SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL PROPERTIES OF NICKEL- AND IRON-CARBOXYLATE COMPLEXES

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL PROPERTIES OF NICKEL- AND IRON-CARBOXYLATE COMPLEXES

by

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic Acid
ROS	Reactive Oxygen Species
IARC	International Agency for Research on Cancer
FT-IR	Fourier Transform Infrared Spectroscopy
CHN	Carbon, Hydrogen, Nitrogen
AAS	Atomic Absorption Spectroscopy
UV-Vis	Ultraviolet-Visible Spectrometry
CV	Cyclic Voltammetry
TGA	Thermogravimetric Analysis
IC_{50}	Fifty percent cytotoxic dose
S.E.M	Standard error of the mean
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
LMCT	Ligand to metal charge transfer
DMSO	Dimethyl sulfoxide
Gly	Glycine
Ala	β-alanine
His	L-histidine
Ni(gly)	Diaquabis(glycine)nickel(II) dichloride
Ni(ala)	Diaquabis(β-alanine-N,O)nickel(II)
Ni(his)	Bis(L-histidinato)nickel(II) monohydrate
2-pic	2-picolinic acid
3,5-dinitro	3,5-dinitrobenzoic acid

4-amino	4-aminobenzoic acid
Ni(2-pic)	Diaquabis(2-picolinato)nickel(II) dihydrate
Ni(3,5-dinitro)	Tetraaquabis(3,5-dinitrobenzoato)nickel(II) tetrahydrate
Ni(4-amino)	Diaquabis(4-aminobenzoato)nickel(II)
Pyr	Pyrazine-2-carboxylic acid
Ni(pyr)	Diaquabis(2-pyrazinecarboxylato)nickel(II)
Fe(pyr)	Diaquabis(2-pyrazinecarboxylato)iron(II) pyrazine hemisolvate
Mal	Maleic acid
Ni(mal)	Tetraaquabis(maleato)nickel(II)
Fe(mal)	Tetraaquabis(maleato)iron(II)
Pdc	Pyridine-2,6-dicarboxylic acid
Ni(pdc)	Dipotassium bis(pyridine-2,6-dicarboxylato)nickel(II) heptahydrate
Fe(pdc)	Hydronium bis(pyridine-2,6-dicarboxylato)iron(III)

SINTESIS, PENCIRIAN DAN SIFAT BIOLOGI KOMPLEKS NIKEL- DAN FERUM- KARBOKSILAT

ABSTRAK

Dalam projek penyelidikan ini, sembilan kompleks Ni(II) telah berjaya disintesiskan daripada tindak balas antara nikel(II) klorida heksahidrat, $NiCl_2.6H_2O$ dengan ligan asid amino (glisina, β-alanina dan L-histidina), ligan asid karboksilik (asid 2-pikolinik, asid 3,5-dinitrobenzoik, asid 4-aminobenzoik dan asid pirazina-2-karboksilik) dan ligan asid dikarboksilik (asid maleik dan asid piridina-2,6-dikarboksilik). Selain itu, dua kompleks Fe(II) dan satu kompleks Fe(III) juga telah berjaya disintesis. Kompleks Fe(II) disintesis daripada tindak balas antara ferum(II) klorida 4-hidrat, $FeCl_2.4H_2O$ dengan ligan asid pirazina-2-karboksilik dan asid maleik masing-masing. Manakala kompleks Fe(III) disintesis daripada tindak balas ferum(III) klorida dengan ligan asid piridina-2,6-dikarboksilik. Nisbah stoikiometri logam kepada ligan yang digunakan ialah 1:2. Kompleks-kompleks ini dicirikan melalui analisis takat lebur, keterlarutan, konduktiviti, Spektroskopi Inframerah (IR), Mikroanalisis CHN, Spektroskopi Penyerapan Atom (AAS), Spektroskopi Ultralembayung Ungu (UV-Vis), Analisis Siklik Voltammetrik (CV), Analisis Termogravimetrik (TGA) dan X-ray Kristalografi. Selain itu, interaksi antara kompleks logam dengan DNA pBR322 juga dikaji melalui eksperimen gel elektroforesis, manakala aktiviti sitotoksik kompleks logam tersebut pula dikaji melalui pengasaian MTT. Analysis keputusan IR menunjukkan kesemua ligan terikat secara monodentat kepada atom pusat Ni dan Fe melalui kumpulan COO^-. Analisis

UV-Vis menunjukkan bahawa keadaan pengoksidaan bagi nikel dalam kompleks ialah +2 dan mempunyai konfigurasi d^8 manakala kompleks Fe(II) dan Fe(III) mempunyai keadaan pengoksidaan +2 dan +3 dan konfigurasi d^6 dan d^5 masing-masing. Analisis CV menunjukkan bahawa semua kompleks mempunyai sifat redoks pembalikan yang penting dalam kegunaan biologi. Hasil kajian eksperimen gel elektroforesis menunjukkan kesemua kompleks logam berjaya membuat pemotongan plasmid DNA dimana pemotongan tersebut menghasilkan jaluran tunggal serta jaluran berganda DNA sebagai produk. Keberkesanan pemotongan plasmid DNA ini bergantung kepada kepekatan kompleks logam tersebut. Kompleks ferum mempamerkan keberkesanan pemotongan yang lebih efisien berbanding dengan kompleks nikel (II). Dalam keputusan pengasaian MTT, kesemua kompleks logam menunjukkan aktiviti sitotoksik terhadap sel turunan kanser HepG2.

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL PROPERTIES OF NICKEL- AND IRON- CARBOXYLATE COMPLEXES

ABSTRACT

In this research, nine Ni(II) complexes were successfully synthesized using nickel(II) chloride hexahydrate, $NiCl_2.6H_2O$ with amino acids (Glycine, β-alanine and L-histidine), carboxylic acids (2-picolinic acid, 3,5-dinitrobenzoic acid, 4-aminobenzoic acid and pyrazine-2-carboxylic acid) and with dicarboxylic acids (maleic acid and pyridine-2,6-dicarboxylic acid). Besides, two Fe(II) complexes and one Fe(III) complexes were also successfully synthesized. Fe(II) complexes were synthesized using iron(II) chloride 4-hydrate, $FeCl_2.4H_2O$ with pyrazine-2-carboxylic acid and maleic acid respectively. While Fe(III) complex was synthesized using ferric chloride anhydrous, $FeCl_3$ with pyridine-2,6-dicarboxylic acid. Stoichiometry ratio of metal to ligand being used is 1:2. These complexes were characterized by determination of melting point, solubility, conductivity measurement, Fourier Transform Infrared Spectroscopy (FTIR), CHN microanalysis, Analysis of Atomic Absorption Spectroscopy (AAS), Ultraviolet-Visible Spectrometry (UV-Vis), Cyclic Voltammetry (CV), Thermogravimetric Analysis (TGA) and X-ray crystallography. Besides that, the interaction between the metal complexes and pBR322 DNA will be investigated by gel electrophoresis experiments, while the cytotoxic activity of the complexes was tested by MTT Assay. FT-IR analysis result shows that the carboxylate group in all of the ligand were coordinated to the central metal ion monodentately. The UV-Vis analysis shows that the oxidation state for nickel complexes is +2 and its configuration system is d^8

meanwhile the oxidation state for Fe(II) and Fe(III) complexes are +2 and +3 and configuration systems are d^6 and d^5 respectively. The CV analysis shows that all the complexes have reversible redox properties which are very important in biological uses. Gel electrophoresis experiments show that all the complexes successfully promote the cleavage of plasmid DNA, producing single and double DNA strand breaks. In MTT assay results, all of the complexes showed cytotoxic activity against HepG2 cancer cell lines.

