IOSR Journal of Pharmacy
e-ISSN: 2250-3013, p-ISSN:2319-4219 www.iosrphr.org
Volume 3, Issue (January 2013), PP.10-17

Thermal, X-Ray Diffraction, Spectral and Antimicrobial Activity of Bivalent Metal (Zn, Cd, Hg, Pb and Ag) Chelates Of 1, 2 Naphthoquinone – 2, Oxime,

[1]N. R. Gonewar, [2]V. B. Jadhav, [3]S.S. Sakure, [4]K. D. Jadhav and [5]R. G. Sarawadekar

1,2,4,5 Bharati Vidyapeeth Deemed University, Pune (India)Yashwantrao Mohite College, Pune 411 038
[4]S.B.B. alias Appasaheb Jedhe College, Shukrawar Peth, Pune 411002.

Abstract:*Five metal chelates of the type M [NQO]* $_2$ *where M = Hg, Cd, Zn, Pb: NQO = 1, 2 napthoquinone – 2, oxime and Ag (NQO) have been synthesized. All chelates have been characterized by modern methods such as elemental analysis, FTIR, Electronic spectra.* ^{1}H *&* ^{13}C *NMR, Thermogravimetry, Differential scanning calorimetry and electron microscopy with EDAX analysis of chelates were carried out. All chelates are found to be coloured and mercury, lead, zinc and cadmium chelates are octahedral while silver chelate has been assigned square planer. These chelates are thermally stable up to* $700^{0}C$ *and all are crystalline in nature. Their particle sizes are in the range of 30-50 nm. The ligand and the metal chelates have been screened for antimicrobial activity on gram positive and gram negative bacteria and fungi and the results are compared with cisplatin as standard.*

Keywords: *1-2 naphthoquinone -2, oxime, X-ray diffraction, IR, NMR, SEM, Antimicrobial activity, Electronic spectra*

I. Introduction:

Al, Zn, Cu and Ni complexes of 1-2-naphthoquinone-2, oxime were synthesized. According to the results of infra red, proton NMR and carbon 13 NMR spectral data, all the complexes in the solid state exists in the quinone oxime form. The authors concluded that the color of the quinone oxime complexes was not related to a quinone oxime or nitrosophenol structure (1). The Fe (III) complex of 1-2-naphthoquinone-2, oxime have been reported and its IR spectra were explained along with electronic spectra (2). The stability constants of the metal chelates of 1-2-naphthoquinone-2, oxime with Mn, Co, Ni, Cu, Zn, VO (II) and UO2 (II) were determined. The stability of the metal chelates was assigned to the fact that the oxygen of a resonating have better basic centre (3). The complexes M (NQO) 2 where M= Mn, Fe, Co, Ni, Cu, Zn, Cd, and Hg: NQO = 1-2-naphthoquinone-2, oxime have been synthesized and their infra red absorption frequencies and electronic transitions have been reported by S. Gurrieri and S. Siracus (4). The present paper describes the synthesis, characterization such as XRD, IR, Electronic spectra, NMR, SEM, TG, DSC and antimicrobial activity of metal chelates of 1,2-naphthoquinone-2,oxime (NQO) with metals like Hg (II), Pb (II), Cd (II) Ag (I) and Zn (II).

II. Materials And Methods

The ligand 1, 2-naphthoquinone-2-oxime or 2-nitroso-1, naphthol is used as it is supplied by AR grade Thomas Baker chemicals. A stock solution of Pb (II), Zn (II), Cd (I), Hg (II) and Ag (I) is prepared by using AR grade chemicals. Deionised water is used during synthesis.

2.1 Preparation of Metal Chelates.

The chelates were prepared by mixing metal salt solution and ligand in 1: 1 proportion for silver chelate and 1: 2 for other metals. The mixture was constantly stirred for one hour on magnetic stirrer. The pH of the mixture was maintained, in between 5.0 – 6.0 by adding ammonia solution to it. Warm the mixture on water bath for about 15 minutes. On cooling it was filtered and found to be coloured.

