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STUDIES ON AN ANTIMICROBIAL ACTIVITY OF METAL (Mn, Fe, Co, Ni, Cu) CHELATES OF 1, 2-NAPHTHOQUINONE 2-OXIME

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ABSTRACT

Five transition metal chelates of the type M $[NQO]_2$ where M = Mn, Fe, Co, Ni, Cu: NQO = 1, 2-napthoquinone 2-oxime have been synthesized. All chelates have been characterized by modern methods such as elemental analysis, FTIR, electronic spectra and X-ray diffraction. The chelate of manganese belongs to triclinic, a = 6.3580, b = 18.9591 c = 7.5697 Å, α = 100.429, β = 74.757, γ = 66.998 and copper chelate belongs to triclinic, a = 7.5528, b = 5.8689 c = 13.6719 Å, α = 63.541, β = 92.028, γ = 103.795, scanning electron microscopy of chelates was carried out. Their particle sizes are in the range of 15-42 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 chemotherapy agent. Mechanism of hydrolysis of metal chelates is discussed.

Key words: 1-2-Naphthoquinone 2-oxime, X-ray diffraction, IR, SEM, Antimicrobial activity, Electronic spectra

INTRODUCTION

Theoretical calculations of infrared, NMR and electronic spectra of 2-nitroso-1, naphthol or 1-2-naphthoquinine-2 oxime and comparison with experimental data have been published by N. R. Gonewar et al.[1] Thermal, X-ray diffraction, spectral and antimicrobial activity of bivalent metal (Zn, Cd, Hg, Pb and Ag) chelates of 1, 2-naphthoquinone-2, oxime, N. R. Gonewar et al.[2] 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

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authors concluded that the color of the quinone oxime complexes was not related to a quinone oxime or nitrosophenol structure[3]. The Fe (III) complex of 1-2-naphthoquinone-2, oxime have been reported and its IR spectra were explained along with electronic spectra[4]. The stability constants of the metal chelates of 1-2-naphthoquinone-2, oxime with Mn, Co, Ni, Cu, Zn, Vo (II) and UO_2 (II) were determined. The stability of the metal chelates was assigned to the fact that the oxygen of a resonating have better basic centre[5]. 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 Gurrieri and Siracus[6].

In this paper, we reported synthesis of bivalent metal chelates of the type M $[NQO]_2$ where M = Mn, Fe, Co, Ni and Cu : NQO = 1, 2-napthoquinone 2-oxime and characterization by XRD, Mid IR, electronic spectra, scanning electron microscopy and antimicrobial activity against microorganisms. A better understanding of the mechanism may also lead to a process of great importance in many biological events.

EXPERIMENTAL

Materials and methods

The ligand 1, 2-naphthoquinone 2-oxime is used as it is a stock solution of Mn (II), Fe (II), Co (II), Ni (II) and Cu (II), is prepared by using AR grade chemicals. Distilled water is used during synthesis.

Preparation of metal chelates

The chelates were prepared by mixing metal salt solution and ligand in 1 : 1 proportion. 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 min. On cooling, it was filtered and compounds were found to be coloured.

Instrumental analysis

Elemental analysis was carried out with a Perkin-Elmer 2400 series for C, H, O and N. The IR spectra are recorded on a Shimadzu FTIR 8400 S Model in a KBr matrix. The XRD patterns of all the samples are recorded on Bruker D_8 diffractometer in the diffraction angle range $(10\text{-}70)^0$ 2θ. SEM was carried out on a JEOL-3SM-5200 scanning electron microscopy.

Antimicrobial activity testing

Test organisms: The antimicrobial activity of ligands, metal salts and synthesized metal chelates is tested 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. ESBL *Escherichia coli*, *Klebsiella Pneumoniae.* The causative agent Cisplatin is chosen as standard chemotherapy agent.

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.

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^{o}C for 48 hrs. for bacteria and 30^{o}C for 5 days for fungi. The zone diameter of inhibition were measured in mm and recorded.

