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# Studies on an antimicrobial activity of metal (Mn, Fe, Co, Ni, and Cu) chelates of 1, 2 naphthoquinone 1-oxime

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***Abstract:*** *Transition metal chelates of the type M [NQO] $_2$ where M = Mn, Fe, Co,Ni, Cu: NQO = 1, 2 napthoquinone 1-oxime have been synthesized. X-ray studies have been carried out for Mn and Cu chelate of 1, 2 napthoquinone 1-oxime and the chelate of manganese belongs to Triclinic, a = 6.3580, b = 18.9591 c = 7.5697 $A^0$, α = 100.429, β = 74.757, γ = 66.998, and copper chelate belongs to triclinic, a = 7.5528, b = 5.8689 c = 13.6719 $A^0$, α = 63.541, β = 92.028, γ = 103.795. Their particle sizes are in the range of 15-42 nm. All chelates have been characterized by modern methods such as elemental analysis, FTIR and Electronic spectra.Scanning electron microscopy of chelates was carried out. The ligand and the metal chelates have been screened for antimicrobial activity on gram positive, gram negative bacteria and fungi. The results are compared with cisplatin as standard chemotherapy agent.Mechanism of hydrolysis of metalchelates is discussed.*

***Keywords****: 1-2 naphthoquinone1-oxime, Antimicrobial activity, Electronic spectra, X-ray diffraction, IR, SEM,*

## I. Introduction:

Theoretical calculations of infra-red, NMR and electronic spectra of 2-nitroso-1, naphthol or 1-2 naphthoquinine-2 oxime and comparisonwith experimental data have been published by N.R. Gonewaret. 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 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 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 (5). The synthesis and characterization of three novel iridium (III) complexes and one rhodium (III) complex with 1-nitroso-2-naphthol chelating as a 1,2-naphthoquinone-1- oximato ligand are described. The X-ray crystal analyses revealed a pseudo- octahedral " piano-stool " configuration through oximato N and naphthoquinone-O, forming a nearly planer five member metallacycle. The remarkable cytotoxicity of the compounds tested may be attributed to nercosis, than to apoptosis, as it is evidenced by the casoase -3/7 activation assay (6). 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 (7). Quantum chemical vibrational study, molecular property and HOMO-LUMO gap energies of zirconium chelate of 1, 2 napthoquinone – 1, oxime have been reported by G.S. Jagtap et.al. (8)

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

## II. Materials and Methods

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

### 2.1Preparation of metal chelates.

The chelates were prepared by mixing metal salt solution and ligand in 2: 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. The mixture was warmed on water bath for about 15 minutes. On cooling it was filtered and compounds were found to be coloured. The chelates were dried in vacuum.

**2.2 Instrumental Analysis.**

Elemental analysis was carried out with a Perkin Elmer 2400 series for C, H, and O& N. The IR spectra are recorded on a Shimadzu FTIR 8400 S model in a KBr matrix.The XRD patterns of all the samples were 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 was 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. The causative agent Cisplatin was chosen as standard chemotherapy agent.

**2.4Maintenance 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.

**2.5Plating**

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 $37^0$C for 48 hrs. for bacteria and incubated at $30^0$C for 5 days for fungi. The zone diameter of inhibition were measured in mm & recorded.

## III. Results and discussion

**3.1 X-ray diffraction:**

The X-ray powder diffraction data was processed by using McMaille computer program for determination of cell parameters and space group (9).1-nitroso-2-naphthol is monoclinic whichis recorded at 300 K. The reported data is comparable which was recorded at 200 K. The
crystal data shows a = 5.4567, b = 9.2861 and c = 15.8238 Å, β = 103.884, volume 778.351 $(Å)^3$, the density was considered as 1.608161g/cm3 and Z = 4.

