CHAPTER ONE

1.1 INTRODUCTION

Ibuprofen was the first member of Propionic acid derivatives introduced in 19 69. It is a popular domestic and over the counter analgesic and antipyretic for adults and children. Ibuprofen has been rated as the safest conventional NSAI D by spontaneous adverse drug reaction reporting systems in the UK. This pro ject summarizes the main pharmacological effects, therapeutical applications and adverse drug reactions, drug-drug interactions and food drug interactions of ibuprofen that have been reported especially during the last 10 years. Ibupr ofen is (2RS)-1[4-(2-methyl propyl) phenyl] propionic indomethacin, are still the most common side effects.1 acid (BP. 2004). Ibuprofen was the first member of propionic acid derivatives to be introduced in 1969 as a better alternative to Aspirin. Gastric discomfort, nausea and vomiting, though less than aspirin o r Ibuprofen is the most commonly used and most frequently prescribed NSAID [2][3] It is a non-selective inhibitor of cyclooxygenase-1 (COX-1) and Cyclooxyg enase-2 (COX-2).[4]. Although its anti inflammatory properties may be weaker than those of some other NSAIDs, it has a prominent analgesic and antipyreti

c role. Its effects are due to the inhibitory actions on cyclo-oxygenases, which are involved in the synthesis of prostaglandins. Prostaglandins have an important role in the production of pain, inflammation and fever.[5]

1.2 CLINICAL PHARMACOLOGY OF IBUPROFEN

Ibuprofen is supplied as tablets with a potency of 200 to 800 mg.[6] The usual dose is 400 to 800 mg three times a day.[7] It is almost insoluble in water havi ng pKa of 5.3.[8] It is well absorbed orally; peak serum concentrations are attai ned in 1 to 2 hours after oral administration. It is rapidly bio-transformed with a serum half life

of 1.8 to 2 hours. The drug is completely eliminated in 24 hours after the last d ose and eliminated through metabolism.[9][10]. The drug is more than 99% pr otein bound, extensively metabolized in the liver and little is excreted unchang ed.[11]

Although highly bound to plasma proteins (90-99%),displacement interactions are not clinically significant, hence the dose of oral anti-cogulants and oral hyp oglycemic needs not be altered.1 More than 90% of an ingested dose is excret ed in the urine as metabolites or their conjugates, the major metabolites are

hydroxylated and carboxylated compounds.[6][12]. Old age has no significant effects on the elimination of ibuprofen.[13]. Renal impairment also has no effe ct on the kinetics of the drugs, rapid elimination still occur as a consequence o f metabolism.[14]. The administration of ibuprofen tablets either under fasting conditions or immediately before meals yield quiet similar serum concentratio ns-time profile. When it is administered immediately after a meal, there is a re duction in t0he rate of absorption but no appreciable decrease in the extent of absorption.[15]