RESULTS AND DISCUSSION

Infrared spectra

IR frequencies of 1-2-naphthoquinone dioxime were calculated by RHF/6-31G* and reported by Gonewar et al.[1] In IR spectra of chelates M $(NQO)_2$ where M = Mn (II), Fe (II), Co (II), Ni (II) and Cu (II) showed a weak γ (C – H) stretching at about 3000-3400 cm^{-1}. The functional group such as C = N and N – O is assigned. The data is given in Table 1. 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) band owing to elongation of this bond 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[7,8]. The data of frequencies are given in Table 1.

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

S. No.	Compound	C – H	C = N	N - O
1	NQO	1668	1551	1069
2	Mn $(NQO)_2$	1584	1499	1112
3	$Fe(NQO)_2$	1606	1541	1089
4	Co $(NQO)_2$	1610	1520	1092
5	$Ni(NQO)_2$	1606	1530	1092
6	Cu $(NQO)_2$	1606	1528	1132

Electronic spectra (UV)

These bands are interpretated as benzenoid electron transfer (BET), quinonoid electron transfer (QET) and combination band, respectively. The third combination band occurring in visible region is composed of n → π* transitions + L to M charge transfer band. The d – d bands, which are expected in this region are not distinctly resolved most probably due to their overlapping in this combination band. The UV spectra of the ligand NQO and its metal chelates M $(NQO)_2$ where (M = Mn (II), Fe (II), Co (II), Ni (II) and Cu (II) were studied in a dimethyl sulphoxide (DMSO) solution and the data is complied in Table 2.

Table 2: Electronic absorption data (λ, nm) of metal chelates in DMSO in the range (200-800 nm)

Compound	π – π* Transitions	π – π* Transitions	n → π* Transitions	Combination of L + d – d Transitions M to L+d–d
NQO	231	304	402	
Mn-2-oxime	258	307	474	
Fe (II)- 2-oxime	288	343	429	
Co (II)- 2-oxime	288	320	564	
Ni (II)- 2-oxime	280	325	450	
Cu (II)- 2-oxime	250	321	505	605

NQO exhibits absorption bands at 231 nm, 304 nm and at 402 nm. These bands are assigned to π to π* and n → π*. The band at 304 nm is originated from the π to π* of the

orthoquinone oxime[9]. The chelates, studied here show two bands which are due to π to π* transition and third one is due to n → π*. In the case of copper chelate, one more band is observed at 605 nm, which can be assigned to combination of ligand to metal or metal to ligand transition with d-d transitions.

X-ray diffraction

The data was processed by using McMaille computer program for determination of cell parameters and space group[10]. 1, 2-naphthoquinone-2, oxime crystallizes in the triclinic group and it has crystallographic parameters, a = 12.0951 Å, b = 6.1556 Å and c = 11.4016 Å

α = 65.964 o, β = 81.864 o, γ = 87.090 o, its volume is 766.887 (Å) 3 and space group H-M symbol P1. Dmin = 3.279487 g/cm^3