CHAPTER 1

INTRODUCTION

1.1 Nickel

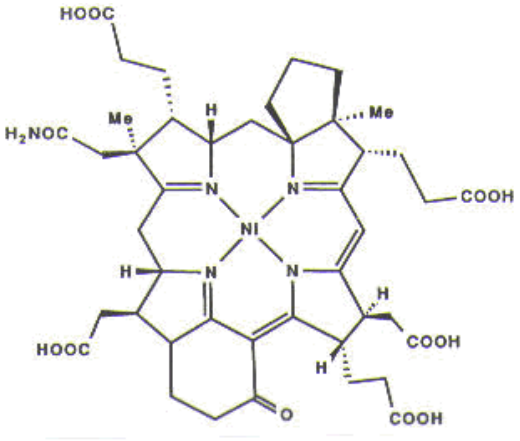
Nickel is a transition metal with the symbol Ni and atomic number 28. It is a silvery white metal that takes on a high polish. It is hard, malleable, ductile, ferromagnetic, and a fair conductor of heat and electricity. Nickel can forms a number of complex compounds. Many of these nickel compounds are water soluble and have a characteristic green or blue color. Nickel and its compounds have no characteristic odor or taste. The most common oxidation state of nickel is +2, though 0, +1, +3 and +4 Ni complexes are observed.

1.1.1 Applications of Nickel

Properties of nickel make it very desirable for combining with other metals to form mixtures called alloys. Some of the metals that nickel can be alloyed with are iron, copper, chromium, and zinc. These alloys are used in making metal coins and jewellery and in industry for making items such as valves and heat exchangers. Nickel is mostly used to make stainless steel. There are also compounds consisting of nickel combined with many other elements, including chlorine, sulfur, and oxygen. Nickel compounds are used for nickel plating, to color ceramics, to make some batteries, and as catalysts to increase the rate of chemical reactions.

Besides, nickel is required for the function of several enzymes in the human body. Nickel is found in factor F430, which is required for methanogenesis, a process used by the archaeobacteria in which the simple gases, such as H_2, CO, and

CO_2, are used to provide both energy and a carbon source (Figure 1.1) (Crabtree, 1988; Lippard and Berg, 1994).



Factor F_{430}

Figure 1.1 Metals in biological systems: nickel in factor F430

1.2 Iron

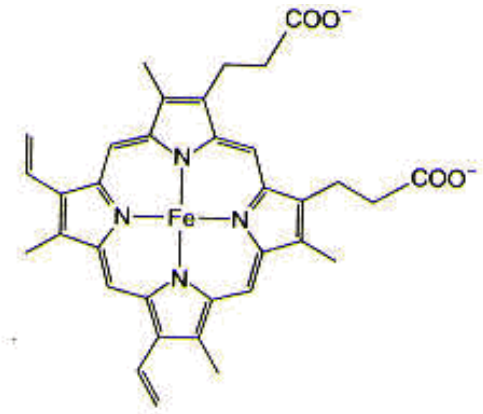
Iron is a chemical element with the symbol Fe and atomic number 26. Iron is a lustrous, ductile, malleable and silver-grey metal. It is one of the few ferromagnetic elements. It is known to exist in four distinct crystalline forms. Iron rusts in dump air, but not in dry air. It dissolves readily in dilute acids. Iron is chemically active and forms two major series of chemical compounds, the bivalent iron (II), or ferrous, compounds and the trivalent iron (III), or ferric, compounds.

1.2.1 Applications of Iron

Iron is the most widely used of all the metals, accounting for 95% of worldwide metal production. Its low cost and high strength make it indispensable in engineering applications such as the construction of machinery and machine tools, automobiles, the hulls of large ships, and structural components for buildings. Since pure iron is quite soft, it is most commonly used in the form of steel. Some of

the forms in which iron is produced commercially include cast iron, wrought iron, carbon steel, alloy steels, iron(III) oxides.

In biological system, iron is found in a variety of iron-sulfur clusters, which are necessary for nitrogen fixation, as well as in heme groups, found in hemoglobin, which is used for dioxygen transport and storage in the body (Figure 1.2) (Crabtree, 1988; Lippard and Berg, 1994).



Heme

Figure 1.2 Metals in biological systems: iron in heme, which is found in hemoglobin

1.3 Transition Metal Complexes

Transition metal complexes have been the subject of thorough investigation because of their extensive applications in wide ranging areas from material sciences to biological sciences (Boerner and Zaleski, 2005). Metal complexes are well-known to accelerate the drug action and the efficacy of a therapeutic agent can often be enhanced upon coordination with a metal ion (Goldstein *et al.*, 1986). The pharmacological activity has also been found to be highly dependent on the nature of the metal ion and the donor sequence of the ligands as different ligands exhibit different biological properties (Delaney *et al.*, 2002). In recent years, the binding studies of transition metal complexes have become an important field in the

development of DNA molecular probes and chemotherapeutics (Dardlier *et al.*, 1997).

The carboxylate group is an important class of ligand in inorganic and bioinorganic chemistry. Metal complexes containing monocarboxylic acids are well known and the publication of many structurally characterised examples of this class of compound has demonstrated the versatility of the carboxylate group as an innersphere ligand (Mehrotra and Bohra, 1983).

The chemistry of nickel (II) complexes with simple carboxylate anions has been widely studied (Sacconi *et al.*, 1987; Oldham, 1968). And several examples of dicarboxylate complexes of this metal have also been described (Krisnamurty and Harris, 1961). The versatility in coordination modes exhibited by these kinds of ligands, together with their ability to form polymeric species make its structural chemistry particularly interesting. Related systems with another function besides the carboxylate group like pyridine-2 carboxylate, glycine and 2-aminobenzoic acid have also received recent attention (D'Avignon and Brown, 1982; Carmona *et al.*, 1990; Zhong *et al.*, 1994; Abbot *et al.*, 1995).