2.2 Instrumental Analysis.

Elemental analysis is carried out with a Perkin Elmer 2400 series for C, H, O & N. The IR spectra are recorded on a Shimadzu FTIR 8400 S model in a KBr matrix. Electronic spectra are recorded on Perkin Elmer UV – visible spectrometer, Lambda-25 model. TGA curves are recorded on Perkin Elmer Pyris. TGA were

recorded on apparatus using 10^0C/min in air and DSC on DSC 800 model. The proton and ^{13}C NMR spectra recorded in DMSO dueterated on Varion. 400 MR powder x ray diffraction patterns are obtained by using Rigaku Miniflex (II). LC – MS scans is carried out on Shimadzu – LCMS 2010 employing electron impact source. Scanning electron microscopy was carried out on Vega 2SB model and EDAX on OXFORD INCA PENTA with TECAN VEGA 2SB.

2.3 Antimicrobial Activity Testing

Test organisms: The antimicrobial activity of ligands, metal salts and synthesized metal chelates is tasted against bacteria [Escherichia coli (NCIM 2065), Bacillus subtilis (NCIM 2063), Staphylococcus aureus (NCIM 2079), Proteus Vulgaris (NCIM 2813), P. aeruginosa
(NCIM 2200), Aspergillius Niger (NCIM 1196) and Candida albicans (NCIM 3471)] strains collected from NCL, Pune India.

2.4 Maintenance of Culture:

The cultures of bacteria and fungi were maintained on Nutrient agent (Hymenia Laboratories Pvt Ltd. Ref. M 002-500G 99% Purity), Mueller-Hinton Agar (Himedia Laboratories Pvt. Ltd Ref. M 173 – 500G, 99% Purity) and subcultured accordingly and preserved at 4^o C. for 24 hours in incubator.

2.5 Plating

The 100 µL cell suspension (108 cell / ml of bacteria & yeasts C. albicans and 100 µL of spore suspension of mold (A niger) were spread on then. Agar (for bacteria) and Mueller-Hinton Agar for fungi were used. Then wells were bored in the media. In the wells DMSO (solvent), ligand, metal salts and metal chelates solutions were poured for each organism, and then incubated at 37^0C for 48 hrs. for bacteria and 30^0C for 5 days for fungi . The zone diameter of inhibition were measured in mm & recorded.

III. Results And Discussion

3.1 Thermal Analysis:

The TG, and DTG data is given in Table 1. The ligand 2-nitroso-1-naphthol or 1, 2 naphthoquinone-2 oxime (NQO) is heated with 10^0C/min in air up to 380^0C and DSC was carried out by heating 10^0C/min from 30^0C to 200^0C in N_2. The TG data of NQO shows first weight loss corresponds to the loss of (N-OH) which is in good agreement in the range of 114-245^0C (16.74% and found as 19.17%). It indicates that the ligand has high chemical stability. DSC data of NQO shows one exotherm. The onset temperature at 146.87^0C and the peak temperature at 152.25^0C and ΔH found to be 332.93 J/g. It can be assigned to loss of N-OH moiety. TG data of metal chelates of Pb, Cd, Zn and Hg show more or equal results for the first step decomposition about 100 -350^0C and weight loss in range of 25 to 35 % and DTG temperatures are in the range of 152 ± 1^0C. Ag (NQO) shows weight loss in the temperature range 146 – 350 ^{0}C upto 22 % and DTG temperature is at 163^0C. DSC data of metal chelates shows that the chelates containing Zn, Hg and Cd exhibits an exotherm starting at about 146^0C and peak temperatures about at 151 ± 2^0C. There is a variation of the absorption of heat as it can be seen from the Table 2. In the case of Ag (NQO) it shows an exotherm with onset temperature at 153.55^0C and peak temperature at 164.38^0C. The enthalpy of the process is recorded as -305.69J/g. Pb (NQO) 2 shows an exotherm which starts at 187.64^0C and peak temperature at 190.76^0C. The heat of decomposition is 130.80 J/g.

Table: 1 Thermo analytical data of ligand and its chelates

Sr.No.	Compound	Temp Range ^{0}C	% loss	DTG ^{0}C
1	NQO	114-150 150-253	10.82 8.35	134 189
2	Zn (NQO)2	95-350	30.0	152
3	Cd (NQO)2	100-350	27.2	151
4	Hg (NQO)2	101-355	35.0	153
5	Ag(NQO)	146-350	22.0	163
6	Pb (NQO)2	110-350	25.0	--

Table: 2 Differential Scanning Calorimetric data of ligand and its chelates

Sr.No.	Compound	DSC EXO		ΔH
		Onset Temp.	Peak Temp.	J/g
1	NQO	146.87	152.25	-332.93
2	Zn (NQO)2	149.64	153.30	-134.29
3	Cd (NQO)2	147.82	152.09	-124.77
4	Hg (NQO)2	146.20	150.75	-139.06
5	Ag(NQO)	153.55	164.38	-305.69
6	Pb (NQO)2	187.64	190.76	-130.80