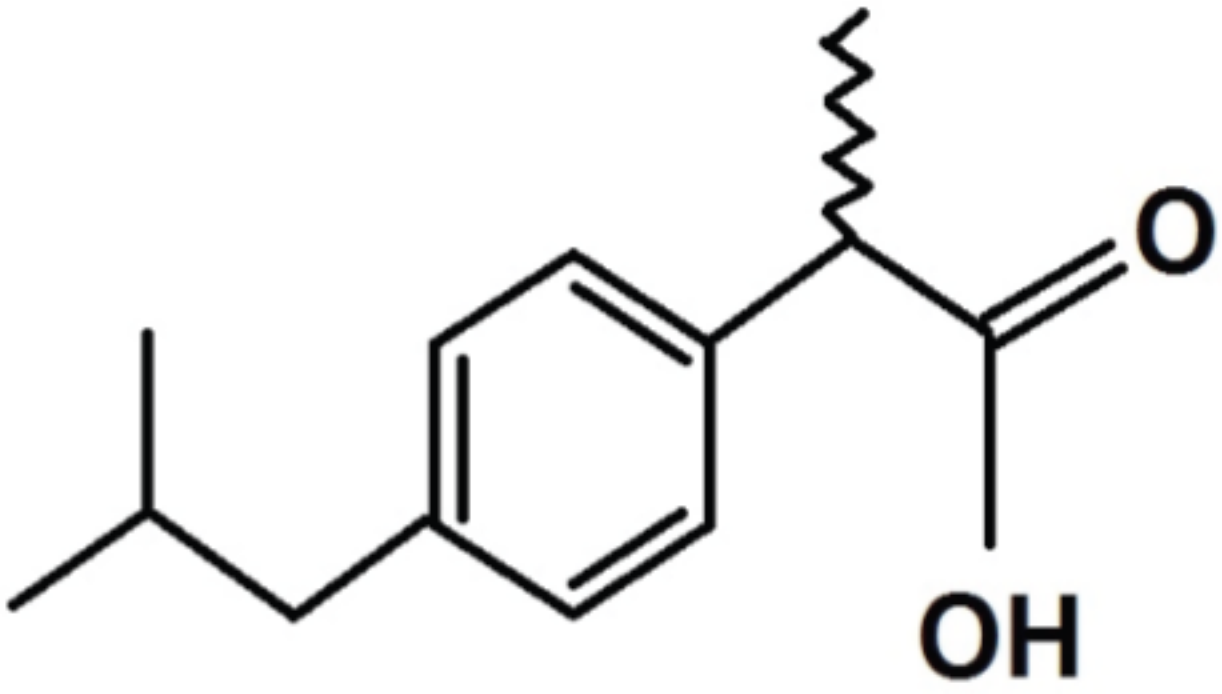

Figure 1. Structural formula of Ibuprofen

1.3 Therapeutic Application of Ibuprofen

A low dose ibuprofen is as effective as aspirin and paracetamol for the indicati ons normally treated with over the counter medications.[16]. It is widely used as an analgesic, an anti inflammatory and an antipyretic agent.[17-19]. Recem ic ibuprofen and S(+) enantiomer are mainly used in the treatment of mild to moderate pain related to dysmenorrhea, headache, migraine, postoperative d ental pain, management of spondylitis, osteoarthritis, rheumatoid arthritis and soft tissue disorder.[20]. A number of other actions of NSAIDs can also be attri buted to the inhibition of prostaglandins (PGs) or thromboxane synthesis, inclu ding alteration in platelet function. (PGI2 and Thromboxane), prolongation of g estation and labor

(PGE2, PGF2A), gastrointestinal mucosal damage (PGI2 and PGE2), fluid and e lectrolyte imbalance (renal PGs), premature closure of ductus arteriosus (PGE 2) and bronchial asthma (PGs).[21]. The main therapeutic applications of ibupr ofen are as follows:

1.3.1 Patent Ductus arterosus (PDA)

This is a frequent complication in premature infants. So far, intravenous indom ethacin is the standard mode of medical therapy.[22]. However, because of ad

verse effects of indomethacin, other PG inhibitors such as ibuprofen have bee n studied for the closure of ductus arteriosus, and results indicated that ibupro fen is

as effective as indomethacin.[23]

1.3.2 Rheumatoid and osteo-arthritis (RA and OA)

Ibuprofen is widely used in the management of numerous inflammatory, musc uloskeletal and rheumatic disorders, because they are highly effective having minimal toxicities.[24],[25]. Ibuprofen 2400 mg per day resulted in rapid impro vement and complete resolution of gouty arthritis within 72 hours.26 In doses of approximately 2400 mg daily, it is equivalent to 4g of aspirin in

terms od anti inflammatory effects.[27] Higher doses, 1200 to 1600

mg per day have been compared with a number of NSAIDs and it

has been found to be as effective and well tolerated.[28]. Osteoarthritis

is very common and treatment involves NSAIDs, particularly ibuprofen.[29],[3 0] For control of joint symptoms, diclofenac, ibuprofen, tolmetin and naproxen are equally effective.[31]. Roughly 1% of rheumatoid arthritis (RA) patients rec eiving NSAIDs are prone to develop major GI bleeds.[32]. With ibuprofen, gastr ic toxicity has been observed in 10 - 32% of patients.[33].

