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Abstract

The water-soluble Ni (II) complex, $[Ni(bpy)_2(phen\text{-}dione)](OAc)_2 .2H_2O$ has been synthesized and characterized by physio-chemical and spectroscopic methods. The binding interactions of this complex with calf thymus DNA (CT-DNA) were investigated using fluorimetry, spectrophotometry, circular dichroism, and viscosimetry. In fluorimetric studies, the enthalpy and entropy of the reaction between the complex and CT-DNA showed that the reaction is exothermic and enthalpy-favored (ΔH = -123.88 kJ mol^{-1}; ΔS = -323.48 J $mol^{-1}K^{-1}$). The competitive binding studies showed that Ni(II) complex could not release Methylen Blue (MB) completely.

The complex showed absorption hyperchromism in its UV–Vis spectrum with DNA. The calculated binding constant, K_b obtained from UV–Vis absorption studies was 2×10^5 M^{-1}. Moreover, the complex induced detectable changes in the CD spectrum of CT-DNA, as well as changes in its viscosity. The results suggest that this nickel complex interact with CT-DNA via a groove binding.

Key Words: Mixed ligand Ni(II) complex; Phen-dione; Bipyridine; DNA groove binding.

Chapter One

Introduction

1. Introduction

1.1 Inorganic medicinal chemistry [1]

Inorganic or metal-containing medicinal compounds may contain either (a) chemical elements essential to life forms—iron salts used in the treatment of anemia—or (b) nonessential/toxic elements that carry out specific medicinal purposes—platinum-containing compounds as antitumor agents or technetium and gadolinium complexes as medical diagnostic tools.

Introducing metal ions into a biological system may be carried out for therapeutic or diagnostic purposes, although these purposes overlap in many cases.

In 1991, Peter Sadler noted that most elements of the periodic table, up to and including bismuth with an atomic number of 83, have potential uses as drugs or diagnostic agents.

Inorganic compounds have found usage in chemotherapeutic agents such as:

1. Anticancer agents like cis-$[Pt(NH_3)_2Cl_2]$
2. The gold-containing antiarthritic drug Auranofin
3. Metal-mediated antibiotics like bleomycin, which requires iron or other metals for activity
4. Technetium-99m and other short-lived isotopes used as radiopharmaceuticals in disease diagnosis and treatment
5. Magnetic resonance imaging (MRI)-enhancing gadolinium compounds
6. Antibacterials, antivirals, antiparasitics, and radiosensitizing agents.

1.2 Nickel

1.2.1Characteristics

Nickel is a chemical element, with the chemical symbol **Ni** and atomic number 28. It is a silvery-white lustrous metal with a slight golden tinge. It is one of the four ferromagnetic elements at about room temperature. Its use has been traced as far back as 3500 BC, but it was first isolated and classified as a chemical element in 1751 by Axel Fredrik Cronstedt**.**

It belongs to the transition metals and is hard and ductile. It occurs most often in combination with sulfur and iron in pentlandite, with sulfur in millerite, with arsenic in the mineral nickeline, and with arsenic and sulfur in nickel galena [2,3,4]. Nickel is commonly found in iron meteorites as the alloys kamacite and taenite.

Similar to the elements chromium, aluminium and titanium, nickel is a very reactive element, but is slow to react in air at normal temperatures and pressures. Due to its permanence in air and its slow rate of oxidation, it is used in coins, for plating metals such as iron and brass, for chemical apparatus, and in certain alloys such as German silver.

Nickel is chiefly valuable for the alloys it forms, especially many superalloys, and particularly stainless steel. Nickel is also a naturally magnetostrictive material, meaning that in the presence of a magnetic field, the material undergoes a small change in length [5]. In the case of nickel, this change in length is negative (contraction of the material), which is known as negative magnetostriction and is on the order of 50 ppm.

The most common oxidation state of nickel is +2 with several Ni complexes known. It is also thought that a +6 oxidation state may exist, however, this has not been demonstrated conclusively. The unit cell of nickel is a face centered cube with a lattice parameter of 0.352 nm giving a radius of the atom of 0.125 nm [6].