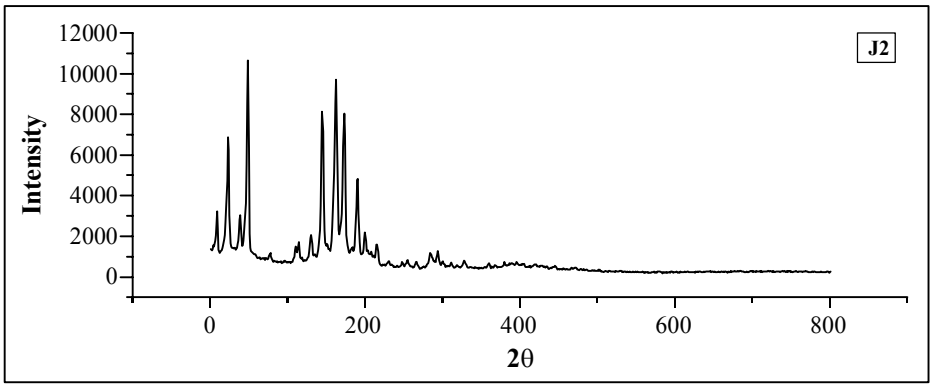


Fig. 1: X-ray diffraction of NQO

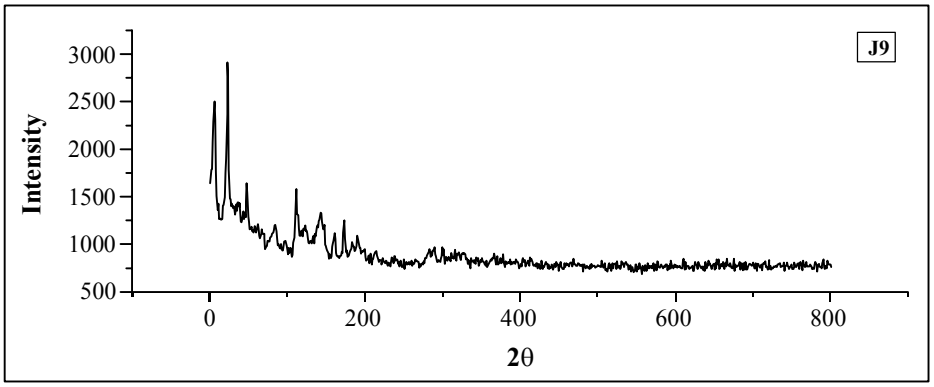


Fig. 2: X-ray diffraction of Mn $(NQO)_2$

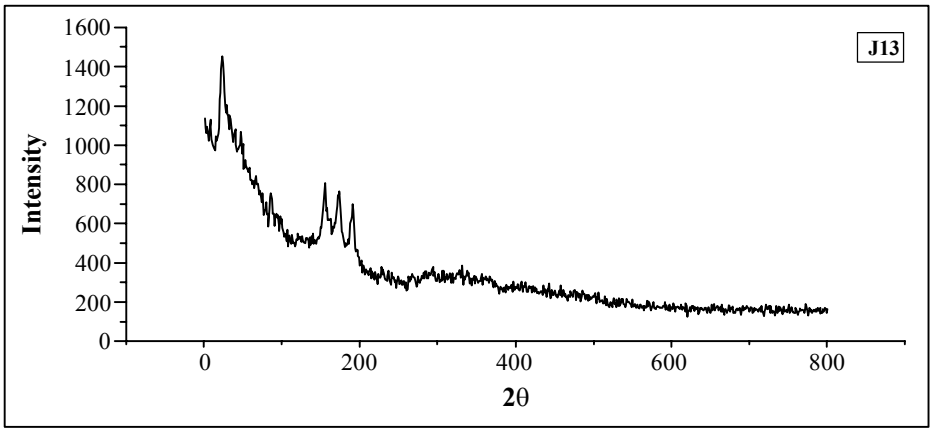


Fig. 3: X-ray diffraction of Cu $(NQO)_2$

The metal chelate of Mn $(NQO)_2$, shows data as per computer code referred above that it belongs to Triclinic, a = 6.3580, b = 18.9591 c = 7.5697 Å, α = 100.429, β = 74.757, γ = 66.998, volume = 767.899 $(Å)^3$ and density calculated as 2.0771 g/cm^3 with Z = 2. Table 3 shows h, k, l data of Mn $(NQO)_2$.