3.1.1 Kinetics

To calculate kinetic parameters of 1, 2-naphthoquinone-2, oxime a separate TG/DTG was run on Shimadzu STA in the ranges 0.0^0C to 800^0C in air. The first step decomposition started at 114.8^0C and ended at 150.09^0C. The observed weight loss was 10.82%. The second step decomposition started at 150^0C and ended at 253^0C. The observed weight loss was 8.35%. The TG data was used to calculate kinetic parameters by using developed software (5). The kinetic parameters for F-5 model calculated using Coates _ Redfern equation which is random nucleation (Mampel unimolecular law). The results are given in Table -3.

Table: 3 Kinetic parameters for decomposition of NQO in air

Sr. No.	Parameter	Step I 114.8 to 150.09 ^{0}C	Step II 150 to 253 ^{0}C
1	n Order of reaction	1	1
2	E Energy of activation	51.46 KJ/Mol	36.83 KJ/Mol
3	R regression coefficient	0.904	0.879
4	Log A Frequency factor	2.9096	1.7101
5	S Entropy of activation	-200.87 J/mol/K	-222.31 J/mol/K
6	G Free energy of activation	190.93 KJ/Mol	186.76 KJ/Mol
7	H Enthalpy of activation	105.70 KJ/Mol	77.5674 KJ/Mol

3.2 X-Ray Diffraction:

X-ray diffraction patterns were obtained using Rigaku X-ray diffractometer (CuK_α radiation) Miniflex 2 and the data such as d values in A^0 and ratio I/I_0values are given in Table – 3 for 1,2 naphthoquinone-2,oxime.

Table: 4 XRD data of 1, 2 naphthoquinone-2, oxime or 2-nitroso-1 naphthol (NQO)

Sr. No.	d A^o	I/I_O
1	10.3696	31
2	9.2437	14
3	8.3547	29
4	7.34347	68
5	6.9974	15
6	6.5342	29
7	6.0787	100
8	5.0693	14
9	4.2630	19
10	4.1674	22
11	3.9004	23
12	3.6747	74
13	3.4268	90
14	3.2996	77
15	3.1773	21
16	3.1016	45
17	3.0075	24
18	2.9247	17
19	2.8590	19

It is observed that all these chelates are crystallite in nature. The data was processed by using McMaille computer program for determination of cell parameters and space group (6). 1, 2 naphthoquinone-2, oxime crystallizes in the triclinic group and it has crystallographic parameters,

$a = 12.0951\ A^o$, $b = 6.1556\ A^o$ and $c = 11.4016\ A^o$

$\alpha = 65.964^o$, $\beta = 81.864^o$, $\gamma = 87.090^o$,

Its volume is 766.887 $(A^o)^3$ and space group H-M symbol P1.

Dmin = 3.279487 g/cm^3

The metal chelate of Zn (NQO)2, shows data as per computer code referred above that it belongs to Triclinic, a = 9.2288, b = 8.1837 c = 10.8027 A^0, $\alpha = 113.503$, $\beta = 112.454$, $\gamma = 72.422$, volume = 680.545 $(A^O)^3$ and density calculated as 2.589280 g/cm^3 with Z = 2.

