University of Babylon / college of pharmacy

Synthesis, characterization and biological activity of azo ligand and complexation with selected metal ions.

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ABBREVIATION

- **L1 : Ligand1:-** 4-((2,4dihydroxyphenyl) diazenyl)- 1,5-dimethyl -2-phenyl -1-*H*- pyrazol- 3(2*H*)-one.

-(DDPPL1):- 4-((2,4dihydroxyphenyl) diazenyl)- 1,5-dimethyl -2-phenyl -1-*H*- pyrazol- 3(2*H*)-one. -

-L2: ligand2:- 4-((2-hydroxynaphthalen-1-yl) diazenyl) -1,5-dimethyl -2-phenyl -1-*H*- pyrazol- 3(2*H*)-one.

-(HDPPL2):- 4-((2-hydroxynaphthalen-1-yl) diazenyl) -1,5-dimethyl -2-phenyl -1-*H*- pyrazol- 3(2*H*)-one .

ABSTRACT

The transition metal complexes of Cu(II) and Ni(II) were synthesized from the Azo ligand derived from 4-aminoantipyrine , the ligand: 4-((2,4dihydroxyphenyl) diazenyl)- 1,5-dimethyl -2-phenyl -1-H-pyrazol- 3(2H)-one, ***(DDPPL1)*** *and 4-((2-hydroxynaphthalen-1-yl) diazenyl) -1,5-dimethyl -2-phenyl -1-H- pyrazol- 3(2H)-one,* ***(HDPPL2)****.The ligand and its metal complexes were characterized by their melting point, infrared and UV-visible spectroscopy. The biological activity also was investigated against nine bacterial sample include: (Gram positive bacteria) and (Gram negative bacteria) :* ***Streptococcus spp and staphylococci aureus, Escherichia coli , Klebsiella pneumonia , proteus spp, Salmonella typhi, Acinetobacter spp , Vibrios cholera , and Yersinia enterocotitica****. The metal complexes were showed higher antibacterial activity compared to the free ligands.*

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Chapter 1

Introduction

Chapter 1

1- Introduction:-

Pyrazolone is a five membered lactam ring compound containing two nitrogen toms and ketone in the same molecule. Fig (1). Lactam ring is an active nucleus in pharmacological activity . Pyrazolone is an active moiety as a pharmaceutical ingredient, especially in the class of non-steroidal anti-inflammatory agents (NSAID) used in the treatment of joint disorder , arthritis and other musculoskeletal. [1] The term pyrazolone sometimes refers to non-steroidal anti-inflammatory agents . Pyrazolone class non-steroidal anit-inflammatory drug (NSAID) includes oxyphenbutazone, phenylbutazone , ramifenazone , and dipyrone . Antipyrine (also called phenazone) is a pyrazolone class analgesic agent in solutions in combination with other analgesic such as benzocaine , and phenylephrine. [2] Pyrazolone derivatives are also used in preparing dyes and pigments [3] . 2, 3-Dimethyl -1-phenyl -5-pyrazolone (antipyrine) has been discovered as antipyretics of the quinoline type [4]. This discovery initiated the beginning of the German drug industry that dominated the field for approximately 40 years. Phenylbutazone , was originally developed as a solubilizer for the insoluble aminopyrine .Fig(2) . It is now used for the relief of many forms of arthritis in which capacity it has more than an analgesic action in that it also reduces swelling and spasm by an anti – inflammatory action.

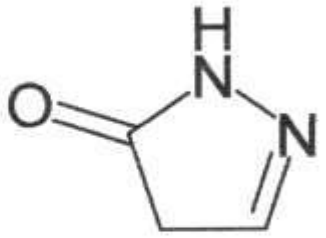
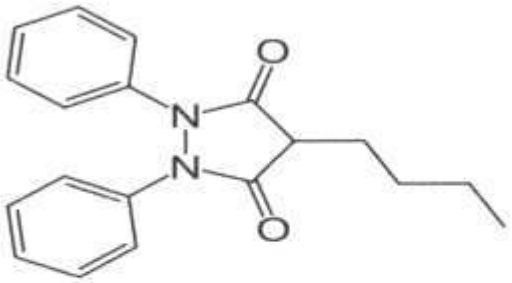


Fig.(1):-The structure of pyrazolone.



Fig(2):- The structure of phenylbutazone.