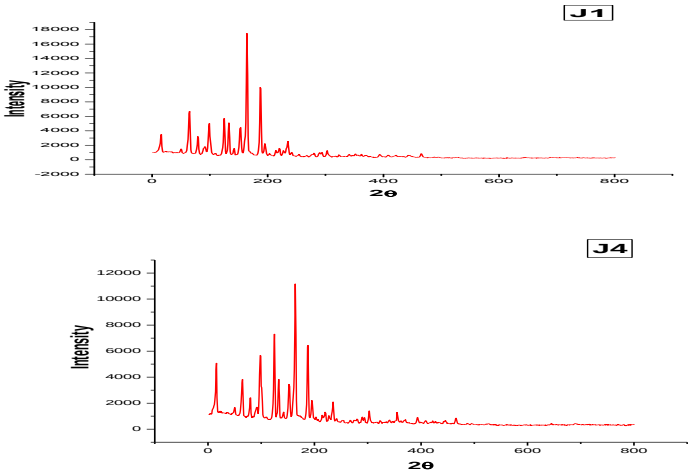



Fig. 2 X- ray diffraction of Mn (NQO) 2

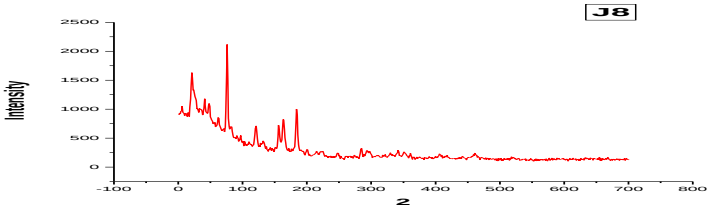



Fig. 3 X- ray diffraction of Cu (NQO) 2

The metal chelate of Mn (NQO)2, shows data as per computer code referred above that it belongs to Triclinic, a = 6.5689, b = 7.9317 c = 12.4753 $A^0$, α = 81.080, β = 105.896, γ = 100.687, volume = 610.549 $(A^O)^3$ and density calculated as 2.0771 g/$cm^3$ with Z = 2. Table-1 shows h, k, l data of Mn (NQO) 2.

Table: 1 h k l values of Mn (NQO) 2,

| h | k | l | TH(Obs) | TH-ZERO | TH(Calc) | DIFF |
|---|---|---|---|---|---|---|
| 0 | 1 | 0 | 11.423 | 11.409 | 11.413 | -0.005 |
| 1 | -1 | -1 | 16.325 | 16.311 | 16.317 | -0.006 |
| 1 | -1 | -2 | 19.000 | 18.986 | 18.991 | -0.005 |
| 0 | 1 | -2 | 19.782 | 19.768 | 19.750 | 0.018 |
| 0 | 0 | 3 | 22.362 | 22.248 | 22.347 | 0.001 |
| 1 | 1 | -2 | 23.171 | 23.157 | 23.151 | 0.005 |
| 1 | -2 | 0 | 25.151 | 25.137 | 25.144 | -0.007 |
| 0 | 1 | -3 | 26.293 | 26.279 | 26.283 | -0.004 |
| 2 | -1 | -2 | 28.635 | 28.621 | 28.624 | -0.003 |
| 1 | -2 | -3 | 29.408 | 29.394 | 29.391 | 0.002 |
| 2 | 0 | 1 | 31.374 | 31.360 | 31.363 | -0.004 |
| 1 | -1 | 3 | 31.81 | 31.867 | 31.849 | 0.018 |
| 2 | -2 | -1 | 32.678 | 32.664 | 32.630 | 0.033 |

The metal chelate of Cu(NQO)2, shows data as per computer code referred above that it belongs to triclinic, a = 7.0141, b = 9.5129 c = 8.2653 $A^0$, α = 67.085, β = 110.427, γ = 111.337, volume = 459.133 $(A^O)^3$ and density calculated as 2.322300 g/$cm^3$ with Z = 2. Table-2 shows h, k, l data of Cu $(NQO)_2$.