Table 1: Doses of Ibuprofen in adult & Children (34)

Patient	Ibuprofen	Doses
Adult	Analgesia	200-400mg. Every 4-6hrs
	Anti- inflammatory	300 mg, Every 6-8 hrs or 400-800 mg 3-4 times daily.
Children	Antipyretic	5-10 mg/kg. Every 6 hrs (max. 40 mg/kg per day).
	Anti-inflamatory	20-40 mg/kg/day in 3-4 divided dose.

1.3.3 Cystic fibrosis (CF)

High dose ibuprofen therapy has also been shown to be effective in decreasing inflammation, probably by decreasing polymorphonuclear cell influx into the lungs.[34]. The risk of developing GI side effects from high dose ibuprofen ther

apy is low in patients with CF.[35],[36]

1.3.4 Orthostatic hypotension

Ibuprofen is useful in the treatment of severe orthostatic hypotension as with other NSAIDs.[37]. Toxic effects are unlikely at doses below 100 mg/kg but ca n be life-threatening or severe above 400 mg/kg.[38].However, large doses do not indicate that the clinical course is likely to be lethal.[39]

1.3.5 Dental pain

Ibuprofen is one of the most effective and widely used NSAID in treatment of dental pain.[40]. Dental practitioners have relied on ibuprofen and other NSAID s to manage acute and chronic orofacial pain.[41] A dose of 400 mg of ibuprof en provides effective analgesic for the control of postoperative pain after third molar surgery.[42]. A liquid gel preparation of ibuprofen 400mg provides faster relief and superior overall efficacy in post surgical dental pain.[27]

1.3.6 Dysmenorrhea, fever and headache

Non-prescription ibuprofen is useful for managing minor aches and pains, redu

cing fever and relieving symptoms of dysmenorrhea.[43-45].Dysmenorrhea is the most common menstrual complain.[46].Ibuprofen was superior to placebo for pain relief and menstrual fluid PGF2 alpha suppression.[47]. Cycloxygenase inhibitors reduce

the amount of menstrual prostanoids release, with concomitant reduction in uterine hyper contractility.[48]. Over-the-counter (OTC) ibuprofen preparations are mainly used for acute indications, such as fever or headaches, especially tension type headache.[49-51.It has been reported that the combined use of paracetamol

and ibuprofen reduce fever very rapidly.[52]. Fever almost invariably accompanies uncomplicated falciparum malaria. In a randomized double-'blind' study, a single dose of ibuprofen was compared with paracetamol for the treatment of fever >38·5 °C due to uncomplicated falciparum malaria. Ibuprofen was significantly more

effective than paracetamol in lowering temperatures throughout the first 4-5 hrs after dosing and thus should be considered as an antipyretic agent in the management of uncomplicated falciparum infections, providing there is no contraindication to its use.[53]. Evers *et al.* in 2006, conducted a double blind study

to investigate the efficacy of zolmitriptan and ibuprofen in the treatment of mi graine in children and adolescents. Pain relief rates after two hours were 28% for placebo, 62% for zolmitriptan and 69% for ibuprofen.[54]

1.3.7 Prophylaxis of Alzheimers disease

The administration of NSAIDs, particularly ibuprofen markedly reduced neurod egeneration.[55],[56]. In some studies, ibuprofen showed superior results com pared to placebo in the prophylaxis of Alzheimer's disease, when given in low doses over a long time. Further studies are needed to confirm the results befor e ibuprofen can be recommended for this indication.[57]

1.3.8 Parkinson's disease (PD)

Inflammation and oxidative stress have been implicated as pathogenic mecha nisms in PD.[58] Epidemiologic evidence showed that regular use of NSAIDs, p articularly non aspirin COX inhibitors such as ibuprofen lower the risk of PD.[5 9],[60]. It induced apoptosis significantly in early and late stages, suggesting t hat these anti-inflammatory agents might inhibit microbial proliferation.61

1.3.9 Breast cancer

Harris et al. in 1999 conducted a study for utilization of NSAIDs in breast canc er. Breast cancer rate was decreased by approximately 50% with regular ibupr

ofen intake and 40% with regular aspirin intake. Results suggested that specifi c NSAIDs may be effective chemo preventive agents against breast cancer.[6 2]

