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# Macrocyclic nickel(II) complexes: Synthesis, characterization, superoxide scavenging activity and DNA-binding

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# ABSTRACT

A new series of nickel(II) complexes with the tetraaza macrocyclic ligand have been synthesized as possible functional models for nickel-superoxide dismutase enzyme. The reaction of 5-amino-3methyl-1-phenylpyrazole-4-carbaldehyde (AMPC) with itself in the presence of nickel(II) ion yields, the new macrocyclic cationic complex, [NiL(NO<sub>3</sub>)<sub>2</sub>], containing a ligand composed of the self-condensed AMPC (4 mol) bound to a single nickel(II) ion. A series of metathetical reactions have led to the isolation of a number of newly complexes of the types  $[NiL]X_2$ ; X = ClO<sub>4</sub> and BF<sub>4</sub>,  $[NiLX_2]$ , X = Cl and Br (Scheme 1). Structures and characterizations of these complexes were achieved by several physicochemical methods namely, elemental analysis, magnetic moment, conductivity, and spectral (IR and UV-Vis) measurements. The electrochemical properties and thermal behaviors of these chelates were investigated by using cyclic voltammetry and thermogravimetric analysis (TGA and DTG) techniques. A distorted octahedral stereochemistry has been proposed for the six-coordinate nitrato, and halogeno complexes. For the four-coordinate, perchlorate and fluoroborate, complex species a square-planar geometry is proposed. The measured superoxide dismutase mimetic activities of the complexes indicated that they are potent NiSOD mimics and their activities are compared with those obtained previously for nickel(II) complexes. The probable mechanistic implications of the catalytic dismutation of  $O_2^-$  by the synthesized nickel(II) complexes are discussed. The DNA-binding properties of representative complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> have been investigated by the electronic absorption and fluorescence measurements. The results obtained suggest that these complexes bind to DNA via an intercalation binding mode and the binding affinity for DNA follows the order:  $[NiLCl_2] \Box [NiL](PF_4)_2$ .

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# 1. Introduction

Superoxide anion  $(O_2^{-})$ , the one-electron reduction product of dioxygen  $(O_2)$ , is a toxic reactive oxygen species [1]. In eukaryotes, its principal source is the mitochondrial inner membrane, both sides of which release superoxide as a byproduct of aerobic metabolism [1]. Superoxide anion and the reactive oxygen species (ROS) that be derived from it is the respiratory chain within the mitochondria [2]. ROS include hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide. They are very transient species due to their high chemical reactivity that leads to broad array of diseases [3] including autoimmune. inflammatory disorders such as rheumatoid arthritis (RA) and ischemic diseases as well as organ failure from species or trauma. There is much evidence implicating oxidative stress and mitochondrial dysfunction in Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis (ALS), and, indeed, in aging and age-associated decline [4]. By catalyzing the conversion of  $O_2^{-}$  to  $H_2O_2$  and  $O_2$ , superoxide dismutase (SOD) represent the first line of defense against  $O_2^{-}$ . Preclinical studies have revealed that SOD enzymes play a protective effect in animal models of several diseases [5]. However, the therapeutic use of the native SOD has several limitations related with low cell permeability and short half-life. In addition, bovine SOD was tested in clinical trials but immunological problems lead to its withdrawal from the market [6]. To overcome this problem, synthetic SOD mimetic compounds have emerged as a potential novel class of drugs. A list of diseases that may be treatable with SOD mimics now includes heart attacks, stroke, autoimmune diseases (such as osteoarthritis), neurodegenerative diseases (including Alzheimer's and Parkinson's) and aging [7]. It is the first time that a molecule is a totally selective SOD catalyst. And by eliminating superoxide, the production of pro-inflammatory cytokines can be also eliminated. Therefore selective non-toxic superoxide substituents for SODs are desirable for medical purposes [8]. Artificial scavengers with various metal ions (Mn, Fe and Cu) have been prepared and tested. Out of these manganese is the most suitable transition metal for pharmaceutical applications due to its relatively low toxicity [9]. However other transition metal complexes are also studied to help elucidating structure/activity relationships and other properties





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influencing SOD activity. Among these SOD-functional models, metal complexes with macrocyclic ligands constitute an important group [10].

Two classes of superoxide detoxification enzymes are known: superoxide dismutase (SOD) enzymes and superoxide reductase (SOR) enzymes [1]. The most recently identified superoxide dismutases is nickel superoxide dismutase (NiSOD). This enzyme has been isolated from a number of Streptomyces bacteria and from cyanobacteria. The crystal structure has been published by two groups [11,12] and show a hexameric structure with a molecular weight of approximately 80,000 Daltons, where each monomeric unit contains what has been described as a "nickel-hook." Each nickel is bound by a small peptide chain with binding ligands that consist of two amides, one from the N-terminal and one from the backbone of cysteine 2, two thiolates, one from cysteine 2 and one from cysteine 6, and an imidazole from histidine 3. This is the first SOD in which the binding ligands are anything other than histidines, aspartates and water/hydroxide. The nickel is redox active but the details of the mechanism are still to be unraveled, in large part because of the complexity of making nickel redox active with superoxide.

One significant difference between NiSOD and the other SODs is the apparent lack of ionic strength effect on the catalytic rate constant [13], suggesting there is limited need to generate an electrostatic funneling mechanism. Thus far, preparation of a short chain peptide to mimic the nickel hook region has failed to produce a functional NiSOD mimic although it has produced spectroscopic mimics [14]. The role of the protein is clearly crucial but the details remain to be elucidated. The enzymatic activity of NiSOD is as high as that of Cu–ZnSOD at about  $10^9 M^{-1} S^{-1}$  per metal center. Recently some low molecular weight nickel(II) complexes mimicking superoxide dismutase (SOD) activity with high catalytic activity have been reported [15].

The design of small molecules that bind and react at specific sequences of DNA under physiological conditions via oxidative and hydrolytic mechanisms has been attracted great interest in the field of bioinorganic chemistry [16-18]. Intercalators are small molecules that contain a planar aromatic heterocyclic functionality which can insert and stack between the base pairs of double helical DNA [19]. Recently interaction of transition metal complexes with DNA has attracted much attention [20]a-e. In general, transition metal complexes can interact non-covalently with DNA by intercalation, via groove binding or external electrostatic binding. Among the factors governing the binding modes, it appears that the most significant is likely to be that of molecular shape. Studies reveal that the ligand modifications in geometry, size, hydrophobicity, planarity, the charge and hydrogen-bonding ability of the complexes, may lead to subtle or to spectacular changes in the binding modes, location, affinity, and to a different cleavage effect [21]. These properties have turned chemical nucleases into useful tools as adjuvant in PCR diagnostics [22], nucleic acid attacking and cleaving agents [23]. Therefore, extensive studies using different structural transition metal complexes to evaluate and understand the factors that determine the mode of binging interactions with DNA and the cleavage mechanisms are necessary. A broad variety of transition metal complexes have been widely exploited for these purposes not only because of their unique spectral and electrochemical signatures but also due to the fact that by changing the ligand environment one can tune the DNA binding and cleaving ability of a metal complex [24]. Several efficient cleaving agents have been developed in course of time. However, most of the studies are dealing about non-cyclized mononuclear metal complexes. In that few of them are reported as macrocyclic mononuclear metal complexes which show greater cleaving efficiency or DNA interaction than the non-cyclized complexes [25].