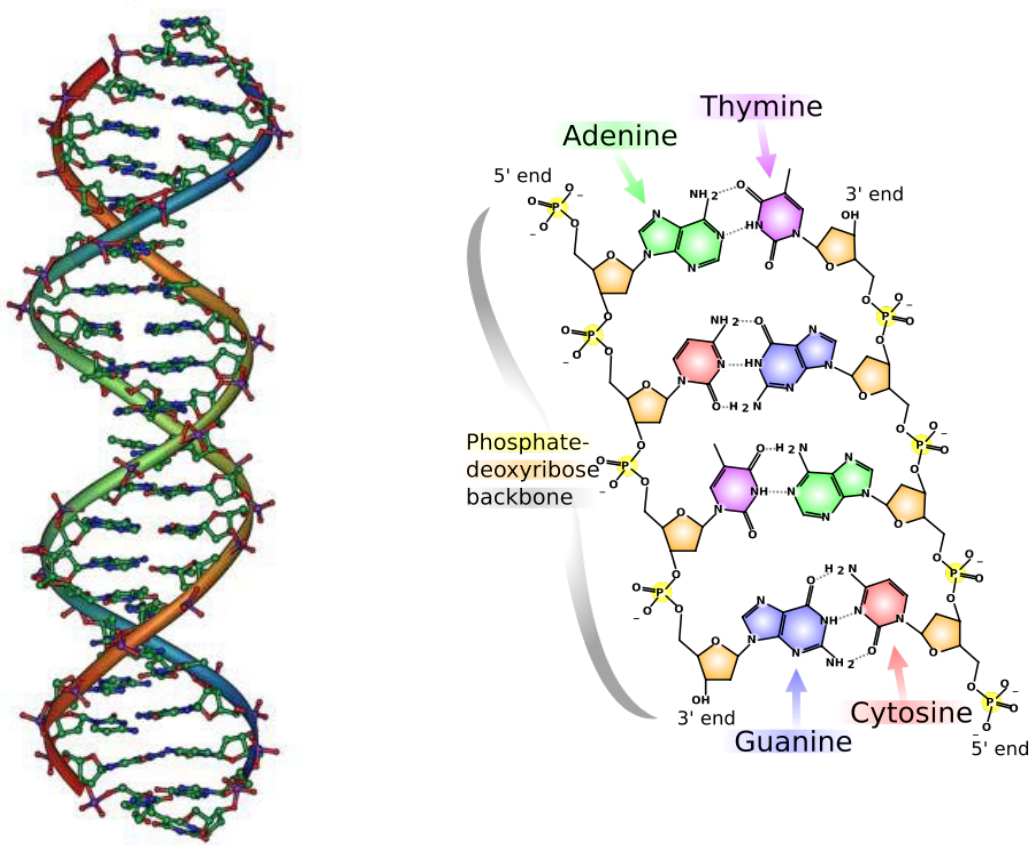
1.4 DNA

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person’s body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA).

The information in DNA is stored as a code made up of four chemical bases: adenine(A), guanine(G), cytosine(C), and thymine(T). Human DNA consists of

about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.

DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix (Figure 1.3). The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules forming the vertical side pieces of the ladder.



(A) (B)

Figure 1.3 (A) The structure of part of a DNA double helix (B) The chemical structure of DNA (http://schools-wikipedia.org/wp/d/DNA.htm)

1.5 The Interaction of Transition Metal Complexes and DNA

Transition metal complexes have been of interest in the field of cancer research. They had been widely studied for their antimicrobial and anticancer properties. This is because they exhibit unique spectral and electrochemical signatures, as well as the ability of their ligands to be modulated to DNA binding and cleaving abilities.

Many biological studies suggest that DNA is the primary intracellular target of anticancer drugs because of the interactions between small molecules and DNA can often cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death (Hecht, 2000). Of those studies, the interaction of transition metal complexes with DNA has gained much attention owing to their possible application as new therapeutic agents (Metcalfe and Thomas, 2003).

A number of transition metal complexes with planar aromatic heterocyclic ligands have been used as probes of DNA secondary structure and therapeutic agents, and the biological activities of these DNA targeted complexes usually increase compared with those of either free ligands or metal ions alone, which may be due to different binding properties of these complexes to DNA (Lewandowski *et al.*, 2005). The type of central metal ions, which is responsible for the geometry of complexes, also has significant influence on the intercalating ability of transition metal complexes to DNA (Chaires, 1997; Mozaffar *et al.*, 2004).

It is believed that the biological activity of antitumor metal complexes is strictly connected to their abilities to bind to DNA, damage DNA structures and impair DNA functions (Burrows and Rokita, 1994). Impairment of DNA function results in inhibition of replication and transcription process and even in cell death, if

eventually the DNA lesions are not rapidly and properly repaired (Sitlani *et al.*, 1992; Li *et al.*, 2005).

Basically, transition metal complexes interact with double helix DNA in either noncovalent or covalent way. Concerning the noncovalent interactions between transition metal complexes and DNA, they can occur by intercalation, groove binding, or external electrostatic binding. Intercalation consists of the insertion of a flat aromatic molecule between two adjacent bases. Groove binding is a stronger type of interaction that takes place when a molecule of proper size enters one of the grooves of DNA. External electrostatic binding refers to those interactions that occur on the DNA surface, mainly governed by electrostatic effects. Among these interactions, intercalation is one of the most important DNA binding modes. It was reported that the intercalating ability appeared to increase with the planarity of ligands.

Specifically we investigated the interaction between the metal complex and DNA by gel electrophoresis**.** When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoiled form will relax to produce a slower moving nicked circular form (Form II) (An *et al.*, 2006). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between (Masataka *et al.*, 1999). Hence, DNA strand breaks were quantified by measuring the transformation of the supercoiled form into nicked circular and linear forms (Figure 1.4).

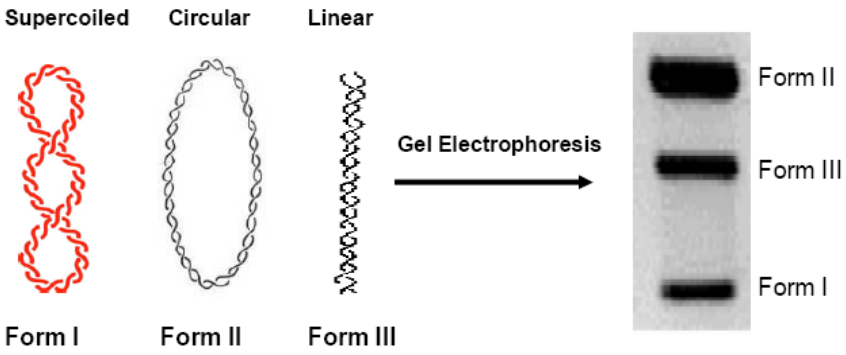


Figure 1.4 Three forms of DNA that can be tracked using agarous gel electrophoresis

1.6 Transition Metal Complexes as Anticancer Agents

In the history of coordination complexes in cancer therapy, the first transition metal to be used successfully as an anticancer agent was platinum. It was used in a compound cis-$[Pt(NH_3)_2Cl_2]$ or cisplatin (Figure 1.5). Its ability to inhibit tumors was discovered by Rosenberg around 1969 (Rosenberg *et al.*, 1969 & 1970). Cisplatin entered clinical trials in 1971 and was approved by the FDA in 1978 (Higby *et al.*, 1974). It has become one of the most widely used drugs in cancer chemotherapy. The platinum drugs such as carboplatin, nedaplatin and oxaliplatin (Figure 1.6) are frequently used in combination therapy for numerous solid tumours, including ovarian, head and neck, testicular, bladder, colorectal, gastric, melanoma and small-cell lung cancer (Giaccone, 2000).

Systemic toxicity of cisplatin gives rise to a number of limitations. Serious side-effects such as nausea, nephrotoxicity, neurotoxicity and ototoxicity occur often (Reedijk, 1996). In addition, cisplatin, which is administered intravenously has limited solubility in aqueous solution (Wong and Giandomenico, 1999). Therefore, these have stimulated a search for other transition metal complexes which have higher activity and reduced side-effects.