1.4 Adverse Reactions

NSAIDs are widely used, frequently taken inappropriately and potentially dang erously.[63]. Nevertheless, ibuprofen exhibits few adverse effects.[64] The ma jor adverse reactions include the affects on the gastrointestinal tract (GIT), the kidney and the coagulation system.[65]. Based on clinical trial data, serious GI T reactions prompting withdrawal of treatment because of hematemesis, pep tic

ulcer,[66] and severe gastric pain or vomiting showed an incidence of 1.5% wit h ibuprofen compared to 1% with placebo and 12.5% with aspirin.[67]. Ibuprofe n was a potential cause of GI bleeding,68],[69] increasing the risk of gastric ulc ers and damage, renal failure, epistaxis,[70-73] apoptosis,[74] heart failure, hy perkalaemia,[75] confusion and bronchospasm.[76]. It has been estimated tha

t 1 in 5 chronic users(lasting over a long period of time) of NSAIDs will develop gastric damage which can be silent.[77]. Other adverse effects of ibuprofen ha ve been reported less frequently. They include thrombocytopenia, rashes, hea dache,

dizziness, blurred vision and in few cases toxic amblyopia, fluid retention and ibuprofen.[78] Effects on kidney (as with all NSAIDs) include acute renal failur e, interstitial nephritis, and nephritic syndrome, but these very rarely occur.[27]

1.5 LIGAND AND COMPLEX FORMATION

A ligand is an ion or molecule that donates two or more lone pairs of electron s to a metal ion to form coordination or dative bond.

According to Lewis Theory, ligand is a lewis base due to the electrons they do nate to empty orbital. They are either anion or polar molecues. Ligands can be classified based on electron pair they donate according to ligand theory. Thes e includes

i. MONODENTATE LIGAND: they donate one lone pair of electron to

neutral metal e.g $pt(Cl)_4$.

ii. Bidentate LIGAND: they donate two lone pair of electron to neutra l matal e.g CH_3NH_3

iii. POLYDENTATE LIGAND: they donate more than two lone pairs of electron i.e the binding site is beyond one e.g tetraethylenediamm ine.

1.6 WHY DRUG-METAL COMPLEX

New development in chemotherapy includes the synthesis and characterizatio n of new drug involving metals. Selective novel metal chelators for the treatm ent of diseases that involves metal or mineral inbalance. Promising research i nvolves some complexes with different metal ions including those of cobalt, c opper, titanium, iron, platinum , gold, molybdenum, tin and manganese. Drug metal complexes are used in treatment of metal deficiency diseases.

1.7 CHEMISTRY OF TRANSISTION METALS USED

1.7.1 NICKEL

Nickel compounds are chemical compounds containing the element nickel wh ich is a member of the group 10 of the periodic table. Most compounds in the group have an oxidation state of +2. Nickel is classified as a transition metal w ith nickel(II) having much chemical behavior in common with iron(II) and cobal t(II). Many salts of nickel (II) are isomorphous with salts of magnesium due to the ionic radii of the cations being almost the same. Nickel forms many coordi nation complexes. Nickel tetracarbonyl was the first pure metal carbonyl prod uced, and is unusual in its volatility. Metalloproteins containing nickel are foun d in biological systems. Nickel forms simple binary compounds with non metal s including halogens, chalcogenides, and pnictides. Nickel ions can act as a ca tion in salts with many acids, including common oxoacids. Salts of the hexaaq ua ion ($Ni \cdot 6\ H_2O^{2+}$) are especially well known. Many double salts containing n ickel with another cation are known. There are organic acid salts. Nickel can b e part of a negatively charged ion (anion) making what is called a nickellate. N umerous quaternary compounds (with four elements) of nickel have been stu died for superconductivity properties, as nickel is adjacent to copper and iron i n the periodic table can form compounds with the same structure as the high-

temperature superconductors that are known.

1.7.1.1 Colour

Most of the common salts of nickel are green due to the presence of hexaaquanickel(II) ion, $Ni(H_2O)_6^{2+}$.