Many synthetic routes to macrocyclic ligand involve the use of the metal ion template to orient the reacting groups of the reacting substrates in the desired conformation for the ring closure. The favorable enthalpy for the formation of metal ligand bonds overcomes the unfavorable entropy of the ordering of the multidentate ligand around metal ion thus promotes the cyclization reaction [26]. This work describes the synthesizes and characterization of a series of new tetraaza macrocyclic nickel(II) complexes. It has been documented in the literature that small molecule SOD mimics exhibit DNA binding ability and nuclease activity [27]. Hence, before studying the DNA interaction of the complexes, we examined the superoxide scavenging activity by phenazine methosulphate (PMS) and nitroblue tetrazolium (NBT) assay. The DNA binding activity was also investigated and is described in this study.

# 2. Experimental

# 2.1. Materials

All chemicals used were of analytical grade. 5-amino-3-methyll-phenylpyrazole-4-carbaldehyde (AMPC) was prepared according to the method described elsewhere [28a].

# 2.2. Synthesis of the tetraaza macrocyclic [Ni(II)L(NO<sub>3</sub>)<sub>2</sub>] complex

A solution of freshly prepared 5-amino-3-methyl-l-phenylpyrazole-4-carbaldehyde (0.024 mol) in absolute ethanol (50 ml) was heated and stirred. While the 5-amino-3-methyl-l-phenylpyrazole-4-carbaldehyde solution was under reflux, a solution of the appropriate amount of nickel nitrate Ni(NO<sub>3</sub>)<sub>2</sub>  $6H_2O$  in 50 ml of absolute ethanol was added. The reaction mixture was boiled under reflux and stirred for 24 h, during which time colored products precipitated. After cooling, the solution was filtered and the isolated solid was washed with ethanol and ether and finally dried *in vacuum* over CaO at room temperature for 2 weeks.

## 2.3. Synthesis of [NiLX<sub>2</sub>] and [NiL]X<sub>2</sub> complexes

Metathetical displacement of the nitrate ion was accomplished by dissolving  $[NiL(NO_3)_2]$  in hot MeOH, and adding an excess of NaX (X = Cl, Br, ClO<sub>4</sub> or BF<sub>4</sub>) aqueous solution. The reaction mixture was stirred for 60 min at room temperature. In most cases the complex salts precipitated after slow evaporation of MeOH. The colored precipitates were isolated by filtration, washed with water, ethanol and finally dried in vacuum at room temperature over CaO.

## 2.4. Physical measurements

The I. R. spectra were recorded using KBr disks in the 4000– 200 cm<sup>-1</sup> range on a Unicam SP200 spectrophotometer. The electronic absorption spectra were obtained in DMF solution with a Shimadzu UV-240 spectrophotometer. Magnetic moments were measured by Gouy's method at room temperature. ESR measurements of the polycrystalline samples at room temperature were made on a Varian E9 X-band spectrometer using a quartz Dewar vessel. All spectra were calibrated with DPPH (g = 2.0027). The specific conductance of the complexes was measured using freshly prepared  $10^{-3}$  M solutions in electrochemically pure MeOH or DMF at room temperature, using an YSI Model 32 conductance meter. The thermogravimetric measurements were performed using a Shimadzu TG 50-Thermogravimetric analyzer in the 25–800 °C range and under an N<sub>2</sub> atmosphere. Elemental analyses (C, H, and N) were carried out at the Micro analytical Unit of Cairo University.

## 2.5. Electrochemical measurements

Cyclic voltammetric measurements were performed by a computerized Electrochemical Trace Analyzer Model 273A-PAR (Princeton Applied Research, Oak Ridge, TN, USA) controlled via 270/250 PAR software was used for the cyclic voltammetry measurements. The electrode assembly (Model 303A-PAR) incorporating of a micro-electrolysis cell of a three electrode system comprising of a hanging mercury drop electrode as a working electrode (area:  $0.026 \text{ cm}^2$ ), an Ag/AgCl/KCl<sub>s</sub> reference electrode and a platinum wire counter electrode, was used. Stirring of the solution in the micro-electrolysis cell was performed using a magnetic stirrer (305-PAR) to provide the convective transport during the preconcentration step. The whole measurements were automated and controlled through the programming capacity of the apparatus. The supporting electrolyte used was 0.1 M n-Bu<sub>4</sub>NClO<sub>4</sub>. The solvents used were sufficiently pure and the concentration of the complexes was 0.001 M.

#### 2.6. Superoxide dismutase assay

SOD mimetic catalytic activity of the investigated copper(II) complexes was assayed by using phenazine methosulphate (PMS) to photogenerate a reproducible and constant flux of  $O_2^-$  at pH = 8.3 (phosphate buffer). Reduction of nitroblue tetrazolium to blue formazan was used as an indicator of  $O_2^-$  production and followed spectrophotometrically at 560 nm. The addition of PMS ( $9.3 \times 10^{-5}$  M), and phosphate buffer (final volume of 2 ml) caused a OD( $\Delta_1$ )<sub>560</sub>/min change of 0.035. The reaction blank samples and in the presence of nickel(II) complexes was measured for 5 min. Each experiment was performed in duplicate and the SOD activity has been defined as the concentration of the tested compound for the 50% inhibition of the NBT ( $1.7 \times 10^{-4}$  M) reduction (IC<sub>50</sub> value) by the superoxide anion radical ( $O_2^-$ ) produced.

## 2.7. DNA-binding studies

#### 2.7.1. Absorption spectra method

DNA binding experiments were carried out in 0.1 M phosphate buffer (pH 7.2) using a solution of calf thymus (CT) DNA which gave a ratio of UV–Vis absorbance at 260 and 280 nm ( $A_{260}/A_{280}$ ) of *ca*. 1.8 indicating that the CT-DNA was sufficiently protein free. The concentration of DNA solution was determined by the absorbance at 260 nm using  $\varepsilon_{260}$  value of 6600 M<sup>-1</sup> cm<sup>-1</sup> at 260 nm as reported in the literature [28b]. Absorption titration experiments of nickel(II) complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> were performed using a fixed complex concentration to which increments of the DNA stock solution were added. Complex solutions employed were 20  $\mu$ M in concentration and calf thymus DNA was added to give a ratio of 8:1 [DNA]/[complex]. Complex-DNA solutions were allowed to incubate for 10 min before the absorption spectra were recorded.

#### 2.7.2. Fluorescence spectra measurements

The fluorescence spectra was employed to determine the relative DNA binding properties of the complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> to CT-DNA in 50 mM Tris–HCl/1 mM NaCl, buffer, pH 7.5. Fluorescence intensities of the examined complexes at 305 nm with an excitation wavelength of 285 nm were measured at different CT-DNA concentrations. Reduction in the emission intensity was observed with addition of CT-DNA. The relative binding tendency of the complexes to CT-DNA was determined from Stern–Volmer plot. Plotting the relative emission intensities ( $I_0/I$ ) against [DNA] yields a linear Stern–Volmer plot that describes the static quenching process.  $I_0$  and I are the fluorescence intensities of complex in the absence and presence of DNA (quencher) respectively. The slope of this plotted line yields  $k_{sv}$ , the static quenching constant or associative equilibrium constant which measure the binding ability of the complexes to CT-DNA.