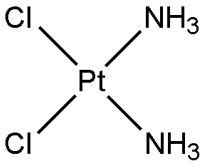
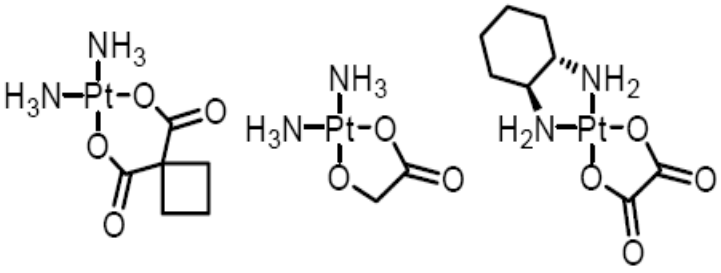


Figure 1.5 Structural formula of cisplatin



carboplatin nedaplatin oxaliplatin

Figure 1.6 Cisplatin analogues used in the clinic

1.7 Mechanism of DNA Cleavage

McCord and Fridovich were the first to discover that the enzyme superoxide dismutase is able to dismutate superoxide anion to form oxygen and hydrogen peroxide (McCord and Fridovich, 1969). Transition metal complexes were found can induce DNA cleavage by generating reactive oxygen species (ROS). These ROS have been found to interact directly with DNA or DNA components via singlet oxygen species (O_2•) and hydroxyl radicals (·OH). One of the methods from which it is produced is the Fenton reaction (Figure 1.7), where a transition metal is oxidised to a higher oxidised state, by donating an electron to a hydrogen peroxide species, resulting in the formation of a hydroxyl radical and a hydroxyl ion. ·OH is one of the most studied reactive biological radicals, and has been implicated in reactions with the nucleic acid bases of DNA. ·OH reacts preferentially with the π-bonds of DNA bases, but can also interact with the sugar units by hydrogen abstraction. ·OH is known to react with each of the four DNA bases, resulting in mutagenic lesions.

While, literature have shown that $O_2^{\cdot -}$ radicals cannot induce DNA damage (Muller and Burrows, 1998). These $O_2^{\cdot -}$ can react with H^+ to generate H_2O_2 which in turn can react with more $O_2^{\cdot -}$ to generate more ·OH via Harber-Weiss reactions.

The ·OH radical will attack the guanine moiety at the C4, C5 or the C8 position. Guanine is the most sensitive base towards oxidative attack. Floyd *et al.* was proving that the major product formed by ·OH and guanine was 8-hydroxyguanine (8-OH-Gua) suggesting that ·OH radicals are involved in the attack of purine bases (Floyd *et al.*, 1986). From Figure 1.8, adduct 1 and 2 revert back to guanine by gaining an electron via thiols generated in the cells. Adduct 3 (8-OH-Gua) can be oxidised to form 8-oxo-Gua and reduced to form formamidopyrimidine (Fapy-G). Cadet *et al.* also implicated the ·OH in tandem DNA base damage (Cadet *et al*., 1999). This damage via the Fenton reaction can be mediated in vivo by labile transition metals, such as iron (Fe), copper (Cu) and nickel (Ni) (Lloyd and Phillips, 1999).

$$X^{2+} + H_2O_2 \rightarrow X^{+} + 2H^{+} + O_2^{\cdot -}$$
$$X^{+} + H_2O_2 \rightarrow X^{2+} + \cdot OH + OH^{-}$$
$$X^{+} + O_2 \rightarrow X^{2+} + O_2^{\cdot -}$$
$$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + 2 \cdot OH$$

$$O_2^{\cdot -} + X^{2+} \rightarrow {}^1O_2 + X^{+}$$

Figure 1.7 Mechanism of Fenton reaction (X represents metal)

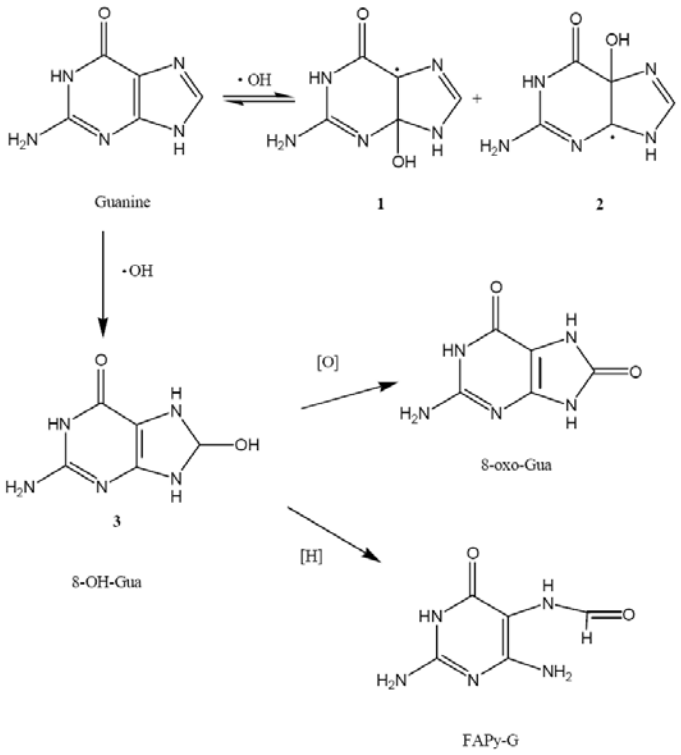


Figure 1.8 Schematic representation for the reaction of guanine with hydroxyl radicals

1.8 Biological Activity of Nickel(II) Complexes

Nickel is a compound that occurs in the environment only at very low levels. However, exposure to high levels of nickel pollution has been linked to nasal and lung cancer and nickel is therefore considered a carcinogen (Matkar *et al.*, 2006).

The Occupational Safety and Health Administration (OSHA) has set an enforceable limit of 1.0 mg nickel/m³ for metallic nickel and nickel compounds in workroom air to protect workers during an 8-hour shift over a 40-hour work week. The Environmental Protection Agency (EPA) recommends that drinking water levels for nickel should not be more than 0.1 mg per liter.

Nickel plays versatile and sometimes controversial roles in living systems (Lancaster, 1998). Biological effects of nickel are closely related to its chemical forms of existence. For example, water-insoluble crystalline Nickel sulfate, NiS and

Nickel(II) oxide, NiO were suspected to induce human lung and nasal cancers (IARC, 1990), while animal studies suggested that water-soluble nickel sulfate did not cause a higher incidence of lung cancer statistically (Dunnick *et al.*, 1995).

As a platinum group metal, nickel(II) may share some similarity with platinum(II). In fact, several nickel complexes have been found to inhibit proliferation of diverse cancer cells (Ferrari *et al.*, 2002; Liang *et al.*, 2004; Matkar *et al.*, 2006). In addition, the ability of certain nickel compounds to interact with DNA and RNA has been exploited for research purposes.

One of the synthetic nickel complexes extensively studied in the past because of its ability to catalyze limited cleavage of nucleic acids contains a ligand known as CR (2,12-dimethyl-3,7,11,17-tetraazabicyclo-[11.3.1]-heptadeca-1(17),2,11,13,15-pentaene). $[NiCR]^{2+}$ (Figure 1.9) has been used in the past as a structure-specific probe for RNA and DNA oligonucleotides in the presence of oxidizing agent but little is known about the biological effects of either complex. Matkar *et al*. show that $[Ni(CR-2H)]^{2+}$ can damage DNA in the absence of an added oxidizing agent, oxone (Figure 1.10) and has an IC_{50} of 70 μM in human breast cancer cells whereas $[NiCR]^{2+}$ and $NiCl_2$ do not exhibit significant cytotoxicity (Figure 1.11). However, both $[NiCR]^{2+}$ and $[Ni(CR-2H)]^{2+}$ bind to the minor groove of double-stranded DNA (Matkar *et al*., 2006).