Table: 2 h k l values of Cu $(NQO)_2$

| h | k | l | TH(Obs) | TH-ZERO | TH(Calc) | DIFF |
|---|---|---|---|---|---|---|
| 0 | 1 | 0 | 10.499 | 10.459 | 10.458 | 0.000 |
| 0 | 0 | 1 | 11.999 | 11.959 | 11.969 | -0.011 |
| 1 | 0 | 0 | 19.999 | 13.958 | 13.956 | 0.002 |
| 1 | -1 | -1 | 14.698 | 14.658 | 14.469 | 0.009 |
| 1 | 0 | -1 | 16.098 | 16.058 | 16.058 | 0.000 |
| 0 | 1 | -1 | 18.198 | 18.158 | 18.154 | 0.004 |
| 1 | -2 | 0 | 21.998 | 21.958 | 21.963 | -0.006 |
| 1 | -1 | 1 | 23.098 | 23.057 | 23.052 | 0.006 |
| 2 | -1 | -1 | 25.497 | 25.457 | 25.462 | -0.005 |
| 1 | -3 | -1 | 28.297 | 28.257 | 28.257 | 0.000 |

The particle sizes of Mn $(NQO)_2$ and Cu $(NQO)_2$ are found to be as 35.51 nm & 38.19 nm respectively which are calculated by using Scherer equation and that of ligand is 41.92 nm.

**3.2 Infrared Spectra**

IR frequencies of 1-2naphthoquinone 1-oxime were calculated by RHF / 6-31G* and reported by N.R. 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 3. 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 (10, 11). The data of frequencies are given in Table: 3

Table: 3 Characteristic ν IR ($cm^{-1}$) bands of NQO and its metal chelates.

| Sr.No. | Compound | C –H | C = N | N - O |
|---|---|---|---|---|
| 1 | NQO | 1619 | 1557 | 1075 |
| 2 | Mn $(NQO)_2$ | 1568 | 1547 | 1116 |
| 3 | Fe$(NQO)_2$ | 1602 | 1550 | 1085 |
| 4 | Co $(NQO)_2$ | 1605 | 1555 | 1138 |
| 5 | Ni$(NQO)_2$ | 1605 | 1519 | 1158 |
| 6 | Cu $(NQO)_2$ | 1605 | 1529 | 1152 |

**3.3 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 4. NQO exhibits absorption bands at 231nm, 304 nm and at 402nm. These bands are assigned to π to π* and n → π*. The band at 304nm is originated from the π to π* of the orthoquinone oxime (12). The chelates, studied here show two bands whichare 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 605nm which can be assigned to combination of ligand to metal or metal to ligand transition with d-d transitions.

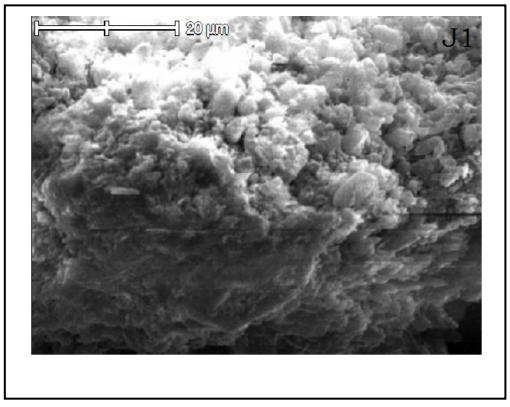
Table: 4 Electronic absorption data (λ nm) of metal chelates in DMSO in the range (200-800 nm).

| Sr. No. | Compound | π – π* Transitions | π – π* Transitions | n → π* Transitions | Combination of L + d – d Transitions M to L+d–d |
|---|---|---|---|---|---|
| 1 | NQO | 224 | 322 | 410 | |
| 2 | Mn-1-oxime | 225 | 325 | 425 | |
| 3 | Fe (II)- 1-oxime | 262 | 343 | 482 | |
| 4 | Co (II)- 1-oxime | 260 | 342 | 522 | |
| 5 | Ni (II)- 1-oxime | 255 | 340 | 505 | |
| 6 | Cu (II)- 1-oxime | 258 | 335 | 422 | 605 |