1.2.2 Applications

Nickel is used in many industrial and consumer products, including stainless steel, magnets, coinage, rechargeable batteries, electric guitar strings and special alloys. It is also used for plating and as a green tint in glass. Nickel is pre-eminently an alloy metal, and its chief use is in the nickel steels and nickel cast irons, of which there are many varieties. It is also widely used in many other alloys, such as nickel brasses and bronzes, and alloys with copper, chromium, aluminium, lead, cobalt, silver, and gold [7].

1.2.3 Isotopes

Naturally occurring nickel is composed of 5 stable isotopes; ^{58}Ni, ^{60}Ni, ^{61}Ni, ^{62}Ni and ^{64}Ni with ^{58}Ni being the most abundant (68.077% natural abundance). ^{62}Ni is the most stable known nuclide of all the existing elements, even exceeding the stability of ^{56}Fe. 18 radioisotopes have been characterised with the most stable being ^{59}Ni with a half-life of 76,000 years, ^{63}Ni with a half-life of 100.1 years, and ^{56}Ni with a half-life of 6.077 days. All of the remaining radioactive isotopes have half-lives that are less than 60 hours and the majority of these have half-lives that are less than 30 seconds. This element also has 1 meta state.

1.2.4 Biological applications of nickel

Indeed, nickel element is an essential element related to the life process. It can promote the absorption of iron element, the increase of red corpus cleand the syntheses of some amino-enzyme in the body [8].The thermal denaturation studies have shown that nickel has a mild protection effect on DNA, similar to that of magnesium [9].

Nickel plays numerous roles in the biology of microorganisms and plants, though they were not recognized until the 1970s [10]. In fact urease (an enzyme which assists in

the hydrolysis of urea) contains nickel. The NiFe-hydrogenases contain nickel in addition to iron-sulfur clusters. Such [NiFe]-hydrogenases characteristically oxidise H_2. A nickel-tetrapyrrole coenzyme, F430, is present in the methyl coenzyme M reductase which powers methanogenic archaea. One of the carbon monoxide dehydrogenase enzymes consists of an Fe-Ni-S cluster [11]. Other nickel-containing enzymes include a class of superoxide dismutase [12] and a glyoxalase [13].

1.3 Heterocyclic dinitrogen aromatic ligands

Heterocyclic such as 2, 2'-bipyridine (bpy), 1, 10-Phenanthroline, terpyridyne are best considered as α-diimines having the group (III) [14]. Pyridine and its chelating analogues bipyridine and phenanthroline are good ligands for transition metals over a range of oxidation states [15].

The replacement of C by N in heterocyclic system lead to more π–electron deficient compounds, there have also been efforts to use polyamines instead of amines as chelate ligand. 2, 2'-bipyridine (bpy) is both a σ-donor and π-acceptor, the lone pair of nitrogen can form σ-bond with the central atom, while the aromatic system can take part in π-back-bonding [16]. In contrast to bpy, phenanthroline is rigidly held in a cis conformation, and is almost always found as a planar ligand [17]. The dipole moment is therefore larger, being 3.64 D as compared to 0.69 D for bipyridyl in benzene at 25°C [18]. Bipyridyl and phenanthroline considered as weak bases [19]. 1,10-phenanthroline and substituted derivatives, both in the metal-free state and as ligand coordinated to transition metals, disturb the functioning of a wide variety of biological systems [20]. 2, 2'-bipyridine (bpy) and 1, 10-Phenanthroline (phen), they can be of better anti-tumor chelators also act as potential anti-tumor agents [21,22]. The complexes of 1, 10-Phenanthroline and other polypyridyls with transition metals have stimulated various researches [23].