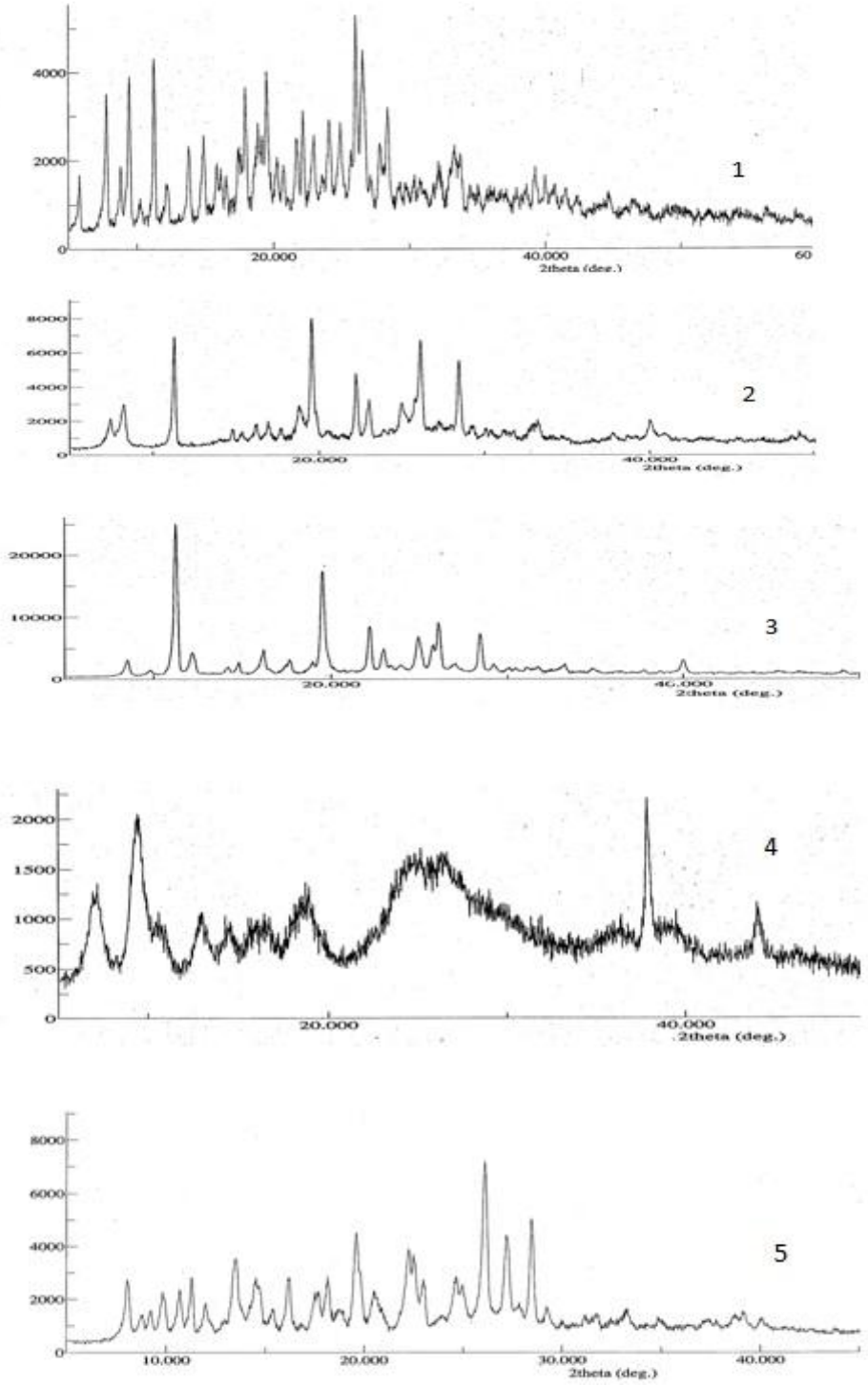

Fig.1 XRD PATTERNS OF METAL CHELATES 1) Pb (NQO) 2, 2) Hg (NQO) 2, 3) Zn (NQO) 2, 4) Ag (NQO) AND 5) Cd (NQO) 2

Table: 5 h k l values of Zn $(NQO)_{2,}$ Th (Obs) and Th (Calc)

Sr.No.	h	k	l	Th(Obs)	Th-Zero	Th(Cals)	Diff.
1	1	0	0	10.520	10.511	10.529	-0.018
2	0	1	0	11.960	11.951	11.971	-0.020
3	0	1	-1	12.640	12.631	12.602	0.029
4	1	1	0	14.500	14.491	14.494	-0.003
5	0	1	1	17.460	17.451	17.439	0.012
6	1	0	-2	18.580	18.571	18.570	0.001
7	1	1	1	20.780	20.771	20.770	0.002
8	2	0	0	21.160	21.151	21.148	0.003
9	1	0	2	24.200	24.191	24.196	-0.004
10	2	2	-2	25.980	25.971	25.960	0.011
11	2	1	-3	26.960	26.951	26.953	-0.001
12	1	2	-3	28.040	28.031	28.040	-0.001
13	2	-1	1	28.720	28.711	28.700	-0.009
14	3	1	-2	29.620	29.611	29.609	0.002
15	3	0	-1	30.460	30.451	30.456	-0.005
16	1	-2	2	32.200	31.191	31.198	-0.007
17	2	3	-2	34.340	34.331	34.335	-0.004

The metal chelate of AgNQO belongs to Triclinic, a = 9.9091, b = 7.4560 c = 12.3020 A^0, α = 90.377, β = 118.382, γ = 99.574, volume = 784.659 $(A^O)^3$ and density calculated as 2.344758 g/cm^3 with Z = 2.

