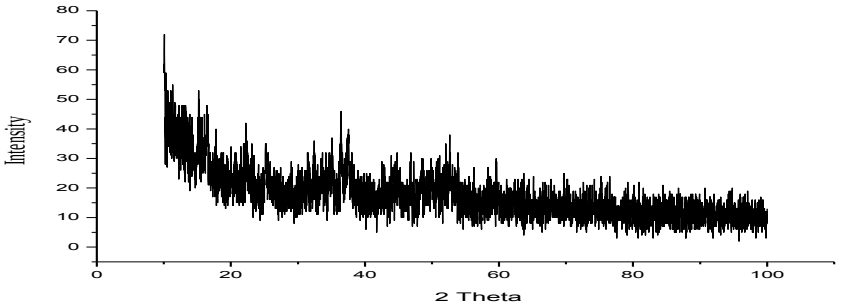
X-ray diffraction patters of Lm. Zn-Lm and Pb-Lm in air are given in Fig. 2



1. Lm



2. Zn-Lm



3. Pb-Lm

Fig.2 XRD Patterns of 1) Lm, 2) Zn-Lm and 3) Pb-Lm

Lm crystallizes in the triclinic group and it has crystallographic parameters,
a = 14.054 A^o, b = 6.450 A^o and c = 7.8378 A^o
$\alpha = 101.80^o$, $\beta = 76.598^o$, $\gamma = 83.800^o$,
Its volume is 669.576 $(A^o)^3$ and space group H-M symbol P1.
Dmin = 1.978477 g/cm^3
The values for miller indices and observed 2 θ for Lm are given in Table-2

Table 2: Miller indices and observed 2 θ for Lawsone monoxime (Lm)

Sr.No.	H	K	L	Observed2θ
1	0	0	1	17.765
2	2	0	0	19.535
3	1	-1	0	24.720
4	2	0	2	36.235
5	3	1	-1	39.610
6	1	1	-2	40.330
7	3	1	2	47.650
8	5	1	0	51.480
9	4	0	3	59.725
10	4	0	-2	61.860
11	3	3	-1	69.900

Zinc Lawsone monoximate (Zn-Lm) crystallizes in the triclinic group and it has crystallographic parameters,
a = 8.0278 A^o, b = 15.6668 A^o and c = 6.6162 A^o
$\alpha = 63.241^o$, $\beta = 111.885^o$, $\gamma = 92.600^o$
Its volume is 683.119 $(A^o)^3$ and space group H-M symbol P1.
Dmin = 2.041 g/cm^3
The values for miller indices and observed 2 θ are given in Table-3.

Table-3: Data for h k l and 2 θ values for Zinc Lawsone monoximate (Zn-Lm)

Sr. No.	h	k	l	TH(OBS)	TH-ZERO	TH(CALC)	DIFF.
1	1	-1	0	21.475	21.507	21.508	-0.002
2	0	2	1	22.855	22.887	22.886	0.001
3	1	1	-1	28.810	28.842	28.842	0.000
4	0	4	1	34.700	34.732	34.730	0.002
5	0	2	-1	37.615	37.647	37.649	-0.002
6	1	-1	1	40.715	40.747	40.744	0.002
7	1	-1	-2	41.950	41.982	41.980	0.001
8	0	4	2	46.720	46.752	46.754	-0.002

Lead Lawsonate monoximate (Pb-Lm) crystallizes in the triclinic group and it has crystallographic parameters,
a = 8.4111 A^o, b = 6.1028 A^o and c = 13.3496 A^o
$\alpha = 110.443$, $\beta = 64.662$, $\gamma = 98.794$,
Its volume is 580.323 $(A^o)^3$ and space group H-M symbol P1.

Dmin = 1.9686 g/cm^3

The values for miller indices and observed 2 θ are given in Table-4.