# 3. Results and discussion

# 3.1. General

The reaction of 5-amino-3-methyl-1-phenylpyrazole-4-carbaldehyde (AMPC) with itself in the presence of nickel(II) ions yields, new macrocyclic cationic complex, [NiL(NO<sub>3</sub>)<sub>2</sub>], containing a ligand composed of the self-condensed AMPC (four mol) bound to a single nickel(II) ion. A series of metathetical reactions have led to the isolation of a number of newly complexes of the types  $[NiL]X_2$ ; X = ClO<sub>4</sub> and BF<sub>4</sub>,  $[NiLX_2], X = Cl and Br (Scheme 1).$  These isolated complexes have been identified and characterized, principally, based on the analytical data as complexes containing the fully cyclized ligand (L). However, attempts to synthesize the corresponding metal-free macrocyclic ligand did not prove successful. All the complexes are microcrystalline in nature, stable to atmosphere and are soluble in the polar solvents such as DMF and DMSO at room temperature. The results of elemental analysis (Table 1) agree well with the proposed mononuclear structure of these Schiff-base macrocyclic complexes. The formation of the Schiff base macrocyclic frame work was further confirmed by IR-spectra and TGA data. The halide or nitrate ions were found to be coordinated to the metal ion as confirmed by thermal analysis data, values of magnetic moments and conductivity measurements. The molar conductivity data for ca.  $10^{-3}$  M of [NiLX<sub>2</sub>] (X = NO<sub>3</sub>, Cl or Br), in DMF solutions at room temperature are in accordance with those expected for a nonelectrolyte, while the measured value for  $[NiL]X_2$  (X = ClO<sub>4</sub> or BF<sub>4</sub>), under the same conditions is as expected for a 1:2 electrolyte [29]. These observations imply that the counter anions in the nitrato and halogeno complex species are coordinated to the nickel(II) ion, while ClO<sub>4</sub> or BF<sub>4</sub> in [NiL]X<sub>2</sub> solution do not participate in coordination. Several attempts failed to obtain a single crystal suitable for X-ray crystallography. However, the analytical, spectroscopic and magnetic data enable us to predict the possible structure of these newly synthesized complexes (Scheme 1).

# 3.2. Infrared spectra

The IR spectra in the (4000–200 cm<sup>-1</sup>) region provide information regarding the coordination mode in the complexes and were analyzed by comparison with data for the free AMPC and the pure isolated complexes. The most relevant bands and proposed assignments for all the complexes are mentioned throughout this section. In the IR spectra of the complexes, the absence of band in the region 3400 cm<sup>-1</sup> corresponding to free primary  $v(NH_2)$  and the absence of free carbonyl function C=O at 1700 cm<sup>-1</sup> suggests, that complete self condensation of amino group by carbonyl group has taken place. A strong intensity band at 1620 cm<sup>-1</sup> attributable to v(C=N) [30] provide strong evidence for the presence of cyclic product. These results strongly suggest that the proposed ligand framework is formed. This has been further corroborated by the appearance of a sharp band in the 450-440 cm<sup>-1</sup> region, which may reasonably be assigned to v(M-N) vibration. Bands observed in the region 1160-1200 cm<sup>-1</sup> may reasonably be assigned to v(C-N) stretching vibrations. The spectral features of these nickel(II) macrocyclic complexes are similar to that reported for the structurally analogous [Pt(TAAP)]<sup>2+</sup>, [Pd(TAAP)]<sup>2+</sup> [31] and [Ni(TAAB)]<sup>2+</sup> [32]. Bands attributed to functional groups in the macrocycles are observed in the following regions (cm<sup>-1</sup>): 3000–3150, aromatic v(C–H), 2900–3000; aliphatic v(C-H), 1610-1620; v(C=C), 1560-1575 and the out of plan bending of phenyl, at ca. 710-745.

The infrared absorption bands of the anions in these complexes show that the perchlorate and fluoroborate are not coordinated to nickel(II) center. This fact was inferred from the strong broad bands occur at 1085 and  $621 \text{ cm}^{-1}$  and a medium broad band at  $412 \text{ cm}^{-1}$  for the perchlorate and at 1050, 620, and  $412 \text{ cm}^{-1}$  for

Table 1	
Molecular formulae, elemental analyses, and physical properties of th	ne tetraaza macrocyclic nickel(II) complexes.

Complex	Color	$\Lambda (\Omega^{-1} \operatorname{cm}^2 \operatorname{mol}^{-1})$	Found (cacld.)			
			С	Н	Ν	М
1. [NiLCl <sub>2</sub> ]	Red	21.10	60.77(61.26)	4.13(4.17)	19.22(19.51)	6.79(6.81)
2. [NiLBr <sub>2</sub> ]	Brown	15.26	55.33(55.54)	3.67(3.79)	17.44(17.68)	5.59(6.17)
3. [NiL(NO <sub>3</sub> ) <sub>2</sub> ] 2H <sub>2</sub> O	Dark red	27.16	55.21(55.53)	4.45(4.21)	17.65(17.68)	6.64(6.17)
4. [NiL](PF <sub>4</sub> ) <sub>2</sub>	Pale brown	145.78	56.97(56.99)	4.17(3.88)	18.11(18.14)	6.51(6.34)
<b>5</b> . [NiL](ClO <sub>4</sub> ) <sub>2</sub>	Greenish yellow	148.65	53.12(53.34)	3.57(3.63)	16.86(16.98)	5.39(5.93)

the fluoroborate. The spectrum of the nitrato complex displays strong bands at about 1410 and 1310 cm<sup>-1</sup> and a band of medium intensity at 1065 cm<sup>-1</sup>. These features indicate that the nitrate anions are coordinated to nickel(II) ion in the monodentate fashion [31]. The coordination of the nitrato group is further supported by appearance of new band at 260 cm<sup>-1</sup>, which may reasonably be assigned to v(M-O) of the O–NO<sub>2</sub> group [33]. The absorptions due to the lattice water molecules are evident in the spectrum of nitrato complex, occurring as a broad absorption at about 3450 cm<sup>-1</sup> and a weak shoulder at 1655 cm<sup>-1</sup> [33]. For the halogeno complexes, [CuLX<sub>2</sub>]; X = Cl or Br, the nonelectrolyte halogens exhibit far IR absorptions at 310–340 cm<sup>-1</sup>, indicating an axial coordinated halide ions [33].

#### 3.3. Electronic absorption spectra

The electronic spectra of the synthesized mononuclear nickel(II) macrocyclic complexes are measured in DMF solutions at room temperature and the results obtained are tabulated in Table 2. The electronic absorption spectra of the six-coordinate complexes show three absorption bands in the regions of 13,460–14,126, 17,564–18,321 and 19,598–20,032 cm<sup>-1</sup>. These absorption bands may be assigned to the  ${}^{3}A_{2g} \rightarrow {}^{3}T_{2g} (v_1)$ ,  ${}^{3}A_{2g} \rightarrow {}^{3}T_{1g} (F)$ ,  $(v_2)$  and  ${}^{3}A_{2g} \rightarrow {}^{3}T_{1g} (P)$ ,  $(v_3)$  transitions, respectively, and reflect that nickel(II) d<sup>8</sup> ions is in a distorted octahedral geometrical environment [34,35]. The d–d absorption bands for these complexes increase toward higher energies in the order Br < Cl < NO<sub>3</sub>, indicating that the presence of these coordinated axial ligands affects the ligand field strength of the complexes. The *Dq* values of [NiL(NO<sub>3</sub>)<sub>2</sub>], [NiLCl<sub>2</sub>] and [NiLBr<sub>2</sub>] are 1412, 1400 and 1346 cm<sup>-1</sup>, respectively.