Table: 6 h k l values of AgNQO, Th (Obs) and Th (Calc)

Sr.No.	h	k	l	Th(Obs)	Th-Zero	Th(Cals)	Diff.
1	0	0	1	8.220	8.205	8.202	0.002
2	1	0	-1	9.600	9.585	9.598	-0.013
3	0	1	0	12.120	12.105	12.087	0.018
4	1	-1	0	14.320	14.305	14.322	-0.018
5	1	-1	-2	18.760	18.745	18.747	-0.002
6	2	0	-2	19.260	19.245	19.264	-0.020
7	2	-1	-1	20.100	20.085	20.071	0.014
8	1	-1	2	24.640	24.625	24.625	0.000
9	2	-1	-3	25.720	25.705	25.675	0.030
10	2	1	0	26.080	26.065	26.056	0.009
11	1	2	-1	27.300	27.285	27.823	-0.010
12	2	-2	-1	27.840	27.825	27.823	0.002
13	3	-1	-1	28.620	28.605	28.605	0.000
14	1	2	-3	32.500	32.485	32.477	0.008
15	2	-2	2	37.180	37.165	37.168	-0.003
16	2	-2	-4	38.020	38.005	38.021	-0.016

The particle sizes were calculated using Debye Scherer Formula and the values found to be for Zn (NQO) 2 as 24.31 nm and for Hg (NQO) 2 as 23.15 nm which indicates that these crystals are nano crystals. Other chelates of Pb (NQO)2, Cd (NQO)2 and Ag (NQO) are crystalline in nature but their crystallographic analysis could not be done , only particle sizes were calculated and found to be 22.63 nm, 24.35 nm and 24.24 nm respectively.

3.3 Electronic Spectra (Uv)

The UV spectra of the ligand NQO and its metal chelates M (NQO) 2 where (M = Zn, Cd. Hg Ag and Pb) were studied in a dimethyl sulphoxide (DMSO) solution and the data is complied in Table 4. NQO exhibits absorption bands at 213.2 nm and at 262.3 nm. These bands are assigned to π to π^*. The band at 262.3 nm is originated from the π to π^* of the orthoquinone oxime (7). The chelates, studied here show only single transition at 262.1 nm which is π to π^* transition.

Table: 7 Electronic absorption data (λ nm) of metal chelates in DMSO in the range (200-800 nm).

Sr.No.	Compound	λ	
1	NQO	213.2	262.3
2	Zn (NQO)2	201.8	260.5
3	Cd (NQO)2	201.4	260.6
4	Hg (NQO)2	260.8	260.8
5	Ag(NQO)	260.4	260.1
6	Pb (NQO)2	263.3	281.2

3.4 Infrared Spectra

IR frequencies of 1-2naphthoquinone 2- oxime were calculated by RHF / 6-31G* and reported by N.R. Gonewar et. al. (8). In IR spectra of chelates M (NQO)2 where M = Pb, Zn, Hg and Cd showed a weak γ (C – H) stretching at about 3000 – 3400 cm^{-1}. The functional group such as C = O, C = N and N – O assigned. The data is given in table 5. It can be seen from the table that the spectrum of NQO can be compared with chelates of metals which clearly shows lower wave numbers for γ(C = N) and V (C = O) bands owing to elongation of these bonds upon coordination. The absorption of γ (N – O) was found at higher wave numbers since this bond was significantly shortened in the chelates. The high position of γ (NO) frequencies indicates that nitroso atom of the oxime group coordinates to the centre (9, 10).

Table: 8 Characteristic ν IR (cm^{-1}) bands of NQO and its metal chelates.