Dipyrone, the most widely used pyrazolone, has been the most studied. The pyrazolidine derivatives, phenylbutazoneand oxyphenbutazone, which are not generally used for analgesia since they differ from the pyrazolones in terms of efficacy and tolerance.. Dipyrone is an inhibitor of cyclo-oxygenase but, unlike aspirin, its effect is rapidly reversible. The inhibition of prostaglandin biosynthesis contributes to the analgesic activity of the pyrazolone derivatives. Peak plasma concentrations of the pyrazolone derivatives generally occur 1 to 1.5 hours after oral administration. Half-lives vary from 1 to 2 hours with propyphenazone, to about 7 hours with dipyrone (2 hours for the active metabolite of dipyrone, 4-methylaminoantipyrine, MAA). Half-life of antipyrine varies considerably between individuals (5 to 35 hours). Unlike the NSAIDs generally, the pyrazolone derivatives antipyrine, aminopyrine and propyphenazone are minimally bound to plasma proteins. The pyrazolones undergo extensive biotransformation, aminopyrine and dipyrone being converted to active metabolites. Dipyrone is the only drug for which results of recent double-blind trials are available. Oral dipyrone has been shown to be more effective than an equal

dose of aspirin or paracetamol in alleviating postoperative pain, and intravenous dipyrone 2.5g was similar in efficacy to pethidine 50 mg. In patients with acute ureteral or biliary colic, dipyrone 2.5g intravenously was similar in efficacy to indomethacin 50 mg or pethidine 50 mg. The most frequently reported side effects of the pyrazolone derivatives are skin rashes. Gastrointestinal side effects are rare. Blood dyscrasias, mostly associated with aminopyrine, have received wide attention in the medical literature, but their true incidence with dipyrone is considerably lower than the often quoted incidence for amidopyrine reported more than 30 years ago [5].

1-1- Antipyrine - ($C_{11}H_{12}N_2O$):-

Antipyrine is a pyrazole derivative of considerable value as analgesics and antipyretics . Its analgesic form is the oldest of the synthetics drugs that relieve pain and reduce fever. It also has a mild anesthetic effect[5]. Antipyrine is longer acting than aspirin (a single dose can give relief from pain for 24 hours) and in most people it has very few side effects. But a small minority of persons are highly allergic to antipyrine and in them the drug can cause severe – skin eruptions, giddiness , tremor , vascular collapse, and even coma and death. In combination with benzocanine, antipyrine is still sometimes used as a topical agent to relieve earache. The use of antipyrine has been greatly reduced since its undesirable side effects have been recognized [6].

1-2- Physical and Chemical Properties:-

Antipyrine is odourless, colourless crystal or a white powder. It is very soluble in water, alcohol or chloroform, less soluble in

ether and its aqueous solution is neutral to litmus paper. However, it is basic in nature . This is due to presence of nitrogen at position 2 . It has a melting point of 110-113^{C} It decomposes when distilled at atmospheric pressures but has a boiling point of 141-141C under high vacuum and 319C at 174mm. Its molecular weight is 188.23. It forms a variety of salts and double salts. Alkylation's at 60C gives mainly the metholalide of the 5 alkoxypyrazole , but meltylation at temperature yields 4-methylantipyrine and 1-phenyl-3-4,4-trimethyl -5-pyrazole. Higher saturated alkyl hadide at 130 -200C give mainly resins, but one or two alkyl or benzyl groups can be introduced in the 4 – position[7]. Bromine yields 4-bromoantipyrine hydro bromide, a yellow salt formerly formulated as a dibromide [8].

It forms a colourless hydrate and readily looses hydrogen bromide, giving 4- bromoantipyrine. Iodination may yield 4-iodoantipyrine or periodides. Nitration gives either 4- nitro- or - 4-,dinitro antipyrine. Sulfonation gives the 4- sulfonic acid , and nitrosation gives 4-nitroso antipyrine . Catalytic hydrogenation of antipyrine over platinum black at room temperature yields the corresponding pyrazolidine (dihydroantipyrine) slowly. Nickel at 160-220C gives either this product or 1-phenyl -2,3, dimethylpyrazolidine, depending on the temperature and the duration of the experiment. Nickel can also bring about ring cleavage to butyranilide. Sodium and alcohol cause slow cleavage of the antipyrine ring to form methylamine and aniline. Phosphorus pentasulphide reduces antipyrine to 1-phenyl -3 methyl pyrozole [9]. The antipyrine ring has been opened by alcoholic potassium hydroxide at 130 to form N-methyl- N^{1}-

phenylhydrazine. Antipyrine is stable to 30% hydrochloric acid at 180, but above 200 it yields aniline, methylamine, and ammonia[10]. Also azo coupling of 3-methyl -1- phenyl 5- pyrazolone or of related compounds give 4-azo pyrazolone, derivatives which are of interest as wool, food, and photographic dyes [11].