The electronic absorption spectra of the four-coordinate perchlorate and fluoroborate complexes display three d–d transitions in the low energy region at, 13,699–14,285, 16,666–16,806 and 19,607–20,000 cm<sup>-1</sup>. These bands are assignable to the spin-allowed d–d bands:  $d_{xy}$  ( $b_{2g}$ )  $\rightarrow dx^2-y^2$  ( $b_{1g}$ ),  $d_z^2$  ( $a_{1g}$ )  $\rightarrow dx^2-y^2$  ( $b_{1g}$ ) and  $d_{xz,yz}$  ( $e_g$ )  $\rightarrow dx^2-y^2$  ( $b_{1g}$ ) corresponding to:  ${}^{1}A_{1g} \rightarrow {}^{1}A_{2g}$ ,  ${}^{1}A_{1g} \rightarrow {}^{1}B_{1g}$  and  ${}^{1}A_{1g} \rightarrow {}^{1}E_{g}$  transitions, respectively. These spectral features of [NiL](ClO<sub>4</sub>)<sub>2</sub> and [NiL](BF<sub>4</sub>)<sub>2</sub> are similar to each other and consistence with the low-spin d<sup>8</sup>-metal ion in the square planer environment [34,35]. The geometry of the reported nickel(II) tetraaza macrocyclic complexes is further supported by measuring their

room temperature magnetic moments which confirmed the proposed stereochemistries for these macrocyclic nickel(II) chelates.

In addition to the ligand field d–d bands, the spectra exhibit strong absorption bands in the high energy region (27,027–27,777 cm<sup>-1</sup>), arising from the charge-transfer transitions, *e.g.*  $M \rightarrow L(\pi^*)$  and  $L \rightarrow M$ . The spectrum of the free AMPC shows a medium band at 26,715 cm<sup>-1</sup> owing to the pyrazole moiety or the aromatic benzene ring bound to the nitrogen of the pyrazole ring, and is assignable to  $\pi \rightarrow \pi^*$ . This band is shifted to slightly higher energies in the spectra of complexes and the extinction coefficient is approximately up to four times as large as the coefficient for AMPC itself. This is a reasonable result since each complex molecule is composed of four AMPC moieties.

#### 3.4. Magnetic moment measurements

The effect of a series of ligands of increasing strength on the spin state of certain transition metal ions is expected to produce high-spin compounds for weak field ligands and low-spin compounds for strong field ligands. Consequently, the magnetic moments of such metal complexes are expected to lie in the region of the two extremes, this indeed being the case with the reported nickel(II) macrocyclic complexes.

The results of room temperature magnetic measurements are recorded in Table 2. These data show an increase of the magnetic moment from diamagnetism to complete paramagnetic as the strength of the axial perturbation is increased. With anions of poor coordinating ability, viz.,  $ClO_4^-$ , and  $BF_4^-$ , the resultant complexes are diamagnetic and therefore have singlet ground states. With anions of greater coordinating ability, viz., NO<sub>3</sub>, Cl<sup>-</sup> and Br<sup>-</sup> the resultant complex exhibits moments consistent with triplet ground state. However, with anions, viz., Cl<sup>-</sup> and Br<sup>-</sup>, the observed magnetic moments are lower than that for nitrato complex. During this change from singlet to triplet ground states, it appears reasonable to assume that the in-plane ligand field remains essentially constant. Visible reflectance spectra on solid samples are consistent with the associated changes in spin of the ground state. The spectra of the  $ClO_4^-$ , and  $BF_4^-$  complexes are consistent with the presence of a planar nickel(II) species, while that of NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup> and Br<sup>-</sup> suggest octahedral or tetragonal nickel(II) structure. These anomalous room temperature magnetic moments of the chloro and bromo

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Electronic absorption spectra (cr	$n^{-1}$ ) of the reported	l nickel(II) complexes
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Complex	$\lambda_{\rm max}~({\rm cm}^{-1})$	$\lambda_{\max}$ (cm <sup>-1</sup> )						
	$^{3}A_{2g} \rightarrow ^{3}T_{2g}$	$^{3}A_{2g}\rightarrow \ ^{3}T_{1g}\left( F\right)$	$^{3}A_{2g}\rightarrow \ ^{3}T_{1g}\left( P\right)$	$\pi \to \pi^*$	Dq	$\mu_{eff}$ BM		
[NiLCl <sub>2</sub> ] [NiLBr <sub>2</sub> ] [NiL(NO <sub>3</sub> ) <sub>2</sub> ] 2H <sub>2</sub> O	13,460 14,000 14,126	17,564 17,986 18,321	19,598 19,805 20,032	32,786 32,258 33,898	1346 1400 1412	3.59 3.56 3.93		
	$^1A_{1g} \rightarrow \ ^1A_{2g}$	$^1A_{1g} \rightarrow \ ^1B_{1g}$	$^1A_{1g} \rightarrow \ ^1E_g$					
[NiL](PF <sub>4</sub> ) <sub>2</sub> [NiL](ClO <sub>4</sub> ) <sub>2</sub>	14,285 13,966	16,806 16,666	20,000 19,607	34,482 33,003		Diamagnetic Diamagnetic		

complex species were interpreted on the assumption of the existence of equilibrium between high and low spin isomers.

It has been reported that for nickel(II) complexes, the relative energies of the singlet and triplet states be very sensitive to the nature of two ligands located axially, above and below the metal atom which is otherwise bound to a planar array of donor atoms [35,36]. Most simply, so long as the axial ligands compare closely in their binding strength to the in-plane ligands, the structure is grossly octahedral and the triplet state lies lowest. As the axial ligand-field strength is decreased, the singlet state drops in energy relative to the triplet. Thus, at some particular point in relative axial-in-plane ligand field strengths, the singlet and triplet states should coexist. On the other hand, an examination of the axial perturbation by anions X, X = Cl, Br and NO<sub>3</sub>, in NiL<sub>4</sub>X<sub>2</sub>, where L<sub>4</sub> represents a planar field produced by four neutral donors, demonstrated that: complexes with anions of negligible or lowcoordinating ability are diamagnetic while those of medium to good coordinating power exhibit room temperature magnetic moments consistent with octahedral or weakly tetragonal nickel(II) complexes. No examples were obtained with intermediate room temperature magnetic moments, the data merely dividing the complexes into two groups, those with singlet ground states and those with triplet ground states as reported in the present study.

# 3.5. Thermal studies

The structure characterization of the synthesized nickel(II) macrocyclic complexes, was further elucidated by employing the thermogravimetric technique and the proposed structures of the reported nickel(II) chelates are confirmed. The thermogravimetric analysis (TGA/DTG), and curves were obtained at a heating rate of 10 °C/min in a nitrogen atmosphere over a temperature range of the ambient temperature to 850 °C. The weight loss corresponding to different stages of pyrolysis were calculated and compared with those of the expelled groups (Table 3).