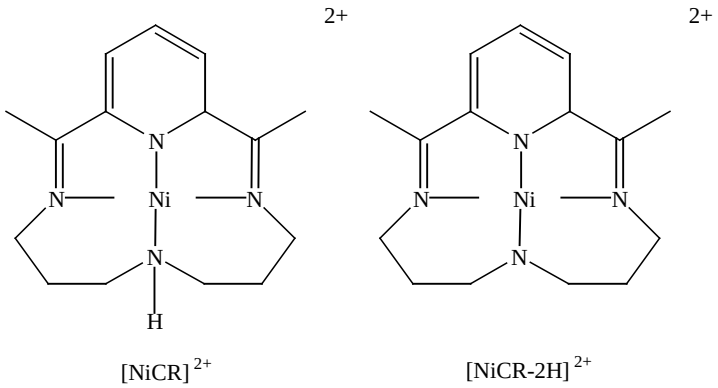


Figure 1.9 The structures of $[NiCR]^{2+}$ and $[Ni(CR-2H)]^{2+}$ (Matkar *et al*., 2006)

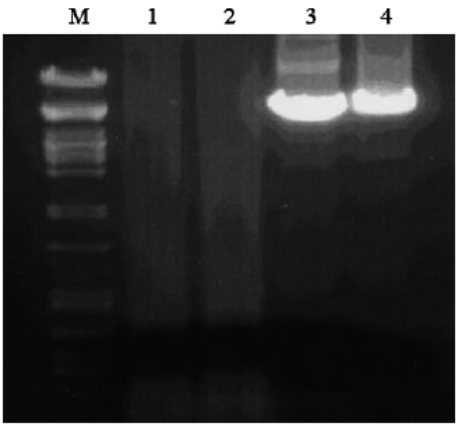


Figure 1.10 $[NiCR]^{2+}$ and $[Ni(CR\text{-}2H)]^{2+}$ caused double-stranded DNA damage in vitro in the presence of oxone. The gel shows the DNA samples after piperidine treatment. Lane 1, linearized plasmid DNA treated with $[NiCR]^{2+}$ and oxone; lane 2, DNA treated with $[Ni(CR\text{-}2H)]^{2+}$ and oxone; lane 3, DNA treated with oxone; lane 4, untreated control; M, marker (PstI-cut lambda DNA) (Matkar *et al*., 2006)

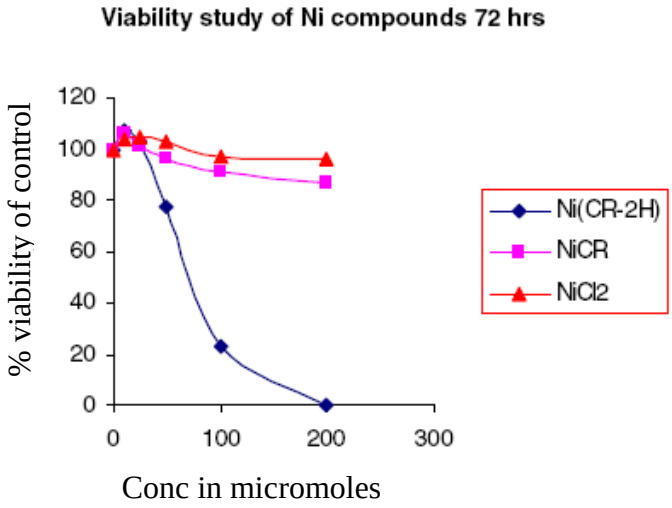


Figure 1.11 Cytotoxicity of $[Ni(CR\text{-}2H)]^{2+}$. MCF-7 human breast cancer cells were treated with 10, 25, 50, 100 or 200 µM $NiCl_2$, $[NiCR]^{2+}$ or $[Ni(CR\text{-}2H)]^{2+}$ and cell viability was determined by MTS assay. Diamonds (♦) stand for $[Ni(CR\text{-}2H)]^{2+}$, squares (■) for $[NiCR]^{2+}$, and triangles (▲) for $NiCl_2$ (Matkar *et al*., 2006)

Besides, Afrasiabi and partners have also done the research on the nickel(II) complexes of naphthaquinone thiosemicarbazone (NQTS) and semicarbazone (NQSC) (Afrasiabi *et al.*, 2005). These two nickel complexes, $Ni(NQTS)_2$ and $Ni(NQSC)_2$ were screened in vitro against MCF-7 breast cancer cell lines for their antiproliferation activity.

Figure 1.12 shows the IC_{50} values (μM) for the two ligands and their nickel(II) complexes. It is observed that complexation with metal ion in both NQTS and NQSC ligands increases the inhibitory action on MCF-7 cell proliferation. The enhancement of antiproliferation activity by metal complexes can be related to an increase in the lipophilicity so they can penetrate into the cells more easily (Petering *et al.*, 1966). It has also been suggested that metal complexation may be a vehicle for activation of the ligand as the cytotoxic agent (Beraldo *et al.*, 2004).

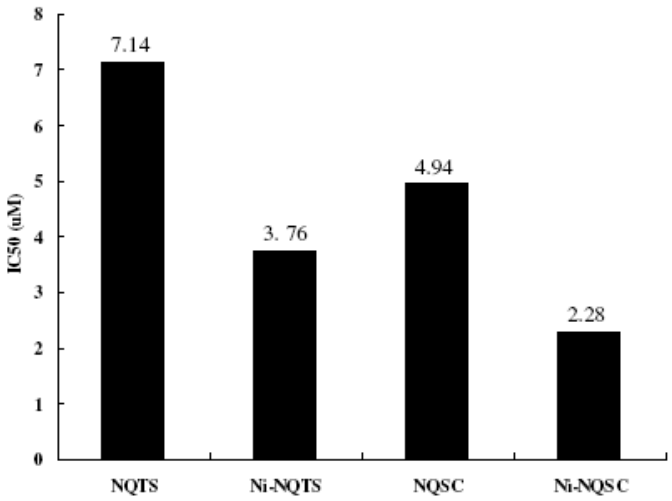


Figure 1.12 Cytotoxic effects of NQTS and NQSC and their nickel(II) complexes on MCF-7 human breast cancer cells (Afrasiabi *et al.*, 2005)

1.9 Biological Activity of Iron Complexes

Iron is one of the most important microelements for human health and is known to interact with numerous other dietary components (Lynch, 1997). Iron often exists in the state of complex in the body, which endows it a great deal of physiological function.

Iron is essential to most life forms and to normal human physiology. It is an integral part of many proteins and enzymes that maintain good health. In humans, iron is an essential component of proteins involved in oxygen transport. It is also

essential for the regulation of cell growth and differentiation. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity.

Iron in the form of complex may take part in the process of transportation and exchange of the oxygen in blood and also participates in other important metabolism as iron enzyme. However, because Fe^{2+} in food has a low biological value it may exchange into the insoluble compounds, which are hard to be adsorbed for its characteristics of being prone to be oxidized and interrupted by oxalate and phosphate (Goldstein and Samuni, 2005). The deficiency of iron micronutrient is one of the most prevalent nutritional problems of humans in developing countries. It has been shown that growing children and women of reproductive age are most vulnerable to the deficiency (Bloem, 1995; Ribaya-Mercado, 1997). Studies show that iron absorption in the body is influenced by several factors, including animal species, dietary factors, i.e. ascorbic acid (Monsen, 1988), pectin content, phytate (Morris and Ellis, 1982), protein sources and amino acids (Martinez-Torres *et al.*, 1981) and the other minerals.