1-3 Application:-

Antipyrine is a pyrazolone class analgesic agent in a liqiud solution (eg Auralgan) in combination with other analgesics such as benzocaine, and phenylepherine as mention above. It has been used as an antipyretic, but replaced due to the possibility of agranulocytosis side effect [12]. Generally antipyrine (2,3-dimethy l-1-pheny l- 5-pyrazolone) and its derivatives have a diversity of application including biological [13]. clinical [14]. and pharmacological areas [15]. .Antipyrines have been also reported to be used as analytical reagents in the determination of some metal ions [16-17].

Also antipyrine containing azo group have been investigated to have significant biological , antifungal, antibacterial activities and some industrial achiviements [18]. Considerable study have been devoted to ligands that derived from either 4-amino or 4 – formylantipyrine[19]. Among the pharmacological application they are used as antipyretics, analgesic , anti –rheumatic and anti-inflammatory drugs.

1-3-1- Antipyretic Drugs :

These are drugs that prevent or reduce fever by lowering the body temperature from a raised state. However, they will not affect the normal body temperature if one does not have fever. It

causes the hypothalamus to override an interleukin – induced increase in temperature. The body will then work to lower the temperature and the result is a reduction in fever. Most are also used for other purposes. Example, the most common antipyretics in the United States are aspirin and acetaminophen (paracetamol), which are used primarily as pain relievers [20].

1-3-2- Analgesic Drugs:

(colloquially known as a painkiller) this is any member of the diverse group of drugs used to relieve pain (achieve analgesia). It acts in various ways on the peripheral and central nervous system: The include paracetamol, the non-steroidal anti-inflammatory drugs (NSAIDS) such as the salicylates, narcotic drugs such as morphine synthetic drugs with narcotic properties such as tramadol and various others. Some other classes of drugs not normally considered analgesics are used to treat neuropathic pain syndromes. These include tricyclic antidepressants and anticonvulsants [21].

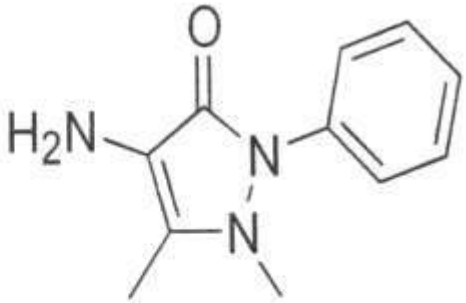
1-3-3- Anti- Inflammatory Drugs :-

This refers to the property of a substances or treatment that reduces inflammation. It makes up one half of analgesics, remedying pain by reducing inflammation as opposed to opioids which affect the brain [22].

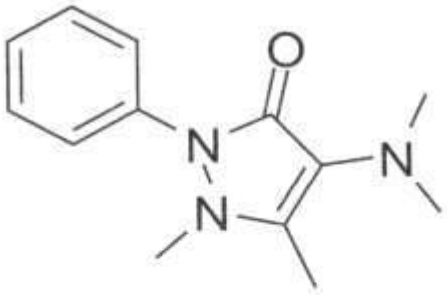
1-4-Aminoantipyrine :-

In this research, the area of interest is on 4-aminoantipyrine . Fig(3) . Its molecular formula is $C_{11}H_{13}N_3O$. It is a pale- yellow crystal with melting point ranging between 106 -110C , and the boiling point 309c . Its IUPAC name is 4-amino-2, 3, dimethyl -

1- phenyl -3- pyrazolin-5-one and other names include: solvapyrin A, aminoazophene, aminoantipyrene, metapyrazone. It has been employed as an antipyretic and analgesic, as in antipyrine, but is somewhat slower in action. Due to the risk of agranulocylosis (toxic or allergic reaction) of ampyrone, its use as a drug is discouraged [23]. Instead it is used as a reagent for biochemical reactions producing peroxide or phenols. Ampyrone stimulates liver microsomes and is also used to measure extra cellular water.



Fig(3) :-The structure of 4-aminoantipyrine.