1,10-Phenanthroline and a number of its derivatives, substituted mainly at the 2,9 ,the 4,7 or the 5,6 positions , play an important role in complex chemistry because of their unique properties as chelating agents [24,25,26]. 1,10-Phenanthroline 5,6 dione (pdon) is of particular interest, since two nucleophilic centers (nitrogen and oxygen lone pairs) are composed in a molecule of such a quinone, with all the atoms, except hydrogens, being of sp^2 hybridization [27]. The presence of two electronegative heteroatoms creates not only the basic properties in the Lewis sense but also, because of the resonance conjugation, make it possible to alter the electron density in different parts of the molecule, especially by the interaction of an external electrophile with the unshared pairs of electrons of the heteroatom [28].

The 1,10-phenanthroline-5,6-dione and 4,7 phenanthroline-5,6-dione are a useful class of heterocyclic *o*-quinone compounds. Historically, in the 1950s, they were first found to be of use because of their activity against protozoa, amoebae and bacteria [29].

1,10-Phenanthroline-5,6-dione is a versatile ligand for the assembly of metal organic materials [30,31]. This ligand has the ability to form stable complexes with a wide variety of metal ions and carries an o-quinone moiety with pH-dependent electroactivity [32].

1,10-Phenanthroline-5,6-dione (pdon) is a chelate ligand containingan o-quinoid moiety which has many interesting characteristics. Owing to its redox activity, pdon in a metal-free state and in complexes with transition metals (ruthenium, cobalt, osmium, iron, and nickel) shows strong electrocatalytic activity for the oxidation of NADH [33,34].

Since pdon can interact via a diiminic binding site and through an o-quinoid group, it acts as a bridging ligand to construct binuclear or multinuclear complexes [35,36].

1.4 Biological roles of nickel complexes

Effect of Ni(II) cystine complex (Fig.1.1) on the enhancement of total peritoneal cells in normal mice are investigated. Treatment with the Ni(II) cystine complex at the doses of 5 mg/Kg and 10 mg/Kg showed some positive effects on enhancement of number of peritoneal cells. The number of macrophages are also increased to some extent. This enhancement [37] might have produced some cytokinetic products such as tumor necrosis factor, interleukim, interferons etc. which in turn may be responsible for killing the tumor cells. In conclusion, Ni(II) cystine complex can be considered as a new compound, having fairly antineoplastic activity [38].

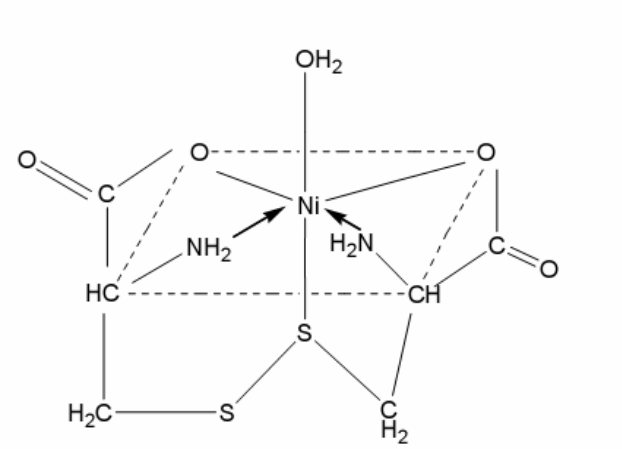
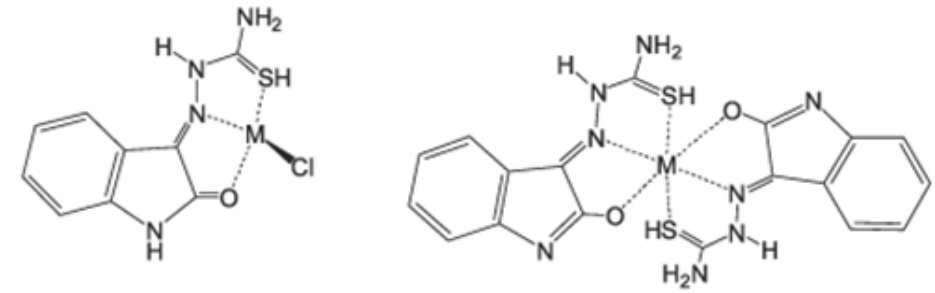


