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Hemolytic and DNA binding studies of divalent transition metal ion based macrocyclic complexes

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New tetra-azamacrocyclic complexes of Ni (II) and Cu(II) have been synthesized by template methodology leading to the formation of complex of type $[MLX_2]$ where L is a macrcocyclic ligand obtained from the condensation of 4-Methyl-*o*-phenylenediamine (DAT) and 1,3-diphenylpropane-1,3-dione (DBM)and X= NO_3^-, Cl^-, and CH_3COO^-. Characterization of newly prepared complexes has been done by using various physico-analytical techniques like UV-visible, IR, ESR, CHN, Magnetic susceptibilities and PXRD.The non-electrolytic nature of the complexes was elucidated by lower value of molar conductance. The data received from various techniques give an indication towards the octahedral geometry of the complexes. The macrocyclic ring is present at the equatorial position whereas the axial positions are occupied by the ligands Cl^-, NO_3^- and CH_3COO^-. Screening of all the complexes has been performed against the pathogenic strains of microbes in order to check their antimicrobial potential. *Invitro*-hemolytic activity reveals about the extent to which lyses of hemoglobin takes place. The Herring fish sperm DNA interaction studies are carried out with the help of UV-absorption spectra. Molecular modeling was done through the assistance of software Chem 3D Ultra that gives the energy calculation and their quantum chemical parameter.

Keywords: Macrocycle, Antimicrobial, Molecular modeling, Hemolytic, Template methodology.

During recent years, much attention has been received towards the chemistry of macrocyclic complexes because of their wide importance in the field of industry, coordination chemistry and medical sciences[1,2]. The more significance of these macrocyclic complexes in chemistry is due to their tendency for chelating towards various transition metal ions depending upon nature, position, number of donor atom sites and size of the metal atom with which they are coordinated[3]. The behavior of macrocyclic complexes can be transformed to compel the central metal ion to acquire uncommon coordination geometry[4]. The best method of their preparation is through the help of metal ion as template for directing the condensation reaction towards ring closure[5, 6]. Large variety of macrocyclic complexes had been efficiently used as oxygen carrier to biomimic intricate living system[7, 8]. However, condensation reaction by template methodology lies within the heart of macrocyclic chemistry[9]. Many macrocyclic molecules that show biological activities like antifungal and antibacterial drug are already reported[10,11]. Because of the lot of requirement of recent metal based antibacterial drug and antifungal macrocyclic compounds, metalloorganic chemistry is turning into a salient space of research[12, 13].On the basis of above considerations and importance , present research work mainly intends to synthesize, characterize, haemolytic, DNA interaction and antimicrobial studies of the macrocyclic complexes.

Experimental Section

Material

The diamine (4-Methyl-*o*-phenylenediamine) and dicarbonyl (1,3-diphenylpropane-1,3-dione) compounds used for the synthesis work was procured from Sigma Aldrich. Blood sample was taken from healthy volunteer. Metal salts were obtained from Merck and Fluca. Analytical grade solvents were used without further purification. DNA was procured from SRL, India.

Synthetic pathway

The tetrazamacrocyclic complexes were synthesized by template condensation. First of all to the hot methanolic solution of 4-Methyl-*o*-phenylenediamine (1.22 g, 10 mmol) added 5mmol of divalent metal salts. The refluxing was continued for about 0.5 hrs. As the change in colour was noticed, there was addition of (2.24 g, 10 mmol) methanolic solution of 1, 3-diphenylpropane-1, 3-dioneand keep

the reaction mixture under refluxing for 6-9 hrs. Now, cool the reaction mixture for overnight, various colour precipitates appeared. Multiple times washings were given to the precipitates with methanol, acetone and diethyl ether and then dried *in vacuo*. The solubility of complexes was found good in Dimethyl sulfoxide and value of molar conductance lies in between 17-28 $ohm^{-1}cm^{2}mol^{-1}$. The scheme of the synthesis is shown in Scheme 1.