1.7.1.2 Geometry

Nickel atoms can connect to surrounding atoms or ligands in a variety of ways. Six coordinated nickel is the most common and is octahedral, but this can be distorted if ligands are not equivalent. For four coordinate nickel arrangements can be square planar, or tetrahedral. Five coordinated nickel is rare.

1.7.1.3 Complexes

Simple complexes of nickel include hexaquonickel(II),yellow tetracyanonickelate $[Ni(CN)_4]^{2-}$, red pentacyanonickelate $[Ni(CN)_5]^{3-}$ only found in solution, $[Ni(SCN)_4]^{2-}$ and $[Ni(SCN)_6]^{4-}$. Halo- complexes include $[NiCl_4]^{2-}$, $[NiF_4]^{2-}$, $[NiF_6]^{4-}$, $[NiCl_2(H_2O)_4]$ $[Ni(NH_3)_4(H_2O)_2]^{2+}$, $[Ni(NH_3)_6]^{2+}$, $[Ni(en)_3)]^{2+}$.[78] Some complexes have fivefold coordination. (tris(N,N-dimethyl-2-aminoethyl)amine); $P(o\text{-}C_6H_4SMe)_3$; $P(CH_2CH_2CH_2AsMe_2)_3$[78] Other ligands for octahedral coordination include PPh_3, PPh_2Me and thiourea.

Nickel tetrahedral complexes are often bright blue and 20 times or more inten sely coloured than the octahedral complexes.[78].The ligands can include sele ctions of neutral amines, arsines, arsine oxides, phosphines or phosphine oxid es and halogens. Several nickel atoms can cluster together in a compound wit h other elements to produce nickel cluster complexes. One example where nic kel atoms form a square pyramid is a nickel hydride cluster complexed by triph enyl phosphine ligands and bonding a hydrogen atom on each edge. Another example has a square planar Ni_4H_4 shape in its core.[79] .Nickel bis(dimethylgl yoximate), an insoluble red solid is important for gravimetric analysis.

1.7.2 COPPER

Copper along with silver and gold belong to group 1B in the periodic table. The se three metals are often referred to as coinage metals. Copper has the electr onic configuration of $1S^2 2S^2 2P^6 3S^2 3P^6 3d^{10} 4S^1$. Copper is a tough, malleable, d uctile metal which resist chemical attack. It has an atomic number of 29, atom ic mass of 63.54 and belongs to period 4 and sub-group1B on the periodic tabl e; it has two natural isotopes with atomic masses between 58 and 68. Copper has a melting point of 1083^0C, boiling point of 259^0C and a density of 8.93g/c

m^3.

1.7.2.1 Deficiency

Because of its role in facilitating iron uptake, copper deficiency can produce an emia-like symptoms, neutropenia, bone abnormalities, hypopigmentation, imp aired growth, increased incidence of infections, osteoporosis, hyperthyroidism, and abnormalities in glucose and cholesterol metabolism. Conversely, Wilson's disease causes an accumulation of copper in body tissues.

Severe deficiency can be found by testing for low plasma or serum copper lev els, low ceruloplasmin, and low red blood cell superoxide dismutase levels; the se are not sensitive to marginal copper status. The "cytochrome c oxidase acti vity of leucocytes and platelets" has been stated as another factor in deficienc y, but the results have not been confirmed by replication.[80]

1.7.2.2 TOXICITY

Gram quantities of various copper salts have been taken in suicide attempts a nd produced acute copper toxicity in humans, possibly due to redox cycling an d the generation of reactive oxygen species that damage DNA.[80][81]. Corres ponding amounts of copper salts (30 mg/kg) are toxic in animals.[82] A minim

um dietary value for healthy growth in rabbits has been reported to be at least 3 ppm in the diet.[83] However, higher concentrations of copper (100 ppm, 200 ppm, or 500 ppm) in the diet of rabbits may favorably influence feed conversion efficiency, growth rates, and carcass dressing percentages.[84]