Fig. 1.1 The structure of Ni(II) cystine complex

The interaction between the $[Ni(RA)_2(H_2O)_2].H_2O$ complex and DNA was studied. By the studies, it was found that this complex is banded master fully with DNA via intercalation mode.The agarose gel electrophoresis studies have been carried out and it was found that the complex can promote the cleavage of plasmid DNA at physiological pH and temperature. The $[Ni(RA)_2(H_2O)_2].H_2O$ complex can more effectively promote the cleavage of plasmid DNA than all-trans retinoic acid and Ni(II) at the same conditions. All of these maybe one of the reasons why the inhibitory effect of $[Ni(RA)_2(H_2O)_2].H_2O$ on the human bladder line EJ cells is much greater than that of retinoic acid. As a result, the Sm,

Y and Cu complexes can also bind to DNA in intercalating mode just like $[Ni(RA)_2(H_2O)_2].H_2O$. By comparing the intercalative degree of these complexes with these studies, the $[Ni(RA)_2(H_2O)_2].H_2O$ complex was the best. The study of the $[Ni(RA)_2(H_2O)_2].H_2O$ with DNA may provide more details about the relation of better anti tumor properties of complexes and the mode of DNA binding [39].

The complexes of manganese(II), iron(II), cobalt(II), nickel(II), copper(II), and zinc(II) with isatin-β-thiosemicarbazone were prepared (Fig.1.2), and the nickel complex was structurally characterized by X-ray crystallography. Investigations of cellular growth and apoptosis induction in the presence of these species indicated that the proligand and the corresponding nickel(II) and copper(II) complexes can inhibit cell proliferation of human leukemia U937cell lines [40]. It was observed that the proligand and its copper complex were equally active, inhibiting the cellular growth by nearly 70% at 20 μg mL^{-1}. With the nickel complex, an inhibition of only 30% was verified at 10 μg mL^{-1}; at higher concentration this complex was cytotoxic [41].



$M(H_2L)_2Cl_2$; M= Co, Ni, Mn, Fe

$M(HL)_2$; M= Cu, Zn, Ni

or $M(H_2L)Cl$; M= Cu

Fig. 1.2 Selective examples of isatin-β-thiosemicarbazone complexes active in the inhibition of cell proliferation

Owing to their useful bactericidal and antifungal activities, transition metal complexes of 1-hydroxypyridine2 (1H)-thione (HPT) have been widely investigated. The complex $[Ni(C_6H_6NOS)_2]$ was synthesized, the structure of $[Ni(C_6H_6NOS)_2]$ complex is shown in Fig.1.3 . Moreover, HPT exhibits unusual versatility in coordinating to metals. On the other hand, the simulation of many metalloenzymes involving the thiolate group has also received considerable attention, particulary the assessment of the antitumor activity of some nickel complexes with chelating ligands [42].

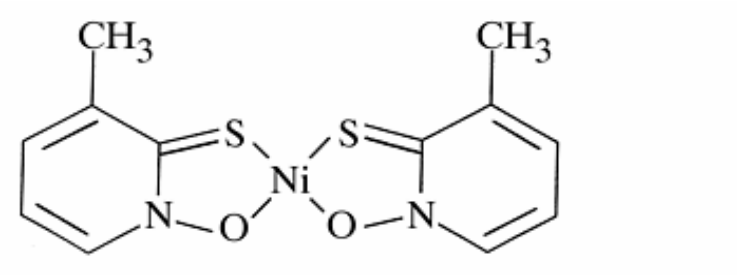


Fig. 13 The structure of $[Ni(C_6H_6NOS)_2]$ complex

1.5 Biological roles of complexes cotaining 1,10-phenanthroline-5,6-dione or 2, 2́-bipyridine as ligand

A series of mixed ligand Cu(II) complexes of 1,10-phenanthroline-5,6-dione containing also 1,10-phenanthroline, 2,2́-bipyridine and 2,2́ ; 6́,2˝-terpyridine as co-ligands have been prepared and characterized. These Cu(II) complexes are particularly attractive species for developing new diagnostic and therapeutic agents that can recognize and cleave DNA [43].