Physical and analytical measurements

Infrared spectra for all the complexes were recorded on Fourier Transformed IR spectrophotometer (Agilent Technologies) from 4000-400 cm^{-}. The electronic spectra in solution state using DMSO as solvent were taken by using instrument Thermo scientific Evolution 201 Spectrophotometer. Melting points were recorded on Automatic melting point apparatus. Analysis of C, H and N was done on Euro Elemental Analyzer at NIT, Kurukshetra. Electron Paramagnetic Resonance spectrum of complex of Cu(II) was taken at room temperature on Model JES FA200 at SAIF, IIT-Madras.

X-ray crystallography

PXRD analysis of the complex was recorded on Pan-Analytical XPERT-PRO instrument by using Cu as anode material and $K\alpha$ = 1.504[Å]. The range of 2θ was taken between 10-80 degrees with step size [°2Th.] = 0.0100. Analysis of the peaks, hkl and d-spacing were done using software Full Proof suite.

Biological studies

All the synthesized complexes were evaluated for various biological activities like haemolytic activity against blood and antimicrobial evaluation against some pathogenic strains of bacteria and fungi. In addition to these, complexes were also exposed for DNA interaction studies.

Antimicrobial screening

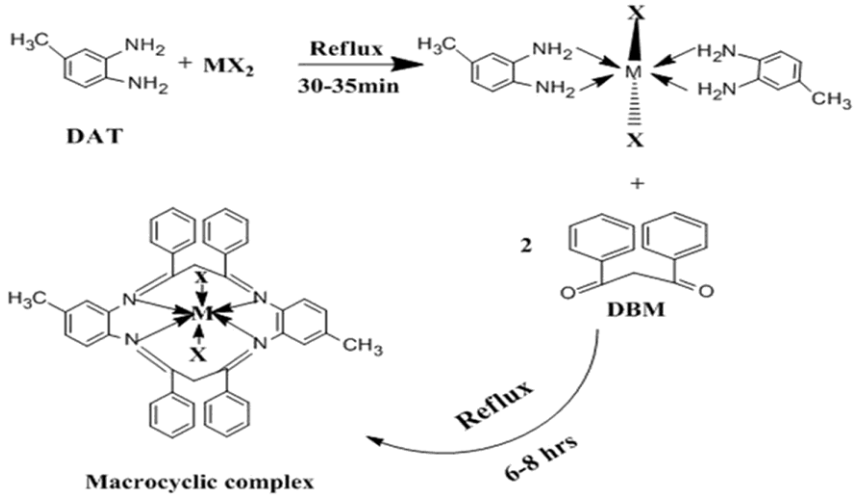
Five strains of pathogenic microbes were taken in the present study. These strains were *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 1652), *Pseudomonas aeruginosa* (MTCC 741), *Candida albicans* (MTCC 9062) and *Saccharomyces cerevisiae* (MTCC). Agar well diffusion method was used for the determining their MIC values.

Haemolytic activity

After collecting the blood sample this was kept for centrifugation at 1000 rpm for 10 min. Now the supernatant was removed and washings were given for 3-4 times with PBS buffer. Centrifugation was continued during washing. Finally PBS was added to make the sample dilute. From the above solution 100 µL were added to the solution of test samples (synthesized macrocyclic complexes) at a different concentration of 36, 72,108,144,180 µg/mL. Millipore water (216) was take as +ve control and PBS (0) was taken as -ve control. Now, the solution was kept for incubation at 37°C for 1 h. After incubation, tubes were centrifuged at 1000 rpm for further 15 min. 200 µL of the supernatant was pipetted out and transferred to the 96 well micro plate reader and absorbance was noted spectrophotometrically at 540 nm.