Table-4: Data for h k l and 2 θ values for Lead Lawsone monoximate (Pb-Lm)

Sr. No.	h	k	L	TH(OBS)	TH-ZERO	TH(CALC)	DIFF.
1	1	0	1	16.450	16.451	16.465	-0.013
2	0	1	-1	22.285	22.286	22.285	0.001
3	1	-1	1	25.210	25.211	25.201	0.001
4	1	1	1	31.475	31.476	31.465	0.011
5	1	-1	3	32.405	32.406	32.402	0.004
6	0	0	3	34.940	34.941	34.945	-0.004
7	1	-1	-1	36.385	36.386	36.383	0.003
8	2	-1	1	37.480	37.481	37.483	-0.002
9	0	2	-1	44.715	44.716	44.729	-0.003
10	0	1	3	48.450	48.451	48.452	0.000
11	1	2	-2	52.700	52.701	52.698	0.003

3.3 Electronic spectra of Lawsone monoxime (Lm) and its metal chelates

The electronic spectra of Lm and its metal chelates were determined in solid state in KBr matrix and in methanol. The data is given in Table-5. In solid state, the first band for Lm is observed at 252 nm whereas in solution state it is at 250 nm which is due to BET. The second band observed at 308nm and 342nm in solid state and in solution state respectively which is due to QET. The third band is observed at 414nm in solid state whereas in solution this band is not observed. It is attributed to $n\rightarrow\pi^*$ transition.

In case of solid state Zn–Lm & Sn-Lm the band is observed at 247 nm for BET which shows hypsochromic effect. In case of Cd-Lm, Hg-Lm & Pb-Lm the bands are observed at 254,267and 288 nm respectively for BET which shows bathochromic effect. In solution they show hypsochromic effect except for Sn-Lm. In case of solid state QET bands exhibit hypsochromic effect for Zn-Lm and Hg-Lm while the bathochromic effect is observed for Cd-Lm, Sn-Lm and Pb-Lm. In case of solution Sn-Lm and Pb-Lm exhibits hypsochromic effect. The third band of $n\rightarrow\pi^*$ transition shows bathochromic effect for all the chelates in solid state while in solution only Zn-Lm and Cd-Lm shows hypsochromic effect.

Table-5 Electronic Spectra (UV-VIS) of Lm and Its metal chelates

Sr. No.	**Compound**	**Principle Band Wavelength in nm**					
		BET		**QET**		**$n\rightarrow\pi^*$**	
		SOLID	SOLUTION	SOLID	SOLUTION	SOLID	SOLUTION
1	Lm	252	250	308	342	414	-
2	Zn-Lm	247	248	307	-	420	406
3	Cd-Lm	254	248	351	-	425	406
4	Hg-Lm	267	236	307	-	438	-
5	Sn-Lm	247	252	356	312	448	-
6	Pb-Lm	288	236	343	342	445	-

3.4 Infra red spectra :

The IR spectra were recorded on a JASCO FTIR in the region of 4000 to 350 cm^{-1} model in a KBr matrix and the data is given in Table-6.

Table-6: Characteristics IR (cm-1) Bands of Lm and its metal chelates

Sr. No.	Comp.	C=O	C=O	C – O	ν_s (M-O)	ν_{as} (M-O)	N – O	C = N
1.	Lm	1629	-	1212	--	--	1050	1552
2.	Zn-Lm	1589		1139	489	373	1093	1553
3.	Cd-Lm	1587	-	1255	531	368	1059	1546
4.	Hg-Lm	1584	-	1256	537	369	996	1526
5.	Sn-Lm	1677	1592	1119	517	303	-	-
6.	Pb-Lm	1654	1586	1161	530	387	1038	1548