On the other hand, excess amounts of iron can result in toxicity and even death (Corbett, 1995). Excessive iron can be toxic, because free ferrous iron reacts with peroxides to produce free radicals, which are highly reactive and can damage DNA, proteins, lipids, and other cellular components. Thus, iron toxicity occurs when there is free iron in the cell, which generally occurs when iron levels exceed the capacity of transferrin to bind the iron. Humans experience iron toxicity above 20 milligrams of iron for every kilogram of mass, and 60 milligrams per kilogram is a lethal dose. The Dietary Reference Intake (DRI) lists the Tolerable

Upper Intake Level (UL) of iron for adults as 45 mg/day while for children under fourteen years old is 40 mg/day.

El Amrani *et al.* have reported $[Fe(3hf)_2Cl(MeOH)]$ complex as shown in Figure 1.13 for their ability to cleave DNA (El Amrani *et al.*, 2006). As shown in Figure 1.14, $FeCl_3$ do not show any DNA cleavage. While at 23 µM of the $[Fe(3hf)_2Cl(MeOH)]$ complex, a mixture of supercoiled plasmid and nicked circular is observed.

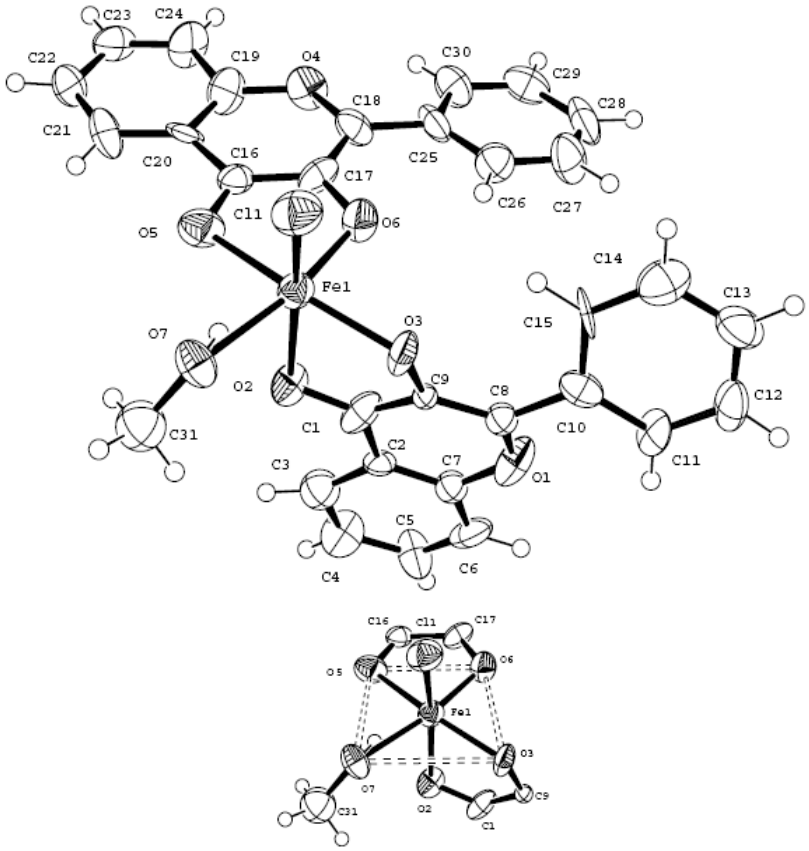


Figure 1.13 ORTEP drawing of the $[Fe(3hf)_2Cl(MeOH)]$ complex (El Amrani *et al.*, 2006)

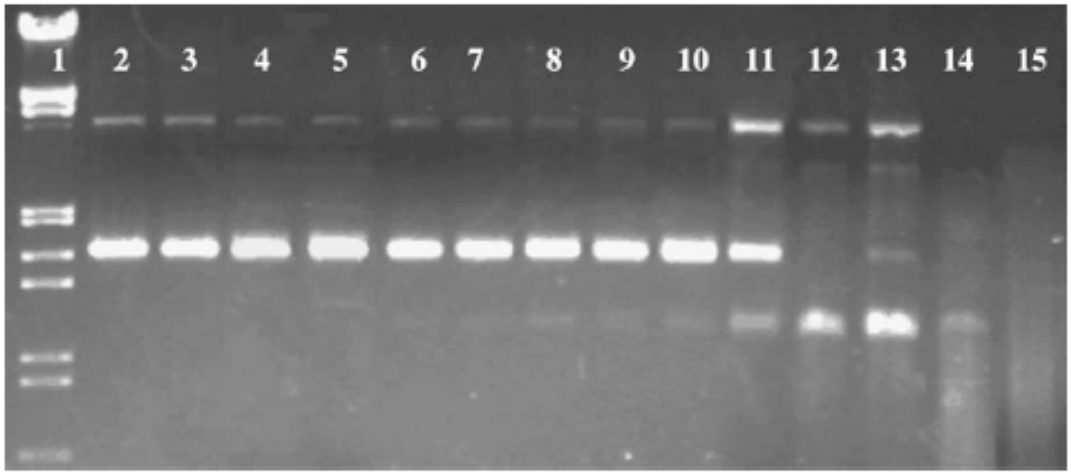


Figure 1.14 Agarose gel electrophoresis of pUC18 plasmid DNA treated with complex, $FeCl_3$, and a mixture $FeCl_3$ + H3hf (1:2) in the presence of the 1 equiv ascorbate/H_2O_2 . Lane 1: λDNA/EcoR1 + HindIII Marker; lane 2: supercoiled DNA; lane 3: supercoiled DNA + ascorbate/H_2O_2; lane 4: 15 µM $FeCl_3$; lane 5: 21 µM $FeCl_3$; lane 6: 27 µM $FeCl_3$; lane 7: 27 µM $FeCl_3$ + 54 µM H3hf; lane 8: 15 µM complex; lane 9: 18 µM complex; lane 10: 21 µM complex; lane 11: 23 µM complex; lane 12: 25 µM complex; lane 13: 26 µM complex; lane 14: 27 µM complex; lane 15: 30 µM complex (El Amrani *et al*., 2006)

Besides, Travnicek and coworkers have tested iron complexes involving N6-benzyladenosine derivatives (L1–L7) of the predominant composition $[Fe(L)Cl_3]\cdot H_2O$ for their in vitro cytotoxicity against human cancer cell lines of malignant melanoma (G-361), osteogenic sarcoma (HOS), chronic myelogenous leukemia (K-562), and breast adenocarcinoma (MCF-7) (Travnicek *et al.*, 2008). All free N6-(benzylamino)adenosine derivatives (L1–L7), used as ligands, showed cytotoxicity with IC_{50} > 50 µM, and $FeCl_3\cdot 6H_2O$ even above 200 µM. The results of selected IC_{50} values is shown in Table 1.1. From the results, complex 2, $[Fe(L_2)Cl_3]\cdot H_2O$ showed significant cytotoxicity against the HOS, K-562, and MCF-7 cell lines, respectively. Moreover, a considerable cytotoxicity has been found for the complex 6, $[Fe(L_6)Cl_3]\cdot H_2O$ on the MCF-7 cell line.

Table 1.1 Selected IC_{50} (μM) values assessed by a calcein AM assay (Travnicek *et al.*, 2008)

Compound	IC_{50} (μM)			
	G-361	HOS	K-562	MCF-7
$[Fe(L_2)Cl_3] \cdot H_2O$ (2)	>50	8	9	16
L_2	>50	>50	>50	>50
$[Fe(L_6)Cl_3] \cdot H_2O$ (6)	>50	>50	>50	4
L_6	>50	>50	>50	>50
$FeCl_3 \cdot 6H_2O$	>200	>200	>200	>200

L_2=N6-(4-fluorobenzyl)adenosine; L_6=N6-(4-trifluoromethoxybenzyl)adenosine. The human cancer cell lines (G-361, malignant melanoma; HOS, osteogenic sarcoma; K-562, chronic myelogenous leukemia; MCF-7, breast adenocarcinoma) were treated with the solution of the tested compound in the 0.5-50 μM range for 72 h, except for $FeCl_3 \cdot 6H_2O$, where the concentration range of 0.5-200 μM was used.