DNA interaction studies

The DNA binding studies of the two synthesized macrocyclic complexes were evaluated by the help



M= Ni(II) and Cu(II), X=NO3-, Cl-, and CH3COO-

SCHEME-1

of UV-visible spectra taken at room temperature. The ratio of absorbance at 260 and 280 nm lies within the range of 1.8-1.9 indicated the purity of DNA and free from proteins. The highly concentrated stock solution (10^{-3}M) of the tested complexes was made by dissolving the complexes in DMSO. The concentration of the DNA was varying taken from 0.00 to 44 × 10^{-10} mol L^{-1}. In the current study, the total volume of the solution is adjusted to 2215-2275 µL by taking 2200 µL buffer and 10 µL DMSO and 5 µL of complex and changing the amount of DNA.

Results and Discussion

Spectroscopic characterisations

Infra red spectra

The showing of medium intensity peak at ~2800-2900 cm$^-$ can be allocated to the stretching frequency of C-H bond. Absence of any peak in the range of ~1700-1720 (ketone) cm$^-$and ~3300-3400 (amine) cm$^-$and presence of a new peak at ~1597 cm^{-1} leads to confirmation that formation of Schiff base has taken place[14] and new band is assigned to azomethine (C=N) linkage[15]. The lowering shift of the frequency is because of drifting of electron density of lone pair of Nitrogen doubly bonded to carbon of the azomethine group to divalent central metal ion[16]. Existence of medium intensity bands at ~1410-1450, ~1300-1325 and ~1015-1030 cm^{-1} in the spectra of the complex shows that the denticity of the NO_3^- group is unidentate with the metal ion of the macrocyclic complexes[17].[Fig. S1]

Physico-analytical data

The elemental analysis data and the analytical data of the synthesized complexes (A1-A6) are given in Table 1 and 2, respectively.

Electron paramagnetic resonance spectrum

Electron paramagnetic Resonance evaluation of the macrocyclic complexes of Copper (II) was taken at X-Band Frequency of 9.4 GHz at ambient room temperature. The field strength (magnetic) used was 3000 Gauss (Fig. 1). The calculated values of g_{II} and $g_{\perp}$ for the complex persuade the condition $g_{II} > g_{\perp} > 2.0023$ that shows the unpaired or odd electron occupy the $d_{x^2-y^2}$ orbital. This manifests distorted elongation of Copper (II) complexes from octahedral symmetry to D_{4h} symmetry. The *G* value reveals significant exchange interaction of the complex as the value is less than 4.

Electronic spectra and magnetic moment

The electronic spectra of all synthesized complexes were determined in DMSO in the absorption region of 250-1000 nm. The entire macrocyclic complex showed different absorption bands corresponding to different electronic transition within the molecule.

Nickel complex

The absorption spectrum of the Ni(II) complex displayed four absorption bands in between ~820-880 nm, ~600-700 nm, ~400-450 nm and ~280-

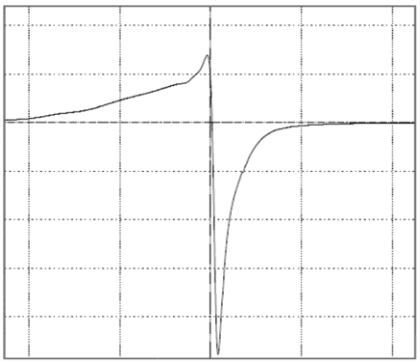


Fig. 1 — EPR spectrum

Table 1 — Elemental analysis data

Exp. (calcd.) %				
Complex	M	C	H	N
A1	7.66(7.82)	70.25(70.43)	4.72(4.84)	7.42(7.47)
A2	7.22(7.36)	72.14(72.28)	5.29(5.31)	7.01(7.02)
A3	7.28(7.30)	65.62(65.77)	4.47(4.52)	10.33(10.46)
A4	8.31(8.40)	69.86(69.97)	4.77(4.80)	7.29(7.42)
A5	7.82(7.86)	65.22(65.38)	4.43(4.49)	10.39(10.40)
A6	7.86(7.92)	71.82(71.85)	5.24(5.28)	6.94(6.98)

Table 2 — Analytical data of the synthesized complexes (A1-A6)