The thermogram of the two halogeno and the fluoroborate complex species are similar and consist of two well defined stages. The first decomposition stage starts at 170-200 °C and continues until 250-300 °C is corresponding to the removal of the two axially coordinated halogeno ions and the electrolytic BF<sub>4</sub> counter ions constituting about 8.11%, 14.59% and 16.55% of the total weight loss (calcd. 8.23%, 14.63% and 16.83%) for the chloro, bromo and fluoroborate complexes respectively. Since the decomposition started above 170 °C, the presence of any solvent/water molecule may be ruled out which are also evident from the elemental analysis. The second stage runs over 300 °C and is consistent with the degradation of the organic part of the complex molecule in successive unresolved steps. Above 800 °C the TGA curve shows no change even when the temperature was raised to 850 °C. This horizontal constant curve may be due to the presence of metal oxides (NiO) residue in the remaining part. The total loss of weight agrees with the corresponding calculated data.

The TG curve of the nitrato complex [NiL(NO<sub>3</sub>)<sub>2</sub>].2H<sub>2</sub>O is shown in (Supplementary material S1), which exhibits three decomposition steps within the temperature range from 75 to 850 °C. The first two steps of decompositions within the temperature range 75– 300 °C corresponds to the loss of water molecules of hydration, and the coordinated nitrate ions with a mass loss of 3.33% (calcd. 3.78%) and 12.34% (calcd. 13.03%). The subsequent step (300– 850 °C) with the maximum decomposition peaks DTG<sub>max</sub> at 650 °C, corresponds to the removal of the organic part of the ligand leaving metal oxide as a residue. The overall weight loss amounts to 91.55% (calcd. 92.65%). The mass losses in this stage are in a good agreement with calculated mass losses and the final product is quantitatively proved to be anhydrous metallic oxide (NiO).

#### 3.6. Electrochemical properties

The electrochemical behavior of the synthesized nickel(II) macrocyclic complexes was investigated, as the redox potential is an important parameter in electron transfer processes in general and also in our catalytic systems. The redox potentials of the catalysts and species involved in the redox cycles have to match some criteria. The oxidation of Ni(II) center to Ni(III) by the substrate  $(O_2^{-})$  and the subsequent reduction of Ni(III) to Ni(II) by another molecule of  $O_2^{-}$  are significant steps in the catalytic cycle. The redox properties of the complexes reported in the present work were studied by cyclic voltammetry in the potential-range -1.0 to 1.5 V in dimethylformamide containing  $10^{-1}$  M tetra (n-butyl) ammonium perchlorate. (Supplementary material S2), depicts the cyclic voltammogram of the nitrato complex [NiL (NO<sub>3</sub>)<sub>2</sub>]. All the complexes undergo reduction and oxidation potentials and their cyclic voltammetric behavior is found to be similar and displayed two well-defined electrode couples. The complexes showed two successive one electron processes and the first electrode couple is safely assigned to the reversible couple Ni<sup>II</sup>/Ni<sup>I</sup> with  $E_{1/2}$  of -417 to  $-526 \text{ mV} (\Delta E_p = 58-70 \text{ mV})$  and represented as follows:

$$[\operatorname{NiL}]^{2+} + e \rightleftharpoons [\operatorname{NiL}]^{+} \tag{1}$$

The second electrode couple with  $E_{1/2} = 420-523$  mV is assigned to the reversible electrode couple Ni<sup>III</sup>/Ni<sup>II</sup> by comparison with analogous Ni(II) complexes [37]. The pertinent redox couple is represented in the following electron transfer series (2):

$$[\operatorname{NiL}]^{3+} + e \rightleftharpoons [\operatorname{NiL}]^{2+} \tag{2}$$

The complexes that undergo reduction are completely regenerated following electrochemical oxidation (the ratio of the peak currents  $i_a/i_c$  is about 1.0), suggesting a chemically reversible  $Ni^{n+} \rightleftharpoons Ni^{(n+1)}$  one-electron transfer processes (1) and (2). However, the value of the limiting peak-to-peak separation ( $\Delta E_n$ ), which lies within the normal values for a reversible one-electron redox process suggests that the heterogeneous electron-transfer process in these complexes is easily reversible ( $\Delta E_n$  60 mV for a reversible one-electron redox process) [38] and not accompanied by stereochemical reorganization [39]. The impact of the electrochemical properties on the catalytic reactivity of these complexes will be discussed below. Table 4, summarizes the half-wave potential ( $\Delta E_p$ ) data calculated as the arithmetic mean of the  $E_{pc}$  and  $E_{pa}$ values. The  $E_{I/2}$  values lie between the potentials of the reduction of  $O_2$  to  $O_2^-$  (-0.16 V vs. NHE at pH 7) and  $O_2^-$  to  $H_2O_2$  (+0.89 V vs. NHE) as it is expected for compounds with a SOD-like activity in dependence on the  $O_2^-$  concentration [40]. However, the similarity in the  $E_{1/2}$  values for the reported nickel(II) complexes show that the axially coordinating and electrolytic anions, have a negligible contribution on the redox potentials.

## 3.7. Superoxide dismutase (SOD) biomimetic catalytic activity

The superoxide dismutase mimetic activity of the reported nickel(II) complexes was assayed by the use of phenazine methosulphate (PMS) as a photogenerator of the superoxide anion radical in association with nitroblue tetrazolium (NBT) chloride as scavenger of the superoxide [41]. Inhibition of the reduction of NBT to formazan (F) by the synthesized nickel(II) complexes was used for detection of the SOD like activity of theses macrocyclic chelates in the phosphate buffer under similar biological conditions as previously described [42]. The chromophore concentration required to yield 50% inhibition of the reduction of NBT ( $IC_{50}$ ) was determined by following the literature method [15b]. A 50% inhibition reduction of NBT (the  $IC_{50}$  value) for complexes 1–5 was obtained from the plot of percentage of inhibition vs the complex concentration

Table 3
Thermogravimetric analysis data of the tetraaza macrocyclic nickel(II) complexes 1-4.

Complex	Step	DTG (°C)	Temp. range (°C)	Mass loss (%) found (calc.)	Species formed
[NiLCl <sub>2</sub> ]	1	180	170-250	08.11(08.23)	NiL
	2	635	250-720	83.25(83.52)	NiO
[NiLBr <sub>2</sub> ]	1	215	190–250	16.55(16.83)	NiL
	2	680	250-720	74.87(75.85)	NiO
[NiL(NO <sub>3</sub> ) <sub>2</sub> ] 2H <sub>2</sub> O	1	90	75-100	03.33 (03.78)	$[NiL(NO_3)_2]$
	2	280	100-300	12.34(13.03)	NiLNiO
	3	650	300-750	75.88(75.84)	
$[NiL](PF_4)_2$	1	260	200-300	14.59(14.63)	NiL
	2	500	300-740	77.12(77.79)	NiO

as shown in Fig. 1 for complex  $[NiL(NO_3)_2]$ . The  $IC_{50}$  values are summarized in Table 5 along with other  $IC_{50}$  values obtained previously for nickel(II) complexes. The data in Table 5 reveal that the  $IC_{50}$  values of reported nickel(II) complexes are in the same level of the other nickel(II) SOD-mimics [15a,15c].