Table 3: h, k, l values of Mn $(NQO)_2$

h	k	l	TH (Obs)	TH-ZERO	TH (Calc)	DIFF
0	2	0	10.700	10.685	10.691	-0.006
0	1	-1	12.200	12.185	12.174	0.010
0	0	1	12.800	12.785	12.781	0.004
1	1	0	14.600	14.585	14.589	-0.005
1	0	1	16.600	16.585	16.592	-0.007
1	-1	1	18.500	18.485	18.475	0.010
1	-3	1	25.499	25.484	25.494	-0.10
1	-1	-1	27.299	27.284	27.283	0.001
2	1	1	28.999	28.984	28.987	-0.002
1	-4	0	32.699	32.684	32.676	0.008
1	2	3	39.299	39.284	39.297	-.013
2	3	3	43.099	430.84	43.073	0.010

The metal chelate of $Cu(NQO)_2$, shows data as per computer code referred above that it belongs to triclinic, a = 7.5528, b = 5.8689 c = 13.6719 Å, α = 63.541, β = 92.028,

γ = 103.795, volume = 525.151 (Å)^3 and density calculated as 2.4178 g/cm^3 with Z = 2. Table 4 shows h, k, l data of Cu $(NQO)_2$.

Table 4: h, k, l values of Cu $(NQO)_2$

h	k	l	TH(Obs)	TH-ZERO	TH(Calc)	DIFF
1	0	0	12.100	12.106	12.096	0.010
1	0	1	13.600	13.606	13.593	0.010
1	0	-1	14.600	14.606	14.613	-0.007
0	1	0	17.400	17.406	17.412	-0.006
1	-1	-2	18.900	18.906	18.905	0.000
0	1	3	20.900	20.906	20.922	-0.016
1	-1	-3	22.899	22.906	22.893	0.013
2	0	0	24.299	24.306	24.330	-0.025
1	0	-3	25.899	25.906	25.904	0.001
2	-1	-2	26.999	27.006	27.003	0.003
2	-1	1	28.800	28.806	28.805	0.001

The particle sizes of Mn $(NQO)_2$ and Cu $(NQO)_2$ are found to be as 28.45 nm and 19.33 nm, respectively which are calculated by using Scherrer equation and that of ligand is 21.93 nm.

SEM studies

The scanning electron microscopy (SEM) of the ligand and their Mn (II), Fe (II), Co (II), Ni (II) and Cu (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. A careful examination of the SEM photographs (shown in Fig. 4) of the ligand and their five metal chelates reveals that all the samples are heterogeneous mixtures of different particle size. The morphology can be explained as -

(i) The ligand NQO is a crystalline particulate matter holding on to form micro groups of particles.

(ii) Mn $(NQO)_2$ shows nanocrystals bound together forming cotton like clusters. The cluster boundaries are well defined.

(iii) Fe $(NQO)_2$ is a continuous phase planer structure with grain boundaries merged together.

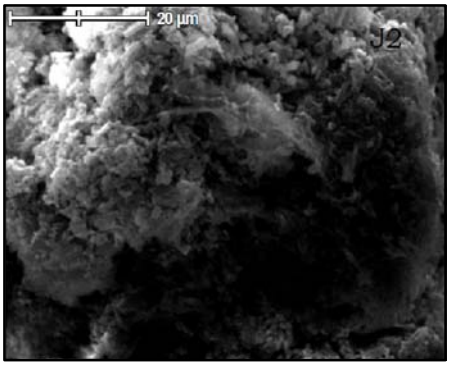
(iv) Co $(NQO)_2$ is with bulk materials and exhibits nonspecific shaped particles.

(v) Ni $(NQO)_2$ shows continuous amorphous phase with Ni homogeneously dispersed.

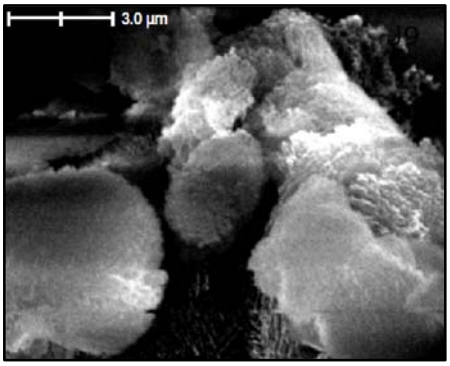
(vi) Cu $(NQO)_2$ shows continuous amorphous phase with Cu homogeneously dispersed.