Complex	M. Formula	M. Wt.	Yield (%)	M.pt	Colour
A1	$[Ni(C_{44}H_{36}N_4)Cl_2]$	750.4	52	243	Green
A2	$[Ni(C_{44}H_{36}N_4)(OAc)_2]$	797.5	64	251	Dark green
A3	$[Ni(C_{44}H_{36}N_4)(NO_3^-)_2]$	803.5	57	240	Yellowish green
A4	$[Cu(C_{44}H_{36}N_4)Cl_2]$	755.2	62	260	Black
A5	$[Cu(C_{44}H_{36}N_4)(OAc)_2]$	808.3	58	265	Greenish black
A6	$[Cu(C_{44}H_{36}N_4)(NO_3^-)_2]$	802.4	49	270	Black

300 nm. (Fig. S2) These bands are in accordance with the three spin allowed transition i.e. $^3A_{2g} \rightarrow {}^3T_{2g}$ (F), (ν_1) $^3A_{2g} \rightarrow {}^3T_{1g}$ (F), (ν_2) and$^3A_{2g} \rightarrow {}^3T_{1g}$ (P), (ν_3) respectively[18] and band at 280-300 nm may be due to π→π* transition. The magnetic moment value of the complex was 2.90-3.00 B.M. showing that presence of two unpaired electrons.

Copper complex

The Magnetic moment value of the Cu (II) metal ion in the macrocyclic complexes is 1.60-1.70 BM. Appearance of three absorption band at ~600-680 nm, ~380-410 nm and ~280-300 nm may be due d-d transition (xy→x^2-y^2 and xz,yz→ x^2-y^2) and higher energy band is due to π→π*, respectively .Distorted Octahedral geometry of the complex was revealed by these transition bands[19].

PXRD

The PXRD data of the complex obtained from the template condensation of 4-methyl-*o*-phenylenediamine(DAT) and 1,3-diphenylpropane-1,3-dione (DBM)reveals that the representative complex possesses the triclinic crystal system. The diffractogram shows peaks that are not very much clear but points towards the slight crystalline nature of the complex. The interspacing and hkl values are shown in Table 3. The calculated cell parameter are a= 4.4928, b = 6.4275, c= 7.3681 and angles are α=105.068, β=101.853, γ= 90.937 *Particle size of the complex*

The size of particle of the complex was determined from its patterns of XRD difffractogram. The high intensity peak was obtained at 24.92 (2θ). By using the Debye-Scherrer formula that can be mathematically represented as:

$D = 0.94 \lambda / \beta \cos\theta$

In the above mentioned equation D = is the apparent particle size

λ = is the incident X-ray wavelength

β = is the full width at half maximum (FWHM) of the given XRD peak (characteristic)

cosθ = position of the particular diffraction peak

From obtained data λ is having the value of 1.54 (A°) and value of β is 0.01676 in radians. The value of cos θ is 0.9764. So calculated results showed that the size of particle in nm is 88.48.

Biological studies

Hemolytic activity

The haemolytic activityand % hemolysis of the two complexes are shown in Fig. 2 and Table 4. It is observed from the figure that macrocyclic complex of Ni(II) was found to have high haemolytic activity as compared with themacrocyclic complex of Cu(II) and the haemolytic activity follows the order Ni(II) >

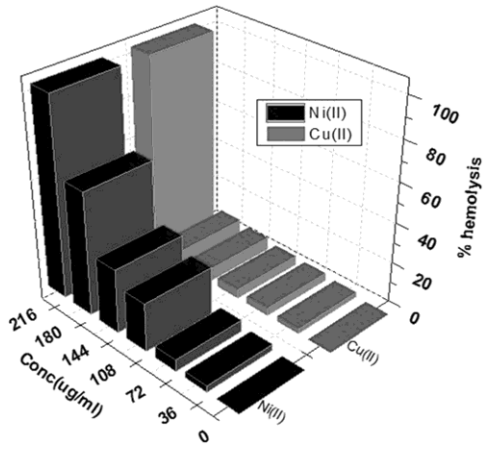


Fig. 2 — Bar graph showing % hemolysis of the synthesized complex

Table 4 — % Hemolysis of the synthesised complex

Conc.(ug/mL)	Ni(II) Complex	Cu(II) Complex
0	0	0
36	3.03122	2.39404
72	5.74149	3.79078
108	23.85291	4.45782
144	31.53262	6.34497
180	61.11679	8.02255
216	99.09545	99.09545