The chemical modification of proteins by the quinine moiety of a 1,10-phenanthroline-5,6-dione (pdon) complex was investigated using $[Ru(pdon)(bpy)_2](ClO_4)_2$ (bpy=2,2́ -bipyridyl) ([1]$(ClO_4)_2$) (Fig.1.4.) and cytochrome c (cyt. c) at various pH (7–9). Mass spectra revealed a peak of cyt. c modified with 1. The reactivity of 1 for cyt. c increased with the pH of the solution. These results suggest that 1 was successfully

attached to cyt. c by a reaction between the quinine moiety of 1 and the amino group or guanidine group of cyt. c. In the reaction at pH 9, the ratio of cyt. c to 1 in the product was estimated to be 1:0.8 by UV–Vis spectroscopy [44].

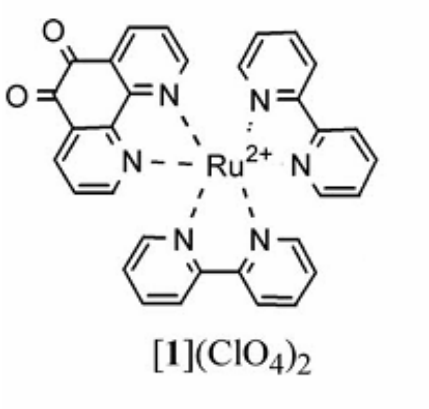


Fig. 1.4 The structure of [Ru(pdon)(bpy)$_2$](ClO$_4$)$_2$ complex

Butyldithiocarbamate sodium salt (Bu-dtcNa) and its two complexes, [M(bpy)(Bu-dtc)]NO_3 (M= Pt(II) or Pd(II) and bpy= 2,2́-bipyridine), have been synthesized. In these complexes, the dithiocarbamato ligand coordinates to Pt(II) or Pd(II) center as bidentate with two sulfur atoms. These complexes show 50% cytotoxic concentration (Cc_{50}) values against chronic myelogenous leukemia cell line, K562, much lower than that of cisplatin [45].

The dinuclear complexes [$Pd_2(L)_2(bipy)_2$](1), [$Pd_2(L)_2(phen)_2$](2), [$Pt_2(L)_2(bipy)_2$](3) and [$Pt_2(L)_2(phen)_2$](4), where bpy= 2,2́ -bipyridine, phen= 1,10-phenanthroline and L=2,2-azanediyldibenzoic dianion) dibridged by H_2L ligands have been synthesized and characterized. The binding of the complexes with fish sperm DNA (FS-DNA) were investigated by fluorescence spectroscopy. The results indicate that the four complexes bound to DNA with different binding affinity, in the order complex 4 > complex 3 > complex 2 > complex 1, and the complex 3 binds to DNA in both coordination and intercalative mode. Gel Electrophoresis assay demonstrates the ability of the complexes to cleave the pBR 322 plasmid DNA. The cytotoxic activity of the complexes was tested

against four different cancer cell lines. The four complexes exhibited cytotoxic specificity and significant cancer cell inhibitory rate [46].

A pair of enantiomers based on the DNA-intercalating Ru(II) poly pyridyl complex have been synthesized. The DNA binding, photocleavage, topoisomerase inhibition, and cytotoxicity of the complexes were studied. As we expect, an intercalative binding mode between the Ru(II) complexes and DNA has been supported by various spectral experiments and hydrodynamic measurements. Δ- $[Ru(bpy)_2(uip)]^{+2}$ was found to possess an obviously greater affinity with DNA than Λ-$[Ru(bpy)_2(uip)]^{+2}$. The synthesized complexes were found to possess high photocleavage activity for plasmid DNA pBR322 under irradiation at 365nm. The inhibition of Topo I and Topo II by the synthesized complexes was studied. The results suggest that both complexes are efficient inhibitors towards Topo II by interfering with the DNA relegation and direct Topo I binding. Moreover, both complexes show moderate antitumor activity towards HELA, hepG2, BEL-7402, and CNE-1 tumor cells. These studies proved that, as good DNA intercalators, Ru(II) polypyridyl complexes are also capable of playing important roles in much wider biologic areas, especially those concerned with DNA intercalation [47].