Antimicrobial activity of causative agents

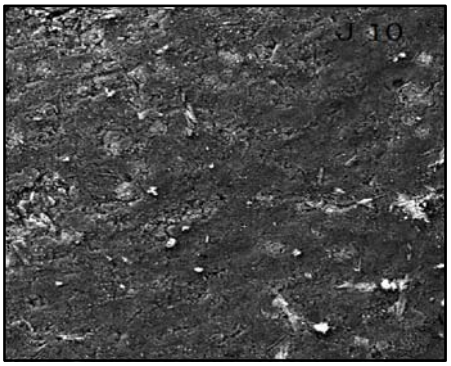
The antimicrobial activity of ligands and their complexes were tested against bacteria and fungi like *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus vulgaries* and *Candida albicans.* The causative agent Cisplatin is chosen as standard chemotherapy agent.



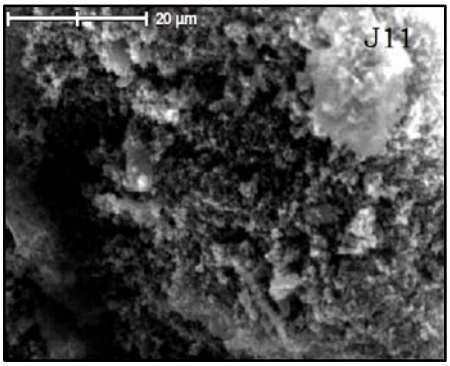
(a) NQO



(b) Mn $(NQO)_2$

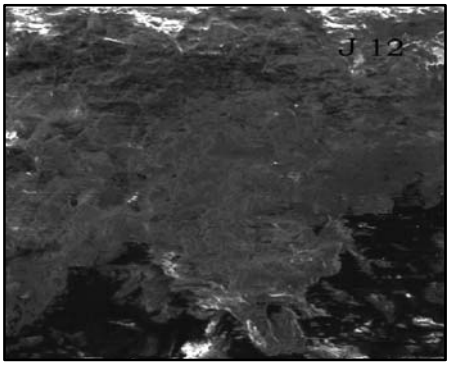


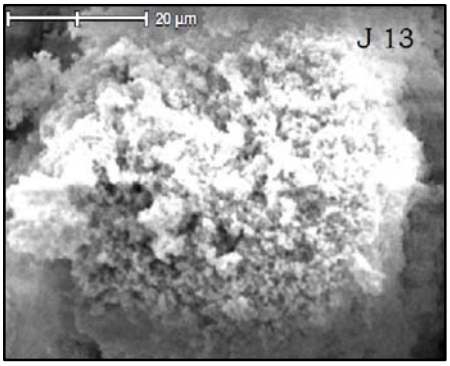
(c) Fe $(NQO)_2$



(d) Co $(NQO)_2$

Cont…





(e) Ni $(NQO)_2$ **(f) Cu $(NQO)_2$**

Fig. 4 : SEM photographes of ligand and its chelates

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 37°C for 24 hours in incubator. The clear zone of inhibition of growth for the organism was measured in mm^2 and the data is given in Table 5.

Table 5: Antimicrobial activities of 1, 2-naphthoquinone dioxime (NQO) and its metal chelates (Inhibition zone area in mm^2)

S. No.	Comp.	*S. aureus*	*B. subtilils*	*P. vulgaris*	*E. coli*	*C. albicans*
1	NQO	572.2	829.1	132.2	397.4	530.6
2	Mn $(NQO)_2$	397.4	615.4	314	254.3	490.6
3	Fe $(NQO)_2$	0.0	0.0	103.81	113.04	0.0
4	Co $(NQO)_2$	314.0	415.2	176.6	153.8	200.9
5	Ni $(NQO)_2$	113.04	346.18	200.96	176.6	226.8
6	Cu $(NQO)_2$	379.9	452.1	226.8	176.6	452.1
7	Cisplatin	314.0	132.6	254.3	254.3	0.0

Antimicrobial activity of the ligands exhibit fairly good activity against the five microorganisms studied. Maximum activity is exhibited by NQO against *C. albicans* (1074.6 mm^2).