Table 3 — X-ray diffraction data

H	K	L	DOBS	DCAL	DOBS-DCAL	2TH.OBS	2TH.CAL	DIF.2TH
1	0	0	6.92219	6.92215	0.00004	12.778	12.778	0.00000
0	1	0	6.17139	6.17141	0.00003	14.340	14.340	0.00000
1	-1	0	5.38456	5.38456	0.00000	16.450	16.450	0.00000
0	1	1	4.37596	4.37597	0.00001	20.277	20.277	0.00000
1	-2	-1	3.58609	3.58608	0.00001	24.808	24.808	0.00000
3	-2	-1	2.29299	2.29299	0.00000	39.259	39.259	0.00000
2	-3	0	2.02790	2.02790	0.00000	44.649	44.649	0.00000
2	-1	-1	1.90818	1.90818	0.00000	47.617	47.617	0.00000

Cu(II). The results are in accordance with the trends already present in the literature[20]. According to ASTM if percentage haemolysis is less than 2 the substance is measured as non-haemolytic, if the percentage haemolysis lies in between 2–5 the substance is very less haemolytic and percentage haemolysis is greater than 5% the complexis considered as haemolytic. So according to ASTM, standards both Ni(II) and Cu(II) complexes were found to be non haemolytic at[21] lower conc. Some compounds have the potential to damage the membrane of the erythrocytes, as they can scathe the cytoskeleton lipids or protein or as they can aggravate a structural destabilization of the membrane by the penetration of the molecule into phospholipids bilayer, consequences of which breakdown of RBC's occur. The % hemolysis is calculated by formula given in Fig. S5

Antimicrobial study

The entire newly synthesized complex possesses fair antimicrobial activities against pathogenic strains of microbes. Complexes A3 and A4 showed good results among all the macrocyclic complexes. Large number of factors like charge, solubility and conductance are influencing the metal ion that might be probable reason for the biological activities of the complexes (Table 5 and Fig. 3). It was also noticed that moieties like C=N linkage when introduced into the compounds showed great extent of biocidal activities that could be answerable for the amplification in the hydrophobic character and lipid solvability of the molecules in crossing the cellular bio membrane of the organism, thus enhancing the biological consumption ratio and activity of such complexes[22].

DNA interactions studies

The most important universally employed methodology for studying the extent and mode of DNA binding is Uv-absorption spectrum studies. The titrations were done by keeping the concentration of the complexes as constant and varying the conc. of DNA.During the absorption studies an equal amount of DNA is added to both reference and compound solution so that absorption due to DNA itself is eliminated. Metal complexes can bind to DNA through various interactions like hydrogen bonding, Vander Walls forces and electrostatic interaction. π-stacking type of interactions of the aromatic group in between the nitrogenous base pairs of DNA are also present[23]. The binding modes are also decided by various factors like type of donor atom, planarity of the ligand and coordination geometry[24]. Titrations were continuously done until the saturation occurred. Upon subsequently increasing the concentration of DNA hyperchromism (Fig. 4) is observed, this showed that the complexes are interacting with DNA may be due to groove binding along the outside of DNA helix or due to electrostatic and hydrogen bonding[25]. The K_b value for Ni(II) and Cu(II) are 0.29×10^5 and $0.31 \times 10^5 M^{-1}$, respectively.