1.6 Characteristics of DNA

The phosphorus- and nitrogen-containing materials that came to be known as nucleic acids were first isolated from cells around 1870 by Friedrich Miescher but were long regarded as something of a curiosity.

Nevertheless, the structures of the monomer units, the nucleotides, were established by 1909 and the correct polynucleotide structure of the chains of DNA and RNA was proposed by Levene and Tipson in 1935.

The nucleotides are made up of three parts:

1. One of the pyrimidine or purine “bases”: uracil, cytosine, adenine, or guanine (Fig.1.5).

All four of these bases are present in RNA, while DNA contains thymine instead of uracil. Atoms in the bases are numbered 1–6 or 1–9.

2. A sugar, either D-ribose or D-2-deoxyribose. Carbon atoms in sugars are numbered 1´–5´.

3. Phosphoric acid

Although the biological synthesis is indirect, we can imagine that nucleotides are formed from these parts by elimination of two molecules of water. In nucleic acids the nucleotides are combined through phosphodiester linkages between the 5'-hydroxyl of the sugar in one nucleotide and the 3'-hydroxyl of another. Again, we can imagine that these linkages were formed by the elimination of water.

The structures of a pair of short polynucleotide strands in DNA are shown in Fig. 1.6. That of a segment of double-helical DNA is shown in Fig. 1.7.

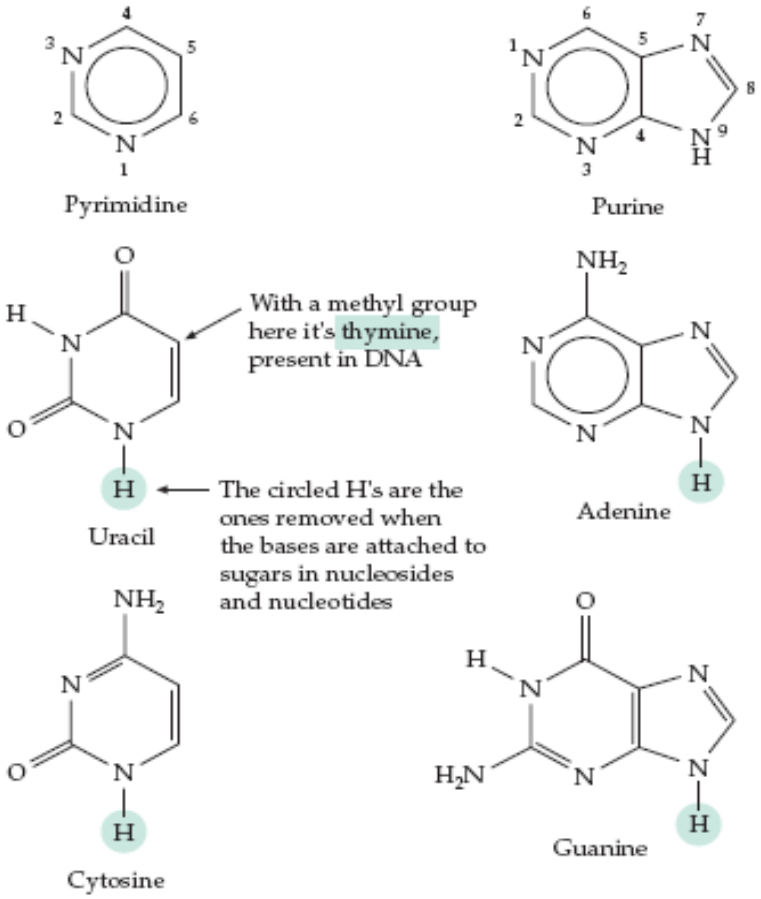


Fig. 1.5 Structures of the major pyrimidine and purine bases of DNA and RNA [45]