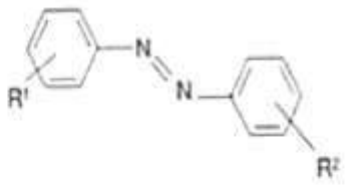


Fig(4) :- The structure of aminophenazone.

1-5- Azo Compounds:-

Azo compounds are compounds bearing the functional group R-N=N-R', in which R and R' can be either aryl or alkyl . IUPAC defines azo compounds as: "Derivatives of diazene (diimide), HN=NH, wherein both hydrogens are substituted by hydrocarbyl groups, e.g. PhN=NPh azobenzene or diphenyldiazene [24]. Azo dyes are important class of organic colorants consists of at least a conjugated chromophore azo (-N=N-) group and the largest and most versatile class of dyes. This chomophoric system is associated with two or more aromatic or heterocyclic rings. The

colour properties of organic dye depend on both the presence of chromophore group and crystallographic arrangement of the molecule in the solid state. There may be more than one azo group present in the dye molecules and thus classified into monoazo , diazo , triazo, and so on according to whether there are one , two , three, four or more azo groups present in the dye molecule. The general structure of azo dye in fig (5):-



Fig(5) :-The general structure of an azo dye.

Coordination chemistry of transition metal complexes with azo ligands is an important and fascinating branch of chemistry. The coordination compounds including azo ligands are of significant importance and play a pivotal role in industry, technology and life processes [25-28]. Due to their potential applications in various fields it has always fascinated and inspired chemists in the world.

This can be evidenced by the vast prolificity and scope of research papers on the subject in recent times and also by the diversity in which it has found applications, Azo group is characterized by a lone pair of orbitals containing two electrons on nitrogen atom, if linked to an aromatic ring carrying an additional donor sites is well suitable for chelation. Majority of these compounds are derived from the coupling of diazotized heterocyclic amines with

aromatic hydroxyl and amino compounds. This type of molecule has several advantages.

To quote a few, the azo group is photochromic, redox responsive, pH-sensitive, stabilizes low valent metal oxidation states due to the presence of a low-lying azo centered π^* molecular orbital , is used as a metal ion indicator in complexometric titration, dyes and pigments in textile industry [29-33].

They are highly coloured and possesses excellent thermal and optical properties and shows important applications such as optical data storage, photo switching and nonlinear optical materials [34-38]. They are involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis and biological activity against bacteria and fungi [39, 41]. The azo compound possesses suitable bonding charactertics due to presence of –N=N- group and can form varities of metal complexes with transition metal ion with unusual structural and magnetic properties [42-47]. Scientists have ventured into the detailed study of these complexes with the help of various physicochemical methods.

1-5-1-Applications of Azo Compound:-

1-5-1-1-. Azo Compound as dyes and pigment:-

Azo compounds have intense colours due to their extensions of the delocalized aromatic π electron system made possible by the presence of the azo group. The azo compounds are widely used as dyes and pigments since almost any colour can be obtained with this class of compounds [48, 49].

1-5-1-2-Applications in analytical chemistry:-

A- Spectrophotometer investigation:

Dyes containing a sulphonic acid group in the 8-position of naphthol ring proved to be less sensitive toward metal ions. Many chromotropic acid derivatives have photometric reagents in the acidic media, contracting colour changes [50, 51].

B-. Extraction photometric applications:

Owing to the low solubility of the metal complexes of 1-(2'-thiazolylazo)-2- naphthaol in aqueous solutions, they can be extracted rather easily into organic solvents. As Co(II) can readily be oxidized to Co(III) in the presence of azo dyes, the complex formed was studied in the presence and absence of ascorbic acid , but there is no evidence of oxidation of Co(II) with 1-(2′-thiazolylazo)-2-naphthol gives rise to a much more contracting colour change which occurs with Co(II). For the determination of nickel in mixtures of metal ions, Co(II) oxidized to its trivalent state and masked by the addition of ammonia, ion is masked with pyrophosphate , the solution is then extracted and Cu, Zn, Cd are removed by back extraction and Ni is best extracted with chloroform solution that is slightly alkaline. The kinetic study of extraction of Ni(II) from aqueous solution showed that formation of 1:1 complex [52, 53].