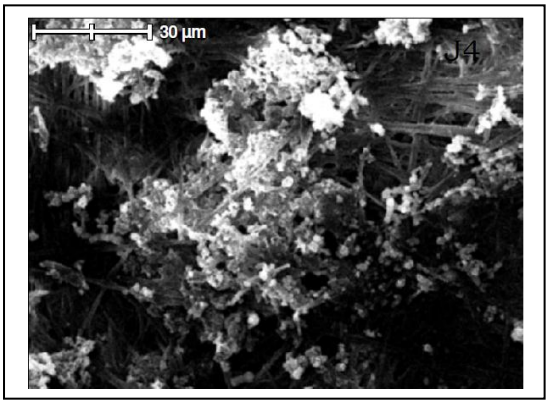
**3.4 SEM studies**

The scanning electron microscopy (SEM) of the ligand and their Mn (II), Fe (II), Co (II), Ni (II) and Cu (II) chelates were 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 morphologycan be explained as

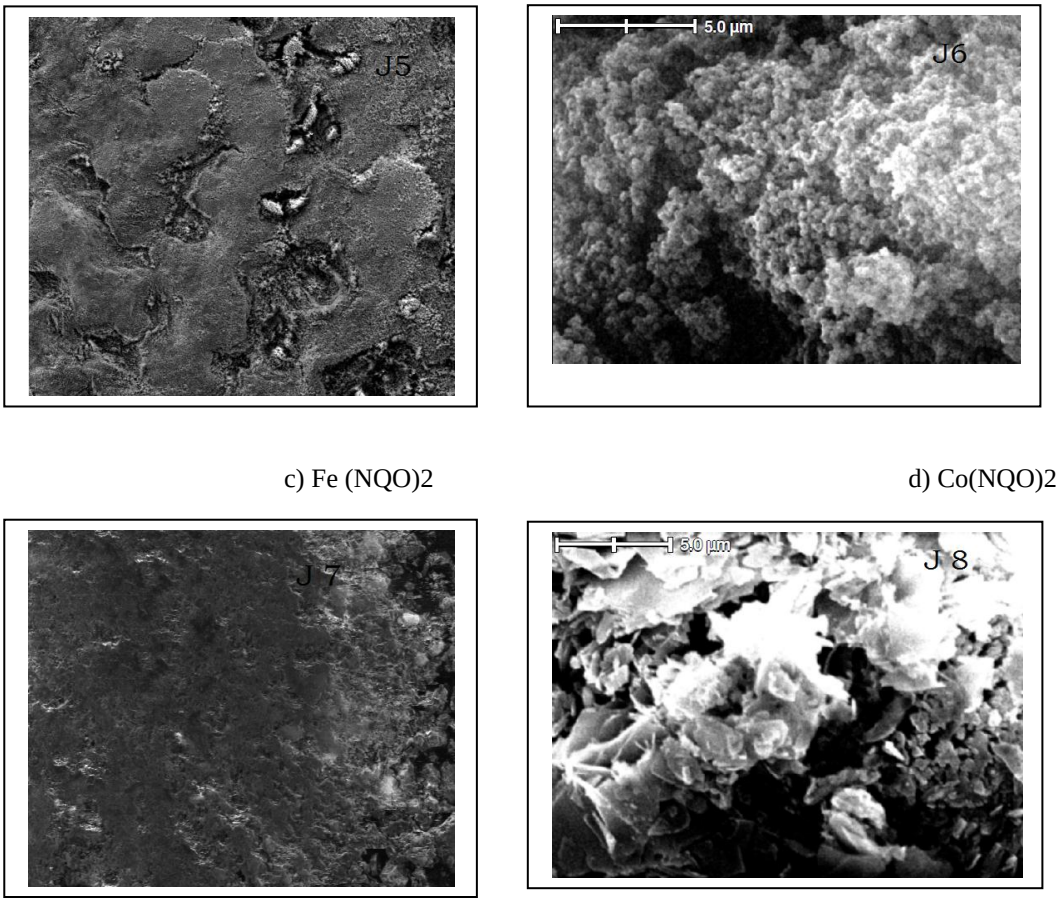
1. The ligand NQO is nanocrystals bound together forming cloud like structure. The cloudy structure is formed by very thin hair like threads firmly woven together.
2. Mn $(NQO)_2$ shows nanocrystalline fine threads protruding out in a bunch .The threads are closed on top by small crystals.
3. Fe $(NQO)_2$ is a continuous phase two planer structures showing a phase like molten mass with craters distributed randomly.
4. $Co(NQO)_2$ is shows particles clustered together like in a bunch of grapes
5. .Ni $(NQO)_2$ shows a continuous phase planer structure with grain boundaries merged together**.**
6. Cu $(NQO)_2$ shows platelet like structure grouping together to form a multilayered leafy structure resembling cabbage leaves like appearance.