The activity of NQO shows decrease in most of the cases. For *B. subtilis*, the trend is not uniform. Here the activity of the ligand is increased from (154.30 mm^2 to 706.5 mm^2). For Ni (II) chelates it is slightly reduced to153.8 mm^2. The variations for this ligand are between zero (minimum) to 1074.6 mm^2 (maximum). The powerful antimicrobial activity of the three 1, 2 NQ 2-oximes and their chelates against the selected microorganisms may results due to the successful competition of these ligands with enzymes to interact with metals. Enzyme also can act as ligands because of the presence of $-NH_2$ groups in protein molecules. This competition might affect the metal enzyme activity, which disturbs the life cycle of microorganisms causing their death or inhibit their growth. The results of metal chelates are comparable with cisplatin complex. A review is presented by Reedijk and Lohman[11] on the mechanism of binding DNA with ciaplatin. The formation of intrastrands cross-links between adjacent guanines to which the Pt $(NH_3)_2^{2+}$ ion is chelated at the n7 atoms, seem to be a very important event.

Hydrolysis reactions of metal chelates

After administration of the drug - usually through injection or infusion in the blood stream a variety of chemical reactions may occur. Hydrolysis process is required to allow fast reactions, with e.g. prote ins, RNA, DNA[12-14]. A significant losses of metal do occur, since 50-70% of the administered metal is excreted within 24 hours[14,15]. The remaining metal chelate eventually diffuses through the walls of (all kinds of) cells. Hydrolysis reactions will take place. Based on work of Martin[16], it is now generally accepted that the hydrolysis operates inside the cells. Among the hydrolysis products Cu $(NQO)_2.H_2O^+$ is the predominant species undergoing reactions with all kinds of molecules present inside the cells (DNA, RNA, proteins). DNA is the most likely target. Early studies by several groups have shown that species specific interaction of metal chelate with DNA is an important event, which may eventually lead to cell killing. The evidence can be summarized as follows -

(i) Induction of filamentous growth indicates that cell division is hampered and cell growth is not[15].

(ii) Induction of lysis in lysogenic bacteria also indicates interaction with DNA. Even a correlation between antitumour activity and prophage induction in lysogenic bacteria was found[17].

(iii) Inhibition of DNA synthesis is selectively inhibited, whereas RNA and protein synthesis are not[18].

Detailed binding studies of metal chelates to DNA and to fragments of DNA, are receiving considerable attention. All bases do have nitrogen's and have been found to be able to co-ordinate transition-metal ions[19]. Investigations have started with studies of the binding preferences of the nuclei bases adenine (A), guanine (G) and cytidine (C). It appeared that adenine might co-ordinate to the metal group through the N_7 atom and through the N_7 atom, where as cytidine can co-ordinate through the N_3 atom. In guanine binding is possible at N_7 and only under alkaline conditions at deprotonated N_l. Detailed kinetic studies and competition studies of about a decade ago have made clear that guanine N_7 has a strong kinetic preference. This has led to the generally accepted view that also in DNA, metal chelate units bind to certain guanine N_7 sites. The site where the second binding occurs cannot be predicted with certainty and at this stage only the several possibilities are briefly mentioned.

(i) Stabilization of the monofunctional binding through hydrogen bonding of the amine ligands and/or the remaining H_2O.

(ii) Chelation to an O_6 group of the same guanine, is possibility has so far not been proved.

(iii) Chelation to a base in the opposite strand of double-helical DNA, which may be a guanine or another base (interstrand cross-linking).

(iv) Chelation to a (next) neighbouring guanine N_7 in the same DNA strand (Intrastrand cross linking).

(v) Chelation to another guanine at the same DNA strand (not a next neighbour).

(vi) Chelation to another base next to guanine in the same strand; likely candidates are adenine (N_7 or N_1) and cytidine (N_3).

(vii) Chelation to a protein side chain residue.