Computational studies

The geometry optimization of the newly synthesized macrocyclic metal complexes were achieved by HF method with 3-21G basis set. The

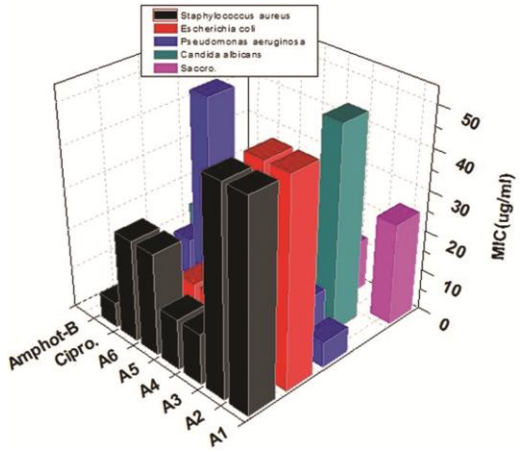


Fig. 3 — Bar graph indicating MIC of the complex

Table 5 — MIC(μg/mL) of the synthesized complexes

Complex	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Saccharomyces cerevisiae
A1	50	50	6.25	--	25
A2	50	50	12.5	50	--
A3	12.5	6.25	6.25	25	12.5
A4	12.5	12.5	6.25	25	6.25
A5	25	12.5	12.5	6.25	25
A6	25	6.25	50	25	--
Ciprofl.	6.25	6.25	12.5	-	-
Amph.-B	-	-	-	12.5	12.5

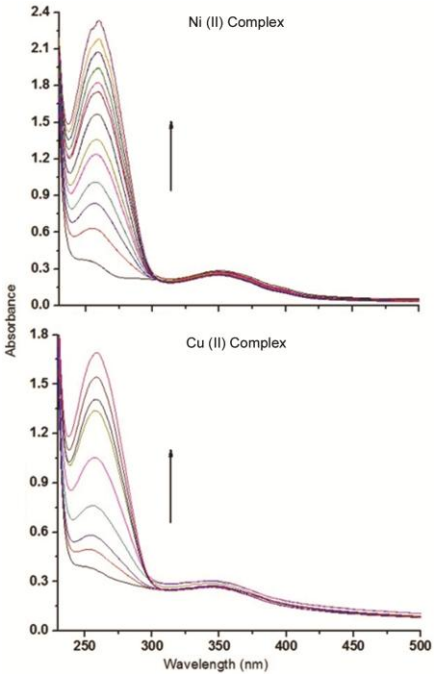


Fig. 4 — Absorption spectrum of the complex with increasing conc. of DNA

structure of the metal complexes were built with the assistance of Perkin-Elmer Chem Bio Draw and optimization was done using Perkin Elmer ChemBio 3D software. The energy gap ΔE (HOMO-LUMO) is a major stability index and is useful for the development of theoretical models for determining the conformational and structural barrier in various molecular systems. The reactivity of the compound can be easily interpreted on the basis of ΔE. The quantum chemical parameter (calculated) is represented in Table 6 and Fig. 5. Several other parameters like absolute hardness (η), global softness (S), electronic charge, ΔN_{max} , global electrophilicity (ω), absolute electro negativity (χ) and separation energy (ΔE) were calculated by the help of following below given equation (1-8)