The SOD-like activity of some metal complexes is a function of several factors, among them is the exchange ability of the axial coordinated ligand, the steric hindrance and flexibility of the metal ion to the geometrical changes. The fast exchange of ligand coordinated axially to the central metal ion and limited steric hindrance to the approach of the  $O_2^{-}$  anion are considered essential requirements for the successful binding of the  $O_2^{-}$ . The flexibility of the metal complex to geometrical arrangement changes, during the redox cycling of  $M^{n+}/M^{(n-1)}$ , which facilitates the interaction of the  $O_2^{-}$  is also important. In addition, the nature of coordinated ligands to the metal ion is also playing an important role in enhancing the SOD like activity of the SOD mimics [43]. The macrocycle framework provides stable and flexible environment similar to that in the active site of the native enzyme, ensuring the stable existence of the complexes at the investigated pH value in aqueous solution. In addition our spectral and electrochemical investigations displayed that this tetraaza macrocyclic ligand system provides these mentioned requirements for its nickel(II) complexes as SOD mimics (see Table 5).

The data in Table 5 show that, all the synthesized nickel(II) complexes exhibit promising SOD mimetic catalytic activity which can be related to the redox potential of the couple Ni<sup>III</sup>/Ni<sup>II</sup> and the



**Fig. 1.** Plot of percentage of NBT inhibition reduction with an increase in the concentration of complex  $[NiL(NO_3)_2]$ .

structural features of these complexes. The overall mechanism has been called a ping-pong mechanism proposed for the dismutation of superoxide anion radical by both the native M-SOD and metal complexes (SOD-mimics) is thought to involve redox cycling of  $M^{n+}$  ion as follows:

$$\mathbf{M}^{(n+1)+}-\mathbf{SOD}+\mathbf{O}_{2}^{-} \rightarrow \mathbf{M}^{n+}-\mathbf{SOD}+\mathbf{O}_{2}$$
(3)

 $M^{n+}-SOD + O_2^{-} + 2H^+ \rightarrow M^{(n+1)+}-SOD + H_2O_2$  (4)

where M = Cu (n = 1); Mn (n = 2); Fe (n = 2); Ni (n = 2). In this reaction the oxidation state of the metal cation oscillates between n and n + 1.

Self-dismutation of O<sub>2</sub><sup>-</sup> at pH 7.4 occurs with a rate constant,  $k \sim 5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ , and is increased more than three orders of magnitude in the presence of SOD [1]. All SOD enzymes, regardless of the type of metal (Mn, Fe, Cu, Ni), have metal-centered reduction potential around +300 mV *vs* NHE, which is midway between the potential for the reduction of O<sub>2</sub> (+890 mV *vs*. NHE) and oxidation of O<sub>2</sub><sup>-</sup> (-160 mV *vs*. NHE). Thus, both processes are thermodynamically equally favored at ~+300 mV *vs* NHE. The data in Table 4 display that the redox potential ( $E_{1/2}$ ) values of the reported macrocyclic nickel(II) complexes are in the allowed ranges of an SOD mimic, and follow the order [NiL](PF<sub>4</sub>)<sub>2</sub>  $\Box$  [NiL](ClO<sub>4</sub>)<sub>2</sub>  $\Box$  [NiLCl<sub>2</sub>]  $\Box$  [NiLBr<sub>2</sub>]  $\Box$  [NiL(NO<sub>3</sub>)<sub>2</sub>] and the SOD mimetic activity of the complexes follows the inverse of this order.

The remarkable appreciable SOD-like activity observed for the coordinately saturated octahedral complexes [NiLCl<sub>2</sub>], [NiLBr<sub>2</sub>] and [NiL(NO<sub>3</sub>)<sub>2</sub>] is explained in term of the exchange ability of one of the axially coordinate ligands with the approaching superoxide anion. A ligand exchange interaction is expected to occur between the highly reactive and more basic  $O_2^-$  and one of the counter anions occupies the axial position of the octahedral complexes [NiLCl<sub>2</sub>], [NiLBr<sub>2</sub>] and [NiL(NO<sub>3</sub>)<sub>2</sub>]. The little higher reactivity of complex [NiL(NO<sub>3</sub>)<sub>2</sub>] compared to [NiLBr<sub>2</sub>], which has  $E_{1/2}$ almost equal to [NiL(NO<sub>3</sub>)<sub>2</sub>] may be attributed to the fact that: the relatively weak axial coordination of nitrato ligands are readily dissociated in solution to provide axial sites on Ni(II) for  $O_2^-$  bonding. The dissociation would also facilitate any necessary geometrical changes induced by  $O_2^-$  bonding during catalysis as in the native SOD.

Remarkably although the square planar complex  $[NiL](ClO_4)_2$ possess very similar redox potential value compared to the hexacoordinate octahedral complex  $[NiLCl_2]$  the later exhibits greater activity. As well as the square-planar complex  $[NiL](ClO_4)_2$  is coordinately unsaturated and already has vacant coordination sites available for  $O_2^-$  bonding. Similar to the previously reported studies of copper(II) SOD mimics, the difference in  $IC_{50}$  values for these complexes should be ascribed to the evident discrepancy in the structures between them, especially in geometry of nickel(II) center. A possible correlation between the strength of the equatorial

#### Table 4

Electrochemical data (mv) for the reported nickel(II) complexes.

Complex	First electrode couple			Second ele	Second electrode couple			
	Epc	$E_{\mathrm{pa}}$	E <sub>1/2</sub>	$\Delta E_{\rm p}$	Epc	$E_{\rm pa}$	E <sub>1/2</sub>	$\Delta E_{\rm p}$
[NiLCl <sub>2</sub> ]	-485	-553	-519	68	553	490	512	63
[NiLBr <sub>2</sub> ]	-480	-550	-515	70	457	390	423	67
[NiL(NO <sub>3</sub> ) <sub>2</sub> ] 2H <sub>2</sub> O	-385	-450	-417	65	450	390	420	66
$[NiL](PF_4)_2$	-495	-557	-526	62	556	490	523	60
[NiL](ClO <sub>4</sub> ) <sub>2</sub>	-490	-548	-519	58	545	485	515	60

#### Table 5

Comparison of the superoxide dismutase biomimetic catalytic activity of the reported nickel(II) complexes with analogous known nickel(II) complexes.

Complex	IC <sub>50</sub> (M)	$k_c ({ m M}^{-1}{ m S}^{-1})$	Reference
[NiLCl <sub>2</sub> ]	$(38.8 \pm 0.15) \times 10^{-6}$	$2.60\times10^5$	This work
[NiLBr <sub>2</sub> ]	$(30.5 \pm 0.81) \times 10^{-6}$	$3.33  imes 10^5$	This work
[NiL(NO3)2] 2H20	$(21.4\pm0.19) imes10^{-6}$	$4.72  imes 10^5$	This work
$[NiL](PF_4)_2$	$(55.38 \pm 1.33)  imes 10^{-6}$	$1.82  imes 10^5$	This work
$[NiL](ClO_4)_2$	$(49.50 \pm 1.10)  imes 10^{-6}$	$2.04\times10^{5}$	This work
Native NiSOD	$pprox\!4.00 imes10^{-9}$	$2.52  imes 10^9$	[15a]
[NiL](BF <sub>4</sub> )	$48.00\times10^{-6}$	$2.10  imes 10^5$	[15c]
[NiL](ClO <sub>4</sub> )	$65.00  imes 10^{-6}$	$1.55  imes 10^5$	[15c]
[NiL <sup>1</sup> ](ClO <sub>4</sub> ) <sub>2</sub> (1)	$40~00  imes 10^{-6}$	$2.52 \times 10^5$	[15a]
$[NiL^2](ClO_4)_2(2)$	$48.00\times10^{-6}$	$2.10  imes 10^5$	[15a]
$[NiL^{3}L](ClO_{4})_{2}(3)$	$43.00\times10^{-6}$	$2.34\times10^{5}$	[15a]
[NiL <sup>3</sup> L'] ( <b>4</b> )	$58.00\times \mathbf{10^{-6}}$	$1.74\times10^{5}$	[15a]