In comparison with Lm spectrum, the spectra of the metal chelates displayed bands ranging between v(C-O) group (9, 10) and confirming the coordination through the phenolic oxygen. The other frequencies of C=O are shown common in all chelates. The main peak for OH from ligand is not observed in chelates. In the region 1700-1200 cm-1, all the chelate showed a number of intensive bands, which are due to normal modes of vibrations of ligand affected by its coordination. The v(C=O) stretching frequency of coordinated carbonyl group is similar that of ligand. All the chelates exhibits v(C=O) band at lower energy. It can be explained on the basis of mono anionic nature of the ligand which acts as a ring with bidendate ligand. In results form a ring with metal ion. A considerable delocalization of the π electron density exhibits such a strong ring formation. This can be explained on the basis of absorption bands by the chelate. The most important region is v 600-300 cm-1 exhibits several absorptions are sensitive to the central metal ion and the structure of the chelate and can be attributed to the normal mode of vibrations of the MO_4 moiety.

Generally the ligand to metal ratio is 1:2 and they have D_2h symmetry and band can be assigned in the region of v 590-420 cm-1 to the ν_{sym} (MO) stretching vibration species B_lu and the other band in the v390- 320 cm-1 to the ν_{asym} (MO) stretching to B_2h. These two vibrations are sensitive to the nature to the central metal ion.

3.5 Metal-Organic Frameworks

Crystalline porous coordination polymers (PCPs), also called Metal-Organic Frameworks (MOFs), are a fascinating class of solid-state inorganic-organic hybrid materials. Research in these compounds is expanding very rapidly owing to their exciting combination of properties for advanced functional materials in gas storage and gas separation, catalysis, chemical sensing, as well as medical applications. Fig 3 shows SEM of 1) Lm b) Zn-Lm 3) Cd-Lm 4) Hg-Lm 5) Sn-Lm 6) Pb-Lm

The crystallite size of chelates is found to be nano range as 53.8, 52.44 and 34.75 nm for Lm, Zn-Lm and Pb-Lm respectively which is calculated using XRD data and Scherrer formula. This difference was attributed to the higher structural defects of the usual material as compared with the coordination modulation nano-MOF. Nevertheless, direct evidence of coordinated oxime groups during crystallization in the framework as a powder has not been reported. Even more generally, the fabrication and characterization of well-defined, stable self-assembled mono layers (SAMs) of organic ligands at a crystal face of a MOF has not been documented to date.

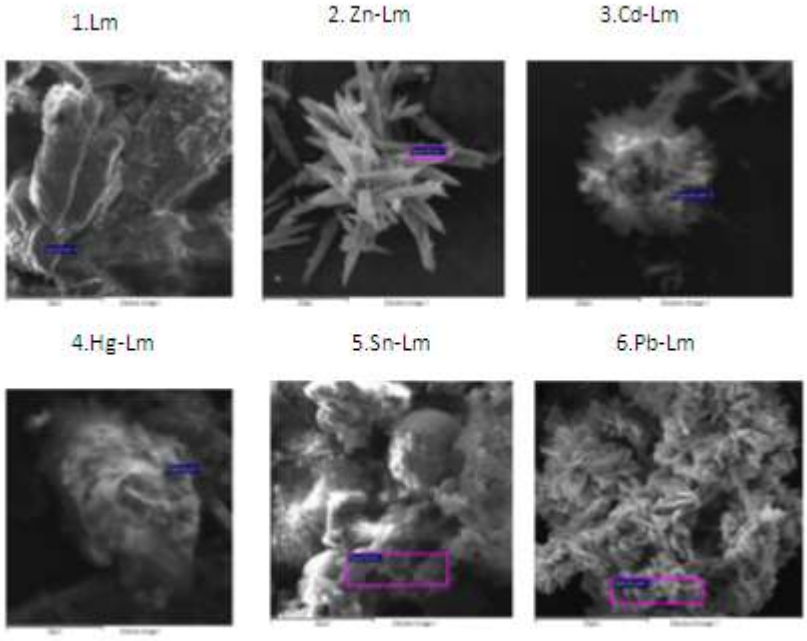


Fig: 3 SEM PHOTOGRAPHS OF LIGAND AND METAL CHELATES

SEM image of Lm shows plates made of various crystals which is nothing but polymerization of crystals through hydrogen bonding. Zn-Lm image clearly shows a needle shaped formation of bulk crystals which are belonging to nano size materials.