$$\Delta E = E_{LUMO} - E_{HOMO} \quad \ldots(1)$$

$$\chi = -(E_{HOMO} + E_{LUMO})/2 \quad \ldots(2)$$

$$\eta = (E_{LUMO} - E_{HOMO})/2 \quad \ldots(3)$$

$$\sigma = 1/\eta \quad \ldots(4)$$

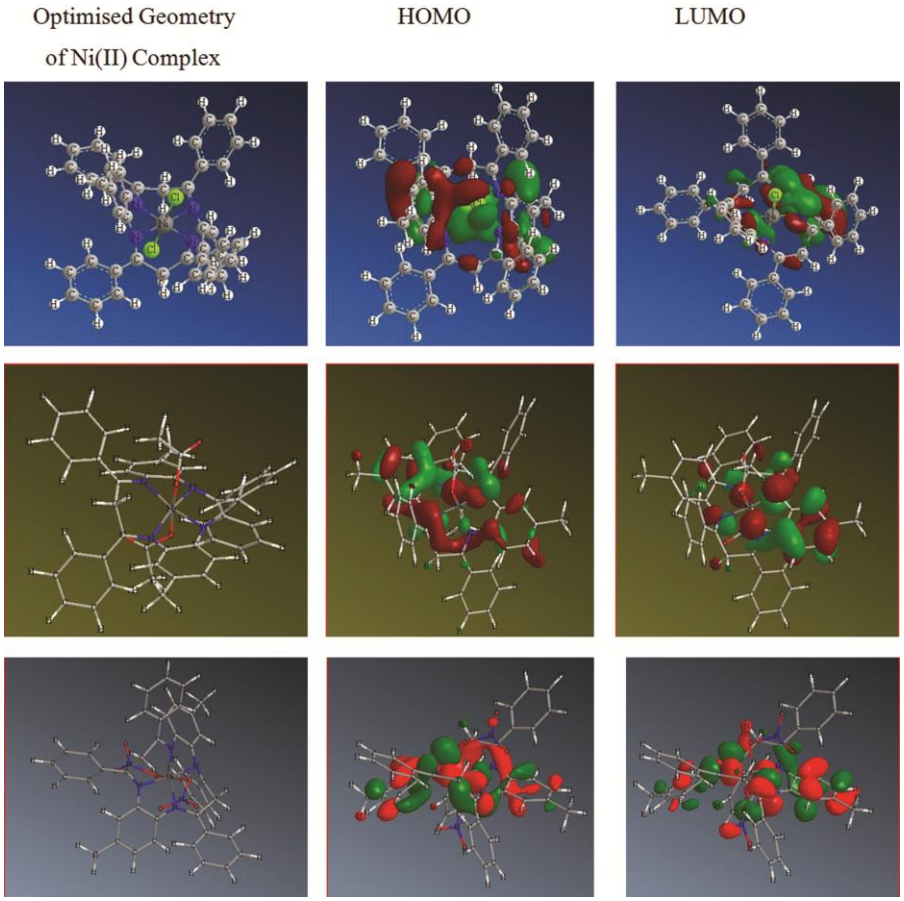


Fig. 5 — Geometry optimised structure and their HOMO & LUMO

Table 6 — Calculated quantum chemical parameter of the synthesized complexes

Comp.	E_{min}.(Kcal/mol)	E_{HOMO}	E_{LUMO}	ΔE	χ	η	σ	Pi	S	ω	N_{max}
A1	314.8	-6.69	-5.91	0.7	6.3	0.39	2.5	-6.30	1.28	50.8	16.1
A2	338.8	-7.35	-5.74	1.6	6.5	0.80	1.2	-6.54	0.62	26.6	8.1
A3	260.2	-7.21	-5.38	1.8	6.2	0.91	1.0	-6.29	0.54	21.6	6.8
A4	512.7	-4.65	-3.60	1.0	4.1	0.52	1.9	-4.13	0.95	16.3	7.9
A5	515.7	-4.64	-4.32	0.3	4.4	0.16	6.1	-4.48	3.07	61.8	27.5
A6	1040.0	-3.89	-3.19	0.7	3.5	0.35	2.8	-3.54	1.42	17.9	10.1

$$Pi = -\chi \quad \ldots (5)$$

$$S=1/2\ \eta \quad \ldots (6)$$

$$(\omega)= Pi^2/2\ \eta \quad \ldots(7)$$

$$\Delta N_{max}=-Pi/\ \eta \quad \ldots (8)$$

Conclusion

On the behalf of various spectroscopic characterization and magnetic moment measurements (μ) six coordinated octahedral geometry have been proposed for all the macrocyclic complexes of divalent transition metal ions. The biological activities reveal that complexes have wide range of bactericidal and fungicidal potentialities. The *in vitro* hemolytic assay showed that complexes exhibited negligible hemolytic activity at lower concentrations. Computational results point towards that complex A3 is most stable among all the synthesized complex.

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