Chronic copper toxicity does not normally occur in humans because of transport systems that regulate absorption and excretion. Autosomal recessive mutations in copper transport proteins can disable these systems, leading to Wilson's disease with copper accumulation and cirrhosis of the liver in persons who have inherited two defective genes.[85]

Elevated copper levels have also been linked to worsening symptoms of Alzheimer's disease.[86][87]

1.7.3 IRON

Iron shows the characteristic chemical properties of the transition metals, namely the ability to form variable oxidation states differing by steps of one and a very large coordination and organometallic chemistry: indeed, it was the discovery of an iron compound, ferrocene, that revolutionalized the latter field in th

e 1950s.[88]. Iron is sometimes considered as a prototype for the entire block of transition metals, due to its abundance and the immense role it has played i n the technological progress of humanity.[89]. Its 26 electrons are arranged in the configuration [Ar]$3d^6 4s^2$, of which the 3d and 4s electrons are relatively clo se in energy, and thus a number of electrons can be ionized.[90]

Iron forms compounds mainly in the oxidation states +2 (iron(II), "ferrous") and +3 (iron(III), "ferric"). Iron also occurs in higher oxidation states, e.g., the purple potassium ferrate (K_2FeO_4), which contains iron in its +6 oxidation state. The anion $[FeO_4]^-$ with iron in its +7 oxidation state, along with an iron(V)-peroxo is omer, has been detected by infrared spectroscopy at 4 K after cocondensation of laser-ablated Fe atoms with a mixture of O_2/Ar.[91] Iron(IV) is a common int ermediate in many biochemical oxidation reactions. Numerous organoiron co mpounds contain formal oxidation states of +1, 0, −1, or even −2. The oxidatio n states and other bonding properties are often assessed using the technique of Mössbauer spectroscopy.[90] Many mixed valence compounds contain bot h iron(II) and iron(III) centers, such as magnetite and Prussian blue ($Fe_4(Fe[C N]_6)_3$).[89] The latter is used as the traditional "blue" in blueprints.[90]Iron is th e first of the transition metals that cannot reach its group oxidation state of +

8, although its heavier congeners ruthenium and osmium can, with ruthenium having more difficulty than osmium.[91]. Ruthenium exhibits an aqueous catio nic chemistry in its low oxidation states similar to that of iron, but osmium doe s not, favoring high oxidation states in which it forms anionic complexes.[91]. I n the second half of the 3d transition series, vertical similarities down the grou ps compete with the horizontal similarities of iron with its neighbors in the peri odic table, which are also ferromagnetic at room temperature and share simila r chemistry. As such, iron, cobalt, and nickel are sometimes grouped together as the iron triad.[89]

Unlike many other metals, iron does not form amalgams with mercury. As a re sult, mercury is traded in standardized 76 pound flasks (34 kg) made of iron.[9 0]

Iron is by far the most reactive element in its group; it is pyrophoric when finel y divided and dissolves easily in dilute acids, giving Fe^{2+}. However, it does not react with concentrated nitric acid and other oxidizing acids due to the formati on of an impervious oxide layer, which can nevertheless react with .[91]. High-purity iron, called electrolytic iron, is considered to be resistant to rust, due to it s oxide layer.

1.7.3.1 DEFICIENCY

Iron deficiency is the most common nutritional deficiency in the world.[92][93] [94] When loss of iron is not adequately compensated by adequate dietary iro n intake, a state of latent iron deficiency occurs, which over time leads to iron-deficiency anemia if left untreated, which is characterised by an insufficient nu mber of red blood cells and an insufficient amount of hemoglobin.[95] Childre n, pre-menopausal women (women of child-bearing age), and people with poo r diet are most susceptible to the disease. Most cases of iron-deficiency anem ia are mild, but if not treated can cause problems like fast or irregular heartbe at, complications during pregnancy, and delayed growth in infants and childre n.[96]