The EDAX data is obtained for elemental analysis and the calculated values are presented in Table-7.

It is observed that this can be a qualitative analysis of the metal chelates.

Table 7: Results of EDAX analysis with calculated data

Sr.No.	Compound	Method	% C	% O	% H	% Metal
1	Lm	Obsd.	50.81	15.11	--	34.07
		Cald.	63.49	25.41	3.70	7.40
2	Zn-Lm	Obsd.	51.31	19.79	--	18.23
		Cald.	54.42	21.73	2.78	6.34
3	Cd-Lm	Obsd.	45.96	39.53	--	9.45
		Cald.	49.18	19.67	2.46	5.74
4	Hg-Lm	Obsd.	55.94	18.44	--	20.22
		Cald.	41.66	16.68	2.08	4.86
5	Sn-Lm	Obsd.	35.48	40.13	--	19.48
		Cald.	48.58	19.45	2.42	5.67
6	Pb-Lm	Obsd.	51.48	16.54	--	29.99
		Cald.	41.16	16.49	2.05	4.80

3.6 Antimicrobial activity studies

Antimicrobial Scanning Results The Lm ligand and its metal chelates are screened for their antimicrobial activities against *Escherichia coli, Bacillus subtilis, Candida albicans, Staphylococcus aureus* and *Klebsiella pneumonie*. The testing against growth of micro-organisms was carried out by using well diffusion method employing Mueller Hinton Agar (MAH) and culture in nutrient broth in each case of micro-organisms. The concentration of ligand Lm and its metal chelates were chosen as 10^{-4}M. the plates were incubated at 35^{o}C for 24 hours in incubator. The clear zone of inhibition of growth for the organism was measured in mm and the data is given in Table-8. Dimethyl sulphoxide i.e. solvent used shows no inhibition for all organisms under studies.

Table: 8 Antimicrobial activities of 2-hydroxy -1, 4- naphthoquinone-1, oxime (Lm) and its metal chelates (inhibition zone diameter in mm)

	E.coli		*Bacillus subtilis*		*Candida albicans*		*K. pneumoniae*		*S. aureus*	
Sample	Zone mm	Area in mm2	Zone mm	Area in mm2	Zone mm2	Area in mm2	Zone mm2	Area in mm2	Zone mm	Area in mm2
DMSO	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Cisplatin	18	254.	13	132.6	Nil	Nil	--	--	20	314
Lm	Nil	Nil	17	226.86	Nil	Nil	13	132.6	12	113.0
Zn-Lm	Nil	Nil	16	200.96	Nil	Nil	Nil	Nil	24	452.1
Cd-Lm	11	94.98	16	200.96	20	314	21	346.18	28	615.44
Hg-Lm	24	452.16	26	530.66	19	283.38	20	314	30	706.5
Sn-Lm	Nil	Nil	14	153.86	14	153.86	Nil	Nil	Nil	Nil
Pb-Lm	Nil	Nil	10	78.5	Nil	Nil	17	226.8	15	176.62

Cisplatin is a standard.

All the metal chelates showed microbial activity against all organisms studied in this work. Hg-Lm showed good activity against all organisms and showed highest activity for S. aureus. The inhibition of the micro-organisms growth for metal chelate was found to be in the following order for *S. sublitis*.

Hg-Lm > Zn-Lm = Cd-Lm > Sn-Lm > Cisplatin > Pb-Lm

The studies demonstrate that metal chelation can increase the antimicrobial activity than metal free ligand. It is responding that metal chelation reduce the polarity of the metal ion mainly due to partial sharing of its positive change with the donor group and possibly the δ electron delocalization occurring within the whole chelate ring system formed during co-ordination and results in increase of the lipophilic nature of the central metal atom (11). It favors for its penetration through the lipoid layer of the membrane. The transition metal

chelates possess high degree of inhibition which can be due to the greater number of δ electrons which increase the electrostatic field around the metal ion.