a)NQO




b) Mn(NQO)2




c) Fe (NQO)2 d) Co(NQO)2

e) Ni(NQO)2 f) Cu(NQO)2

Fig. 4. SEM photographes of ligand and itschelates

**3.5 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.

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 $37^0$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.

Table5: Antimicrobial activities of 1, 2 naphthoquinone 1- oxime (NQO) and its metal chelates (Inhibition zone area in $mm^2$)

| Sr. No. | Comp. | S.aureus | B.subtilils | *P.vulgaris* | *E.coli* | *C.albicans* |
|---|---|---|---|---|---|---|
| 1 | NQO | 660.1 | 907.4 | 490.6 | 415.2 | 1163.5 |
| 2 | Mn(NQO)2 | 510.4 | 706.5 | 415.2 | 346.18 | 1074.6 |
| 3 | Fe(NQO)2 | 0 | 0 | 0 | 0 | 0 |
| 4 | Co(NQO)2 | 113.04 | 200.9 | 0 | 0 | 0 |
| 5 | Ni (NQO)2 | 0 | 213.7 | 132.6 | 94.9 | 176.6 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 | Cu(NQO)2 | 176.6 | 244.4 | 132.6 | 94.98 | 132.6 |
| 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 againstC. *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 J.Reedijk and P.H.M. Lohman (13) on the mechanism of binding DNA with ciaplatin. The formation of intrastrands cross-links between adjacent guanines to which the $Pt\ (NH3)_2^{2+}$ 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. Proteins, RNA, DNA (14-16). A significant losses o f metal do occur , since 50-70% of the administered metal is excreted within 24 hours (17). The remaining metal chelate eventually diffuses through the walls of (all kinds of) cells. H ydrolysis reactions will take place. Based on work of Martin (18). DNA is the most likely target. Early studies by several groups have shown that species specific interaction of metal chelate with D N A is an important event , which may eventually lead to cell killing. Induction of filamentous growthindicates that cell division is hamperedand cell growthis not (17). Induction of lysis in lysogenic bacteria also indicates interaction with DNA. Even a correlationbetween antitumour activity and prophage induction in lysogenic bacteria was found (19).Inhibition of DNA synthesis is selectively inhibited, whereas R N A and proteinsynthesis are not (20). 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 (21).In vitro studies with salmon sperm DNA by Fichtinger et.al.(22-23) 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 (24). Binding to CG, GC, GA, TG or GT units could not be demonstrated ( T = thymidine).Victoria Cepeda et.al.reported a current picture of the known facts pertaining to the mechanism of action of the drug, including those involved in drug uptake, DNA damage signals transduction, and cell death through apoptosis or necrosis. A deep knowledge of the biochemical mechanisms, which are triggered in the tumor cell in response to cisplatin injury not only may lead to the design of more efficient platinum antitumor drugs but also may provide new therapeutic strategies based on the biochemical modulation of cisplatin activity (25).

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