The brain is resistant to acute iron deficiency due to the slow transport of iron through the blood brain barrier.[97] Acute fluctuations in iron status (marked b y serum ferritin levels) do not reflect brain iron status, but prolonged nutritional iron deficiency is suspected to reduce brain iron concentrations over time.[98] [99] In the brain, iron plays a role in oxygen transport, myelin synthesis, mitoc hondrial respiration, and as a cofactor for neurotransmitter synthesis and met abolism.[100] Animal models of nutritional iron deficiency report biomolecular changes resembling those seen in Parkinson's and Huntington's disease.[100] [102] However, age-related accumulation of iron in the brain has also been link ed to the development of Parkinson's.[103]

1.8 AIM OF PROJECT

The aims of this research work are

(i) To synthesis novel complexes of ibuprofen

(ii) To characterize the resulting complexes using standard analytical tec hniques such as solubility, melting point, infrared and ultraviolet .

CHAPTER TWO

2.1 MATERIALS AND METHODS

2.1.1 APPARATUS

The following apparatus were used in the formation of the complexes and furt her analytical studies were carried out.

APPARATUS	MANUFACTURER
Beakers	Pyrex scientific Ltd. England

Conical Flasks	Simax company Ltd. England
Capilary tube	Silber brand Ltd, England
Dessicator	Moncrief Scientific, England
Electrothermal melting point	Gallenkamp Ltd ,England
Round bottom flasks	Pyrex scientific Ltd, England
Hot plate with magnetic stirrer	Gallenkamp Ltd, England
Measuring cylinder	Technico scientific Ltd, England
Plastic condenser	
Reflux condenser	Moramber (Mbc) Ltd
Test tube	Pyrex scientific Ltd, England
Thermometer	Uniscope scientific Ltd, England
Infrared	Duck V Scientific 500 infrared
Ultraviolet	Jenwoy 6405 UV spectrophotometer.

2.1.2 REAGENTS

The following reagents were collected from chemistry department, Kwara Sta te Polytechnic.

Reagent	Manufacturer
Nickel Sulphate Hexahydrate Molecular Formular- $NiSO_4.6H_20$ Molecular mass =262.71g/mol	J.T Barker Chemical C.O Phillburg
Copper Sulphate Molecular formula = $CuSO_4$. Molecular Mass= 159.60g/mol	Eagle Scientific England
Iron Sulphate Heptahydrate Molecular Formular= $FeSO_4.7H_20$ Molecular Mass =278.02g/mol.	East Aglia, Chemicals Hadheigh
Ethanol	East Aglia, Chemicals Hadheigh

Methanol	East Aglia, Chemicals Hadheigh
Distilled water	

2.1.3 MATERIALS

Ibuprofen

Molecular formular= $C_{13}H_{18}O_2$

Molecular Mass=206.29g/mol

Source	BIORAJ Pharmaceuticals, Ilorin.

2.2 EXPERIMENTAL PROCEDURES

All apparatus were cleaned thoroughly before use. The retort stand was set pr operly and a round bottom flask clamped to it. This was further positioned ont o the hotplate with a magnetic stirrer. A reflux condenser was fixed into the ro und bottom flask containing the solution and clamped onto the retort stand. W ater pipes were connected, one into the inlet and the other into the outlet with both connected to full bucket of water and an empty bucket respectively.

2.2.1 IBUPROFEN Ni (ii) COMPLEX FORMATION

3.52g of ibuprofen was weighed using a digital pocket scale and was then diss olved in 10cm3 of distilled water using a clean dried round bottom flask.

2.38g of $NiSO_4.6H_2O$ was also weighed using a digital pocket scale and was th en dissolved in 10cm3 of distilled water in a beaker.

The Nickel solution was added to the ibuprofen solution and shaken vigorousl y for it to mix well. The magnetic stirrer was placed in the mixture and it was r efluxed for 1 hour after which the solution was allowed to cool and a light blue color was observed. The resulting solution was filtered using a filter paper. Th e precipitate was then washed off with methanol.

The complex obtained was weighed and put in a container which was labeled IBNi(ii) complex.

The weight of the complex obtained was 1.7g of methanol.

2.2.2 IBUPROFEN Cu (ii) COMPLEX FORMATION

3.52g of ibuprofen was weighed using a digital pocket scale and was then diss olved in 10cm3 of distilled water using a clean dried round bottom flask.