1.10 Objectives and Scope of Study

The objectives of this research is to synthesize a series of complexes formed by the reaction of carboxylic acids with nickel(II) chloride hexahydrate, $NiCl_2.6H_2O$, iron(II) chloride 4-hydrate, $FeCl_2.4H_2O$ and ferric chloride anhydrous, $FeCl_3$ respectively. The acids utilised were amino acid, carboxylic acid and dicarboxylic acid derivatives and are listed in Table 1.2.

Table 1.2 Carboxylic acids utilised in this study

Acids	**Name**
Amino acid derivatives	Glycine; β-alanine; L-histidine.
Carboxylic acid derivatives	2-picolinic acid; 3,5-dinitrobenzoic acid; 4-aminobenzoic acid; pyrazine-2-carboxylic acid.
Dicarboxylic acid derivatives	Maleic acid; pyridine-2,6-dicarboxylic acid

The complexes that are successfully synthesized will be characterized by determination of melting point, solubility, conductivity measurement, elemental microanalysis (C, H, N), Atomic Absorption Spectrometry (AAS), Fourier

Transform Infrared Spectroscopy (FTIR), Ultraviolet-Visible Spectrometry (UV-Vis), Cyclic Voltammetry (CV) and X-ray crystallography.

Besides that, the interaction between the metal complexes and pBR322 DNA will be investigated by gel electrophoresis experiments, while the cytotoxic activity of the complexes will be tested against human hepatoma cell line (HepG2).

CHAPTER 2

MATERIALS AND METHODS

2.1 Reagents

Table 2.1 and Table 2.2 show the features and the suppliers for all the chemicals and biochemicals which were used in this research. All of these chemicals were used without further purification.

Table 2.1 Chemicals used in this research

Chemicals	Molecular Weight (g/mol)	Purity (%)	Supplier
Nickel(II) chloride hexahydrate $NiCl_2.6H_2O$	237.70	97%	Riedel-deHaen
Iron(II) chloride 4-hydrate $FeCl_2.4H_2O$	198.81	98%	BDH Chemicals Ltd.
Ferric chloride anhydrous $FeCl_3$	162.21	98%	GCE Laboratory Chemicals
Glycine $C_2H_5NO_2$	75.07	100.11%	Fisher Chemicals
β-alanine $C_3H_7NO_2$	89.09	≥ 99%	Fluka Chemika
L-histidine $C_6H_9N_3O_2$	155.16	99%	BDH Chemicals
2-picolinic acid $C_6H_5NO_2$	123.11	98%	Fluka Chemika
Pyrazine-2-carboxylic acid $C_5H_4N_2O_2$	124.10	99%	Aldrich
3,5-dinitrobenzoic acid $C_7H_4O_6N_2$	212.12	99.5%	BDH Chemicals Ltd.
4-aminobenzoic acid $C_7H_7NO_2$	137.14	99.5%	G.P.R
Maleic acid $C_4H_4O_4$	116.08	≥ 99%	Fluka Chemika
Pyridine-2,6-dicarboxylic acid $C_7H_5NO_4$	167.12	≥ 98%	Fluka Chemika
Potassium Hydroxide KOH	56.11	85%	Systerm
Sodium Hydroxide NaOH	40.00	99%	Systerm
Nitric Acid HNO_3	63.01	65%	Systerm

Table 2.2 Biochemicals used in biology part

Biochemicals	Supplier
pBR322 DNA	Fermentas
GeneRuler 1kb DNA Ladder	Fermentas
6X Loading Dye Solution	Fermentas
1X TAE buffer	BIO-RAD Laboratories
Tris-HCl	AMRESCO
DMSO	Fluka Chemika
Tert-butanol	QReC
D-mannitol	R & M Chemicals
Potassium Iodide, KI	Fisher Scientific
Hydrogen Peroxide, H_2O_2	Fluka Chemika
Sodium Azide, NaN_3	Fisher Scientific
Ethidium Bromide, EtBr	Fluka BioChemika
HepG2	ATCC, Manassas, VA, USA
Eagle's Minimum Essential Medium, MEM	Invitrogen (GIBCO), USA
Fetal Bovine Serum, FBS	Invitrogen (GIBCO), USA
Phosphate Buffered Saline, PBS	Invitrogen (GIBCO), USA
Trypsin	Invitrogen (GIBCO), USA
MTT	Invitrogen (GIBCO), USA
Penicillin	Invitrogen (GIBCO), USA
Streptomycin	Invitrogen (GIBCO), USA

2.2 Instrumentation

The instrumentation used for the quantitative and qualitative characterization of the carboxylate complexes are listed in Table 2.3.

Table 2.3 Instruments used for the quantitative and qualitative characterizations

Instruments	Model
Melting Point Apparatus	Gallenkamp Variable Heater
Conductivity Measurement	CyberScan 500 instrument
FT-IR Spectrophotometer	Perkin-Elmer System 2000
Elemental Analyzer (CHNS/O)	Perkin-Elmer Series II 2400
Atomic Absorption Spectroscopy	Perkin-Elmer AAS model 3100
Ultraviolet-Visible (UV-Vis) Spectrometry	Model Jasco V-530
Cyclic Voltammetry (CV)	BAS Epsilon EC-20
Thermogravimetric Analysis (TGA)	Mettler Toledo TGA/SDTA851e
X-Ray Crystallography	Bruker SMART APEX2 CCD

2.3 Experimental

Nickel(II) chloride hexahydrate was used as the starting material to prepare the complex. The molar ratio of starting material:ligand was in 1:2 ratio in the synthesis of the complexes. Nickel(II) chloride hexahydrate (1 g, 4.2070 mmol) was dissolved in 30 mL of distilled water. The green solution was put into a two-neck round flask. All the ligands were dissolved in 30 mL of distilled water before mixed into the two-neck round flask. Then the mixture was refluxed with constant stirring over an oil bath at 80 ºC for 3 hours. In alkaline condition, the pH of the mixture was adjusted to pH 8 with 2M KOH before heating it under reflux. After that, the solution was filtered while it was still hot. Then the filtrate was left to evaporate to dryness at room temperature. The crystals were obtained after a few days of evaporation. The synthesis methods for the complexes of iron(II) chloride 4-hydrate(1 g, 5.0299 mmol) and ferric chloride anhydrous (1 g, 6.1648 mmol) were same as the method above.

2.4 Methods of Characterization

All the characterization analysis had been done in School of Chemical Sciences, Universiti Sains Malaysia.

2.4.1 Determination of the melting point of complexes

Melting point for each complex was determined using Gallenkamp Variable Heater in a capillary tube. The complex was observed through a window of the heater. The temperature was recorded when it was melted.

2.4.2 Conductivity Measurement

The complexes were dissolved in warm distilled water. The measurement was conducted on molarity 10^{-3} M of every complex solution by using CyberScan 500 instrument.

2.4.3 Analysis of Fourier Transform Infrared Spectroscopy (FT-IR)

The infrared spectra of the complexes were obtained by using Perkin-Elmer System 2000 in the region of 4000-400 cm^{-1} at room temperature.