In vitro studies with salmon sperm DNA by Fichtinger-Schepman et al.[20,21] have shown that the most predominant lesion is the intrastrand chelation with two neighbouring guanines (a so-called GG chelate). Also AG chelation has been found in significant amounts[22]. Binding to CG, GC, GA, TG or GT units could not be demonstrated (T = Thymidine).

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REFERENCES

1. N. R. Gonewar, V. B. Jadhav K. D. Jadhav and R. G. Sarawadekar, Research in Pharmacy, **2(1)**, 18-25 (2012).
2. N. R. Gonewar, V. B. Jadhav, S. S. Sakure, K. D. Jadhav and R. G. Sarawadekar, IOSR J. Pharm., **3**, 10-17 (2013).
3. A. P. Avdeenko, G. M. Glinyanaya and V. V. Pirozhenko, Russian J. Org. Chem., **35(10)**, 1480-1487 (1999).
4. B. Foretic and N. Burger, Monatshefte Fur Chem., **127**, 227-230 (1996).
5. P. Lingaiah and E. V. Sundaram, Current Science, **45(2)**, 51-52 (1976).
6. S. Gurriari and G. Siracus, Inorganica Chimica Acta., **5(3)**, 650-654 (1971).
7. V. R. Constantino, H. E. Toma, L. F. C. Olivenia and P. S. Santos, J. Raman Spectroscopy, **23**, 629-632 (1992).
8. C. Natarajan, Nazeer Hussain A., Indian J. Chem., **22A**, 527-531 (1983).
9. C. J. Milios, T. C. Stamatatos and S. P. Perlepes, The Coordination Chemistry of Pyridyl Oximes, Polyhedron, **25**, 134-194 (2006).
10. A. Le Bail, Powder Diffraction, **19**, 249-259 (2002).
11. J. Reedijk and P. H. M. Lohman, Cisplatin, Synthesis, Antitumor Activity and Mechanism of Action, Pharm. Weekbl [Sci.], **7**, 173-180 (1985).
12. S. J. Lippard, New Chemistry of An Old Molecule: Cis $Pt(NH_3)_2Cl_2$, Science, **218**, 1075-1082 (1982).
13. A. Pinto and S. J. Lippard, Binding of the Antitumor Drug Cis- diamminedichloro-platinum(II) (Cisplatin) to DNA Biochem. Biophys. Acta., **780**, 167-80 (1985).
14. A. W. Prestayko, S. T. Crooke, S. K. Carter, Eds. Cisplatin, Current Status and New Developments, New York: Academic Press (1980).
15. B. Rosenberg, Some Biological Effects of Platinum Compounds, Plat. Met. Rev., **15**, 42-44 (1971).
16. R. B. Martin Hydrolytic Equilibria and n7 Versus n1 Binding in Purine Nucleosides of Cisplatin, in, S. J. Lippard, Ed. Platium, Gold and other Metal Chemotherapeutic Agents: Chemistry and Biochemistry, Washington Am. Chem. Soc., 231-244 (1983) (ACS Symposium Series, Vol. 209).

17. S. Reslova, The Introduction of Losogenic Strains of *E. coli* by Cisplatin Chem. Biol. Interact., 66-70 (1972).

18. H. C. Harder R. G. Smith and A. F. Leroy, Cancer Res., **36**, 3821-3829 (1976).

19. R. W. Gellert and R. Bau. Metal Ions in Biological Systems, **Vol. 8**, New York, Marcel Drkker, **1** (1971).

20. A. M. J. Fichtinger-Schepman, P. H. M. Lohman and J. Reedijk, Nucl. Acid Res., **10**, 5345-56 (1982).

21. A. M. J. Fichtinger-Schepman, J. L. Van Der Veer, J. H. J. Den Hartog, P. H. M. Lohman and J. Reedijk, Biochemistry, **24**, 707-13 (1985).

22. J. C. Chottard, J. P. Girault, G. Chottard, J. Y. Lllemand and D. Mansuy, J. Am. Chem. Soc., **102**, 5565-5572 (1980).

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