Sr.No.	Compound	C-H	C = O	C = N	N - O
1	NQO	3156	1594	1668	--
2	Zn (NQO)2	3156	1593	1667	1068
3	Cd (NQO)2	3186	1589	1666	1068
4	Hg (NQO)2	3078	1593	1665	1067
5	Ag(NQO)	3029	1589	1645	1072
6	Pb (NQO)2	3072	1560	1627	1068

3.5 Proton Nmr

NQO shows chemicals shift at 9.26 and 9.22 ppm for N-OH group. The chemical shift for hydroxyl proton is not observed in the chelates of Ag, Zn and Cd. While Pb chelate exhibits chemical shift at 9.30 ppm and chelate Hg exhibits at 9.28 and 9.26 ppm and remaining chemical shifts are similar to ligand molecule. (11, 12).13C NMR chemical shifts of C_1 and C_2 where –N- on C_1 is bonded. NQO exhibits chemical shifts at 147.71 and 182.68 and $CpCl_3$ for C_1 and C_2. The chemical shift for C1 is observed at 182 ppm for the chelates of Zn, Pb and Hg while the chemicals Ag and Cd are not observed. For C_2 the chemical shifts are shown at 149 ppm for all the chelates studied here. The chemical shifts of remaining carbons are in good agreement (13, 14)

3.6 SEM Studies

The scanning electron microscopy (SEM) of the following isomeric ligands and their Zn (II), Cd (II), Hg (II), Ag (I) and Pb (II) chelates was carried. In general, the average crystallite size of the metal chelates is smaller than the crystallite size of the parent ligand. These results of SEM investigations support the results obtained from XRD investigations. The XRD patterns were found to be composed of overlapped sharp lines as well as broad bands indicative of both small crystalline of nano crystalline type and extremely small crystallite size tending to amorphous nature. Secondly a careful examination of the SEM photographs (shown in Fig.2) of the ligand and their five metal chelates reveals that all the samples are heterogeneous mixtures of different particle size. The crystallite size is calculated as 24 ± 2 nm.

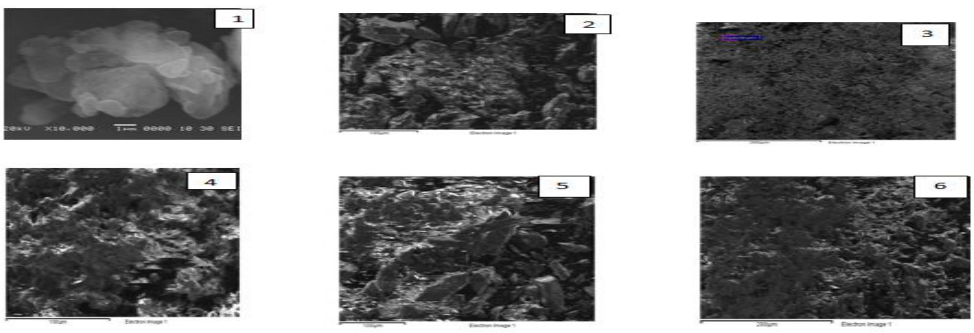

Fig.2 SEM DATA OF 1) NQO, 2) Hg(NQO)2, 3) Ag(NQO), 4) Pb(NQO)2, 5) Zn(NQO)2 and 6) Cd(NQO)2

3.7 Antimicrobial Scanning Results

The causative agent Cisplatin is chosen along with NQO and its metal chelates which are screened for their antimicrobial activities against E.coli, B. subtilis, S. auerus, P. vulgaris, P. aeruginosa, A. niger and C.albicans. The testing against growth of micro-organisms was carried out by using well diffusion method employing Mueller Hinton Agar (MHA) and culture in nutrient broth in each case of micro-organisms. The concentration of NQO and its metal chelates were chosen as 10^{-4}M. The plates were incubated at 35^0C 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: 9 Dimethyl sulphoxide i.e. solvent used shows no inhibition for all organisms under studies.

Table 9: Antimicrobial activities of 1, 2 naphthoquinone-2, oxime (NQO) and its metal chelates (Inhibition zone diameter in mm)

Sr. No.	Compound	*E. coli*	*B. subtilis*	*S. aureus*	*Proteus vulgaris*	*P. aeruginosa*	*A. niger*	*C. albicans*
1	DMSO	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	NQO	23.0 b	26.0 Sh	22.0 b	29.0 Sh	16.0 s	20.0 s	20.0 s
3	Zn (NQO)2	23.0 b	26.0 Sh	19.0 s	26.0 Sh	13.0 w	21.0 b	18.0 s
4	Cd (NQO)2	20.0 s	23.0 b	21.0 b	25.0 b	13.0 w	17.0 s	17.0 s
5	Hg (NQO)2	22.0 b	21.0 b	25.0 b	26.0 Sh	15.0 w	24.0 b	29.0 Sh
6	Ag(NQO)	16.0 s	21.0 b	19.0 s	21.0 b	13.0 w	20.0 s	21.0 b
7	Pb (NQO)2	19.0 s	22.0 b	18.0 s	21.0 b	11.0 w	17.0 s	15.0 w
8	Cisplatin	18.0 s	13.0 w	20.0 s	18.0 s	--	--	18.0 s