Every dry analyte (complexes, ligands and salts of the ligands) was ground with potassium bromide (KBr) in the ratio of 1:10 to obtain fine and evenly ground powder. The fine powder was then pressed into pellets at 7 tonne of pressure by vacuum pump. Spectra were recorded from 400 to 4000 cm^{-1}.

2.4.4 CHN Microanalysis

Microanalysis for the elements of carbon, hydrogen and nitrogen were carried out by using CHNS/O Analyzer (Perkin-Elmer Series II 2400).

2.4.5 Analysis of Atomic Absorption Spectrometry (AAS)

This analysis was conducted to determine the nickel and iron content in each complex. It was carried out on a Perkin-Elmer Atomic Absorption Spectroscopy model 3100. The preparation of standard solution and sample solution was described as below:

1) Preparation of standard solution

The stock solution of nickel and iron were prepared from 1000 ppm of standard solution. While the stock solution of potassium was prepared from 10 ppm

of standard solution. Stock solution was pipetted to a 100 mL volumetric flask by using micropipette. Then the solution was diluted to 100 mL by using 2 % of nitric acid.

A series of nickel, iron and potassium standard solution were prepared in the range of 1-5 ppm. The calibration curve for their total content was constructed based on the absorbances that were exhibited by a series of standard solutions as in Appendix. The calibration curve for nickel, iron and potassium are depicted in Figure 2.1, Figure 2.2 and Figure 2.3 respectively.

2) Preparation of sample solution

The stock solution of nickel complexes were prepared by dissolved 0.02 g of complex in 15 mL of concentrated nitric acid and diluted to 100 mL in 100 mL volumetric flask by using 2% of acid nitric. After that, 10 mL was pipetted from the volumetric flask into a 100 mL volumetric flask and diluted to 100 mL with 2% of nitric acid. The preparation for stock solution of iron complexes was the same as nickel.

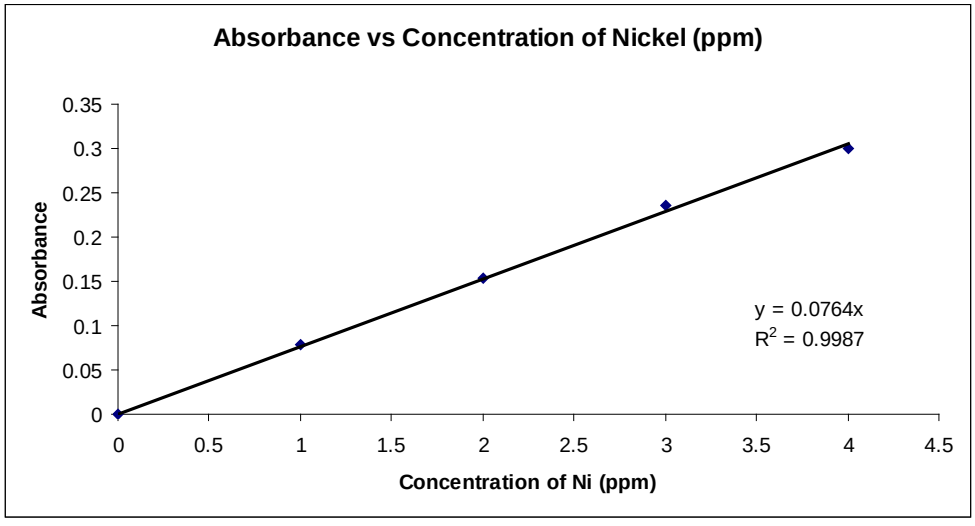


Figure 2.1 The AAS calibration curve for nickel

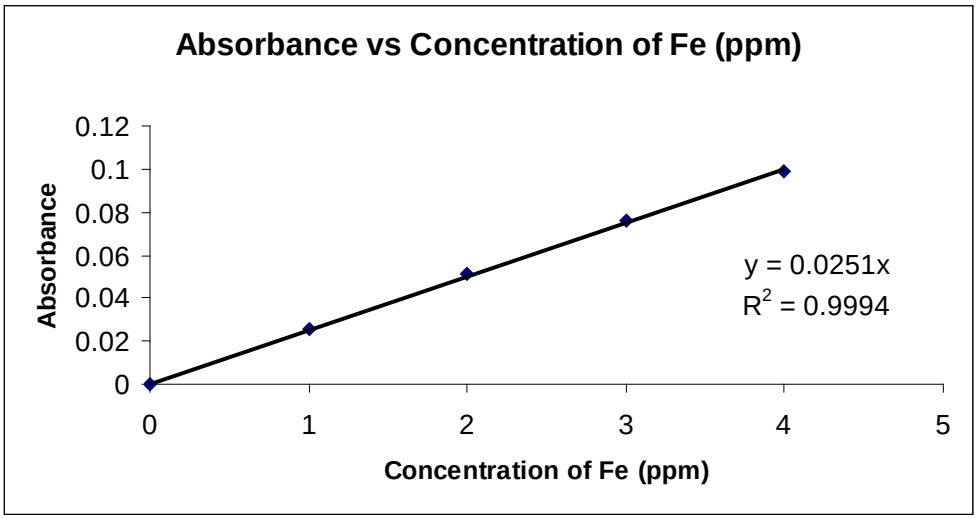


Figure 2.2 The AAS calibration curve for iron

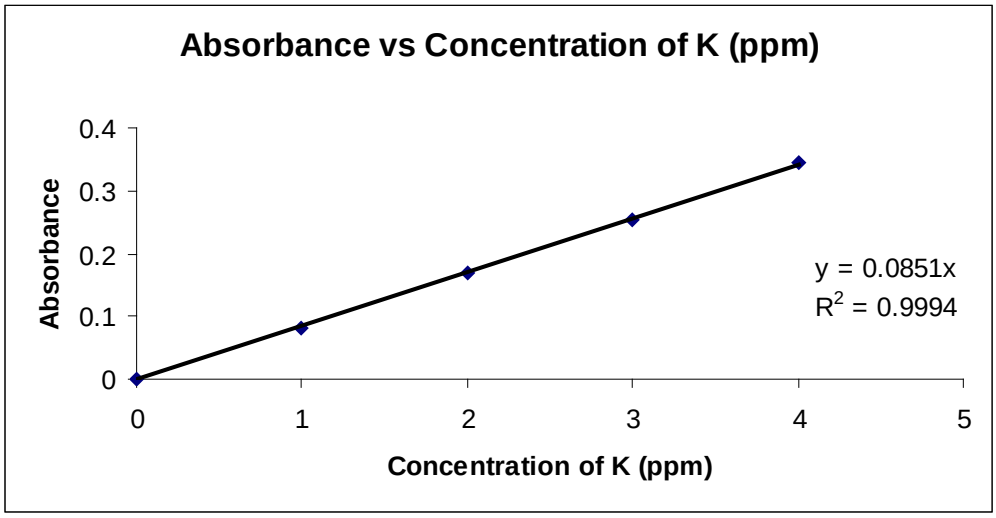


Figure 2.3 The AAS calibration curve for potassium

2.4.6 Analysis of Ultraviolet-Visible (UV-Vis) Spectrometry

UV-Visible spectra were measured using Model Jasco V-530. Approximately 0.03 g of sample was dissolved in distilled water. Dilution process was carried out to obtain clear spectra. The measurement wavelength was set in the range of 200-1000 nm.