field and the SOD activity was suggested [44]. It has been previously reported that a strong equatorial ligand field opposes the interaction of the complexed copper with  $O_2^{-}$ , disfavoring the probable formation of the intermediate copper superoxide adduct [45]. More over, a strong equatorial ligand field is a factor which disfavors the reduction process from copper(II) to copper(I) during the catalytic cycle. Coordination of the fifth ligand to copper(II) ion in the five coordinate square pyramidal or trigonal bipyramidal complex may reduce the strength of the equatorial field experienced by the copper(II) centers as compared to the four coordinated square planar complexes. Another important factor in the catalytic process is the ability of the five-coordinate complex to change its geometry as the geometry of copper in the CuSOD enzyme also changes during the catalytic dismutation process [46]. This hypothesis finds support from Patel and co-workers, they reported that the higher SOD activity of the five coordinate copper(II) complexes is due to the presence of the fifth ligand in the axial position [47]. A great interaction between the superoxide anion radical and copper(II) ion in the five coordinate geometry is induced due to the strong axial bond which results in an increased catalytic activity [48]. This augment may clarify the difference in the SOD like activity between the reported octahedral complex [NiLCl<sub>2</sub>] and the square-planar complex  $[NiL](ClO_4)_2$ .

The reaction mechanism for the disproportionation of the toxic superoxide radical to molecular oxygen and hydrogen peroxide by the nickel-dependent superoxide dismutase (NiSOD) has been studied using the B3LYP hybrid DFT method [49]. The obtained results demonstrated that: Ni-SOD is believed to act like other SODs according to the formal Eqs. (3) and (4), of the dismutation reaction, where Ni(III)-SOD and Ni(II)-SOD represented the oxidized and reduced metal center. The catalytic cycle consists of two half-reactions, each initiated by the successive substrate approach to the metal center. The first (reductive) phase involves Ni(III) reduction to Ni(II), and the second (oxidative) phase involves the metal reoxidation back to its resting state. The Cys2 thiolate sulfur serves as a transient protonation site in the interim between the two half-reactions, allowing for the dioxygen and hydrogen

peroxide molecules to be released in the reductive and oxidative phases, respectively.

The proposed mechanism for catalysis of  $O_2^-$  disproportionation to molecular oxygen and hydrogen peroxide by the reported nickel(II) macrocyclic SOD functional models may proceed similarly. For the six coordinated octahedral complexes an exchange interactions between the highly reactive superoxide anion radical with the good leaving group X, X = Cl, Br or NO<sub>3</sub>), will lead to the formation of a [superoxo-nickel(II)LX] adduct. As a consequence of this interaction, and due to the excessive build up of the electron density on nickel(II) center and according to the electro neutrality principal, nickel(II) undergoes rapid oxidation to nickel(III) with the concomitant formation of the peroxide anion  $O_2^{2-}$ . The protonation of  $O_2^{2-}$ prior to its release from the complex is required, because the peroxide anion is highly basic and thus too unstable to be released in its unprotonated form. Owing to the high basicity of O<sub>2</sub><sup>2-</sup>, the six-coordinate [peroxo-nickel(III)-X] adduct becomes highly unstable because of the excessive build-up of the electron density on the metal ion. At this stage, probably, the axially coordinated X could be forced to dissociate from the complex in the solution as HX. Dissociation of the axial X offers the possibility of hydrogen ion  $(H^{+})$ bonding. The hydrogen proton can then coordinate to the coordinately unsaturated [peroxo-nickel(III)-X] adduct which rearranges to give the perhydroxyl radical  $HO_2^-$ . The basic perhydroxyl (hydroperoxide) formed is further protonated as a result of electrophilic attack by a proton on the oxygen-bonded nickel(III) with release of  $H_2O_2$  and generation of the catalyst in its oxidized inactive form. In a fast reaction a second superoxide anion radical  $(0^{-}_{2})$  will coordinate to nickel(III) center in the five coordinate [Ni(III)LX] intermediate. As a consequence of this interaction, nickel(III) undergoes rapid reduction to nickel(II) with the release of O<sub>2</sub>, and regeneration of the catalyst in its original reduced active form. This assumption mechanism finds supports from the following facts:

- (1) The superoxide anion radical (O<sub>2</sub><sup>-</sup>) has strong reducing and oxidizing power, and consequently can behave as a good oxidizing and as well reducing agent.
- (2) The fact that  $O_2^-$  can reduce and subsequently reoxidize the nickel center requires this center to possess a redox potential between those of the couples  $(O_2^-/O_2)$  and  $(O_2^-/H_2O_2) \approx 300 \text{ mV } vs$  NHE [1]. Our electrochemical results demonstrated that the macrocyclic framework provides the ability of the nickel(II/III) redox couple to reach this desire potential. The probable reaction sequence for the catalytic disproportionation of  $O_2^-$  can be represented as shown in Scheme 2.

# 3.8. DNA-binding

# 3.8.1. Electronic spectral studies

Electronic absorption spectroscopy has been widely employed to determine the binding ability of metal complexes with DNA [50a–d]. The binding ability of the synthesized nickel(II) complexes [NiLCl<sub>2</sub>]



For complex 1, X = Cl, n = 0; 2, X =Br, n = 0; 3,  $X = NO_3$ , n = 2.

Scheme 1. Structure of nickel(II) tetraaza macrocyclic complexes 1-5.



**Scheme 2.** The proposed mechanism of the catalytic dismutation of O<sub>2</sub><sup>-</sup> by nickel(II) tetraaza macrocyclic complexes. X = Cl, Br and NO<sub>3</sub> in the hexa-coordinate octahedral Ni(II) complex species (Scheme 1).

and [NiL](PF<sub>4</sub>)<sub>2</sub> with calf thymus (CT) DNA were assayed by measuring the spectral changes of their electronic spectra during the interaction with DNA. Complex binding with DNA through intercalation usually results in hypochromism and bathochromism, due to intercalative mode involving a strong stacking interaction between an aromatic chromophore of the bound ligand and the base pairs of DNA [51,52]. In the present investigation the interaction of macrocyclic nickel(II) complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> in DMF solutions with CT DNA have been investigated. Absorption titration experiments of nickel(II) complexes in buffer were performed by using a fixed concentration of nickel(II) complex to which increments of the DNA stock solution were added. The binding of nickel(II) complexes  $[NiLCl_2]$  and  $[NiL](PF_4)_2$  to duplexes DNA led to decrease in the absorption intensities (hypochromism) with a small amount of red shifts in the UV-Vis absorption spectra (bathochromism). After intercalating the base pairs of DNA, the  $\pi^*$  orbital of the intercalated ligand can couple with the  $\pi$  orbital of the base pairs, thus decreasing the  $\pi$ - $\pi$ \* transition energy and resulting in the bathochromism [53]. On the other hand, the coupling  $\pi^*$  orbital is partially filled by electrons, thus decreasing the transition probabilities and concomitantly resulting in hypochromism. To compare quantitatively the affinity of the two complexes toward DNA, the binding constants  $k_b$  of the two complexes to CT DNA were determined by monitoring the changes of absorbance at 305 nm for complex [NiLCl<sub>2</sub>] with increasing concentration of DNA (Supplementary material S3). The appreciable decrease in absorption intensity and red shift of the  $\lambda_{max}$  band at 305 nm of [NiLCl<sub>2</sub>] is suggesting that the complex binds to DNA strongly [20]. From the absorption titration data, the binding constant ( $k_b$ ) was determined using the following equation [54]:

$$\frac{[\text{DNA}]}{\varepsilon_a - \varepsilon_f} = \frac{[\text{DNA}]}{\varepsilon_b - \varepsilon_f} + \frac{1}{k_b(\varepsilon_b - \varepsilon_f)}$$
(5)

where [DNA] is the concentration of CT-DNA in base pairs,  $\varepsilon_a$  corresponds to the extinction coefficient observed ( $A_{obs}/[complex]$ ),  $\varepsilon_f$  is the extinction coefficient of the free complex,  $\varepsilon_b$  is extinction coefficient of the complex fully bound to CT-DNA, and  $k_b$  is the intrinsic binding constant. The ratio of slope to intercept in the plot of [DNA]/ ( $\varepsilon_a - \varepsilon_f$ ) vs [DNA] (Fig. 2) gave the value of  $k_b$ .

The binding constants ( $k_b$ ) of complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> are calculated as  $10.83 \times 10^4$  and,  $6.50 \times 10^4$  M<sup>-1</sup>, respectively. The obtained results display that both complexes exhibited hypochromism but of varied degree. Slight bathochromic shift was observed with complex [NiLCl<sub>2</sub>], which was negligible with complex [NiL](PF<sub>4</sub>)<sub>2</sub>. Comparing the intrinsic binding constant of the two



**Fig. 2.** Plot of the ratio  $[DNA]/(\varepsilon_a - \varepsilon_f)$  vs. DNA concentration for the reaction between DNA and complexes  $[NiLCl_2]$  and  $[NiL](PF_4)_2$ .

complexes with those of DNA-intercalative macrocyclic binuclear copper(II) complex, such as  $[Cu_2(L)](ClO_4) (1.72 \times 10^4)$  [55], we can deduce that complex  $[NiLCl_2]$  and  $[NiL](PF_4)_2$  bind strongly to DNA by intercalation. These spectral characteristics obviously suggest that the two complexes in the present study interact with DNA most likely through a mode that involves a stacking interaction between the aromatic chromophore and the base pairs of DNA.

#### 3.8.2. Fluorescence spectral studies

The fluorescence spectroscopy provides insight of the changes taken place in the microenvironment of DNA molecule on ligand binding. To further clarify the interaction of the complexes with DNA, the fluorescence measurements were performed to determine the binding ability of the metal complexes with DNA. The binding of these compounds with calf thymus DNA, were studied by monitoring the changes in the intrinsic fluorescence of these complexes at varying DNA concentration. Fig. 3 shows the representative fluorescence emission spectra of complex [NiLCl<sub>2</sub>] upon excitation at 285 nm. Increment additions of DNA at constant concentration of complex solution caused a gradual decrease in the fluorescence emission intensity with a conspicuous change in the emission spectra (Fig. 3). The spectra illustrate that an excess of



Fig. 3. Fluorescence emission spectra of [NiLCl<sub>2</sub>] complex in the absence and presence of increasing amount of DNA.



**Fig. 4.** Plot the ratio of  $I_0/I$  vs. DNA concentration for the reaction between DNA and complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> at 296 K.

DNA led to more effective quenching of the f1uorophore molecule fluorescence. The reduction of the emission intensity gives a measure of the DNA binding propensity of the complexes and stacking interaction (intercalation) between the adjacent DNA base pairs [56]. The fluorescence quenching curve of DNA-complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> Fig. 4, illustrates that the quenching of complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> is in a good agreement with the linear Stern–Volmer Eq. (6), which provides further evidence that the complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> bind to DNA [57]:

$$I_0/I = 1 + k_{sv}[Q]$$
(6)

where  $I_0$  and I are the fluorescence intensities of complex in the absence and presence of DNA (quencher) respectively.  $k_{sv}$  is the Stern–Volmer constant and [Q] is the concentration of DNA. Plotting the relative emission intensities ( $I_0/I$ ) against [Q] for static process should yield a linear Stern–Volmer plot that describes the static quenching process. The slope of this plotted line yields  $k_{sv}$ , the static quenching constant or associative equilibrium constant. The  $k_{sv}$  values for complexes [NiLCl<sub>2</sub>] and [NiL](PF<sub>4</sub>)<sub>2</sub> are  $35.0 \times 10^3$  M<sup>-1</sup> (R = 0.998) and  $23.75 \times 10^3$  M<sup>-1</sup> (R = 0.9898), respectively. This data suggests that the interaction of complex [NiLCl<sub>2</sub>] with CT-DNA is stronger than that of complex [NiL](PF<sub>4</sub>)<sub>2</sub>, which is consistent with the above absorption spectral results.

The quenching of the complexes fluorescence clearly indicated that the binding of the DNA to complexes molecule changed the microenvironment of fluorophore residue. The shift in emission peak further depicts effective interaction at higher DNA concentration, which is more prominent in case of [NiLCl<sub>2</sub>]. The reduction in the intrinsic fluorescence of these synthesized complex molecules upon interaction with DNA could be due to masking or burial of compound fluorophore upon interaction between the stacked bases within the helix and/or surface binding at the reactive nucle-ophilic sites on the heterocyclic nitrogenous bases of DNA molecule.

# 4. Conclusion

The reaction of 5-amino-3-methyl-1-phenylpyrazole-4-carbaldehyde (AMPC) with itself in the presence of nickel(II) ions yields, new macrocyclic complex,  $[NiL(NO_3)_2]$ , containing a ligand composed of the self-condensed AMPC (four mol) bound to a single nickel(II) ion. A number of newly complexes of the types  $[NiL]X_2$ ; X = ClO<sub>4</sub> and BF<sub>4</sub>,  $[NiLX_2]$ , X = Cl and Br (Scheme 1) have been prepared via series of metathetical reactions. A square planar geometry was deduced for the four-coordinate complex species  $[NiL](PF_4)_2$  and  $[NiL](ClO_4)_2$ , while an octahedral environment with slight distortion in nitrato and halogeno complexes was inferred on the bases of electronic and magnetic moment data. The catalytic activity for the SOD reaction of the title complexes has been demonstrated by in vitro measurements. Taking into account the reduction potentials of the couples  $O_2^{-}/O_2$  and  $O_2^{-}/H_2O_2$  (-0.16 V and 0.89 V, vs. NHE at pH 7, respectively) any redox pair with an intermediate potential value between these limits can act as a catalyst for the SOD reaction. The observed redox processes of the reported nickel centers lie in the middle of this range. Therefore, the electrochemical behavior observed for these complexes is in agreement with their SOD mimetic activity. The absorption and fluorescence studies demonstrated a considerable interaction between complexes  $[NiLCl_2]$  and  $[NiL](PF_4)_2$  with calf thymus DNA.

# Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2012.01.048.

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