(10-15) Weak; (16-20) Small; (21-25) Broad; (26-above) Sharp zone

NQO has shown highest activity for P.vulgaris as well as with remaining organisms. The other results are as follows:

i) Zn (NQO)2 showed good activity against all organisms and showed highest activity for S. *subtilis*
ii) Pb $(NQO)_2$, Ag (NQO), Hg $(NQO)_2$ and Cd $(NQO)_2$ showed highest activity for P.vulgaris. The inhibition of the micro-organism growth for metal chelates was found to be in the following order.

Zn = Hg > Cd > Ag = Pb > Cisplatin

Cisplatin was the first member of a class of platinum-containing anti-cancer drugs. Cisplatin is particularly effective against testicular canThe studies demonstrate that metal chelation can increase the anti microbial activity than metal free ligand. It is reported that metal chelation reduces the polarity of the metal ion mainly due to the partial sharing of its positive change with the donor group and possibly the δ electron decolization occurring within the whole chelate ring system formed during co-ordination and results in increase of the lipophilic nature of the central metal atom (15). It favours 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 increases the electrostatic filed around the metal ion.

IV. Conclusions

All these chelates are crystalline in nature and generally belong to triclinic. The coordination ability of NQO towards M (II) chelates were examined by different spectroscopic methods that unequivocally determine the coordination sites of NQO. It is observed that the ratio of metal to NQO is 1:2 for chelates of Zn, Pb, Cd and Hg while in case of Ag the ratio is 1:1. Biological activity screening proved the good antimicrobial activity of NQO and its metal chelates. The antimicrobial activity is explores on the bases of overtone concept of cell permeability. These results are better than standard Cisplatin chemotherapy agent.

Acknowledgement

We thank Prin. K.D. Jadhav, Principal, Bharati Vidyapeeth Deemed University, Yashwantrao Mohite College, Pune for permission to Publish this work.

References

[1]. A.P. Avdeenko, G.M. Glinyanaya and V.V. Pirozhenko, Russian Journal of Organic Chemistry, 35(10)1480-1487 (1999).
[2]. B. Foretic and N. Burger, Monatshefte Fur Chemie, 127, 227-230 (1996).
[3]. P. Lingaiah and E. V. Sundaram, Current Science Vol. 45, N0.2, 51-52 (1976).
[4]. S. Gurriari and G. Siracus, Inorganica Chimica Acta., Vol. 5 No.3, 650-654 (1971).
[5]. R.G. Sarawadekar and I.B. Gade, Software for Kinetic Analysis of non isothermal thermogravimetry (TG) Data, HEMRL REPORT , PYRO, TR 253 (2006).
[6]. Le Bail, A**.,** Powder Diffraction, 19,249-259 (2002).
[7]. A.Buraway, M. Cais, J. T Chamberlain, F. Livesedge and A. R. Thompson, J. Chem. Soc 3277 (1955).
[8]. N.R. Gonewar, V. B. Jadhav K.D. Jadhav, and R.G. Sarawadekar, Research in Pharmacy 2(1) : 18-25, 2012
[9]. Constantino V. R. Toma H. E., Olivenia LFC and P. *J. Raman Sepctroscopy*, 23, 629 (1992).
[10]. Natarajan C., Nazeer Hussain A. *Indian J. Chem.* 22A, 527 (1983).
[11]. A. Krzan and J. Mavri., *Chemical Physics*, 277, 71-76 (2002).
[12]. V. Enchev, G. Ivanova, A. Vgrinov, G. D. Neykov., *J. Mol, Struct.* 508, 149 (1999).
[13]. G. Ivanov, V. Enchev. *Chem... Phys.* 264, 235 (2001).
[14]. T. Shono, Y. Hayashi and K. Shinra., *Bull. Chem. Soc. of Japan*., 4, 3179-3181 (1971).
[15]. Z.H. Chohan, C. T. Superan and A. Scozafava, J. *Enzyme Inhihitian and Medicinal Chemistry*, 20, 303 (2005).