IV Conclusions:

The metal chelates are thermally stable up to 500^{o}C which is a unique characteristic property. All these chelates are crystalline in nature and generally belong to triclinic. The coordination ability of ligand Lm towards M (II) chelates were examined by different spectroscopic methods that unequivocally determine the coordination sites of ligands Lm. It is observed that the ratio of the metal chelates is 1:2 for chelates of Zn, Cd, Hg, Sn and Pb. Biological activity screening proved the good antimicrobial activity of ligand Lm and its metal chelates. The antimicrobial activity explored on the basis of overtone concept of cell permeability. The antimicrobial activity of all metal chelates is compared with standard Cisplatin complex and the results are encouraging.

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References

[1] J. Lecomte and P. Fischer, Physiological properties of quinones and of oxidative derivatives of adrenaline, Archives Internationales de Physiologie 56, 35 - 44 (1048).
[2] R. K. Sharma, N.S. Jha and S. K. A study of chelation behavior of bivalent metal complexes of biologically active 2- hydroxyl-1,4-naphthoquinone monoxime (HNQM) in dioxane-water mixtures, Sindhwani, Thermochimica Acta., 156(2), 267- 73 (1989).
[3] S.B. Jagtap, S.G. Joshi, G.M. Litake, V.S. Ghole and B. A. Kulkarni, C3 substituted lawsonmonoximates of holmium (III): Synthesis, characterization and antimicrobial activity, Metal Based Drugs., 7(30, 147-150 (2000).
[4] A. B. Pawar, A. A. Killedar, K. D. Jadhav and R. G. Sarawadekar, X-ray diffraction, spectral and antimicrobial activity of bivalent metal (Zn, Cd, Hg, Pb and Sn) chelates of 2-hydroxy 1-4 naphthoquinone
[5] International Journal of Chem. Tech. Research, Vol.4, No.3, pp 882-890, July-Sept 2012.
[6] N.J. Sonwane, Ph.D. Thesis, University of Poona (1981).
[7] C.R. Joshi, Ph.D. Thesis, University of Poona (1975).
[8] V. D. Kelkar, R.R. Gokhale, and H. R. Gholap, Synthesis, characterization and antimicrobial studies of cobalt (III) chelates of some Lawson derivatives. Synthesis and Reactivity in Inorganic and Metal Organic Chemistry, 28(8), 1253-1264 (1998).
[9] J.B. Foreman, E. Frish, in Frish, E. (Ed.), Exploring Chemistry with Electronic Structure Methods, A Guide to using Gaussian, Gaussian, Pittsburg PA (1996).
[10] Gupta K.C. H.K. Abdulkadir, S. Chand,Metallation of tetra dentate N2O2 Schiff base with Mn (II), Co(II), Cu(II) and Zn(II):synthesis, characterization and formation constants measurement, *J. Molecular catalysis A: Chemical*. 202, 253 (2003).
[11] El-Bindary A.A. Spectral studies on metalligand bonding in complexes of 1-acetyl-2-(coumariniminecarboxamide-3-yl)hydrazine ,*Spectrochimica Acta., Part A*, 57 49 (2001).
[12] Z.H. Chohan, C. T. Supuran and A. Scozzafava, Unsymmetrical 1,1′-disubstituted Ferrocenes: Synthesis of Co(ii), Cu(ii), Ni(ii) and Zn(ii) Chelates of Ferrocenyl -1-thiadiazolo-1′-tetrazole, -1-thiadiazolo-1′-triazole and -1-tetrazolo-1′-triazole with Antimicrobial Properties, *J. Enzyme Inhihitian and Medicinal chemistry*, 20, 303 (2005).