1-5-1-3-. Applications as indicators:-

The derivatives have been used as indicators for the direct titration with EDTA solution, colour changing from that of the metal indicator complex to that of the dye itself. At pH 3 only few cations react with 1-(2′-thiazolylazo)-2-naphthnol, which act as relatively

selective reagent for the chelatometric titration of copper and bismuth and if a back titration is carried out, lead and nickel can also be determined at this pH. As the pK OH value of 1- (2-thiazolylazo)2-hydroxy-3-naphthoic acid is greater than that of 1-(2'-thiazolylazo)2-naphthol, the former can be used as metal ion indicator in alkaline solution at pH values of upto 9 and sharp end point is attained in the titration of copper and nickel with EDTA solution. Also the use of this compound as an indicator is favoured because of formation constant of 1:2 complexes are smaller than those for other thiazolylazo dyes and this factor is advantageous for the titration of nickel. Addition of another blue dye such as methylene blue or bromocresol green improves the contrast of the colour change [54, 55].

1-5-1-4. Pharmaceuticals

Azo compounds are also used in the pharmaceutical industry. Azo compounds show herbicidal , anti-inflammatory, antimicrobial, or antiparasitic activity, antiulcer drug, antifungal, antibacterial, antitubercular, antibiotics [56, 57].

Furthermore, thiazole is found in the vitamin B1 molecule and in the coenzyme cocarboxylase. The penicillin molecule also contains a thiazolidine ring. 2-aminothiazole are known mainly as biologically active compounds with a broad range of activities and as intermediates in the synthesis of antibiotics, well known sulfa drugs, and some dyes [58, 59]. Polyfunctional ligands based on benzazoles are relevant due to their biological activity as fungicides, antibiotics and pesticides.

1-5-1-5. Other applications

Azo dyes are currently being used in the industries such as textiles, leather, plastics, cosmetics and food for dying of various materials. Furthermore, dyes and there complexes have been used in field such as biomedical studies, advanced applications in organic synthesis and high technogical areas like lasers, liquid crystalline displays, inking printers, dying of textile fiber and colouring of different materials [60 , 61]. Thiazolylazo compounds are a fascinating class of molecules that have wide applications in trace metal analysis, metal signaling, constructions of optical devices and sensor molecules [62, 63]. In recent year π conjugated metal complexes have found applications in many areas of chemistry and material science e.g. luminescent metal compounds can function as emitters in light emitting device they are known to be very stable to intense irradiation in the infrared region and have been investigated as promising optical limiting materials [64, 65]. Recenly azo metal chelates have also attracted much attention due to their interesting electronic and geometrical features in connection with their applications for molecular memory storage, nonlinear optical elements and printing system.

In recent years a considerable amount of work has been done on the coordination chemistry of copper(II) complexes with Schiff base ligands to model the physical and chemical behaviour of biological copper systems. Schiff bases of 4-aminoantipyrine and its complexes have a variety of application in biological, clinical, analytical and pharmacological areas [66,67]. Studies of new kinds of chemotherapeutic Schiff bases are now attracting the attention of

biochemists [68,69]. Earlier work reported that some drugs showed increased activity, when administered as metal complexes rather than as organic compounds [69,70]. A literature search reveals that much work has been done on the transition metal complexes of 4-aminoantipyrine derivatives, but less has been carried out on the chemistry of transition metal complexes and biological behaviour involving the amino group of 4-aminoantipyrine. It is found that no work has been carried out on the synthesis of Schiff base and its transition metal complexes involving the carbonyl group of 4-aminoantipyrine.

In some researches the transition metal complexes Cr(III), Co(II), Ni(II) and Cd(II) were synthesized with the ligands 4-aminoantipyrine (4-AAP) and thiocynate ion. The complexes were characterized by elemental analysis, electrical conductivity measurement, IR, ^{1}H-NMR and ^{13}C-NMR spectra. The antimicrobial, antifungal activities of 4-aminoantipyrine and its complexes were tested against the microorganisms such as *staphylococcus aurous, E.coli, Pseudomonas aeruginosa* and *Candida albicance* by disc diffusion method. The antimicrobial studies of the ligand 4-aminoantipyrine and its metal complexes indicate that the metal complexes showed greater antimicrobial activity than the free ligand[71].

On the other hand, the newazo compound 1-[(3-Trifluromethylphenyl) azo]-2-naphthol (TFMAN) was prepared from coupling reaction of trifluromethylphenyldiazonium chloride with 2-naphthol as coupling component in a alkaline medium. The prepared ligand was characterized by elemental and thermal analyses as well as FT-IR, UV-Vis and GC-mass spectroscopic technique. Metal chelate

complexes of this azo ligand with metal ions Co(II), Ni(II), Cu(II) and Zn(II) were prepared by reacting (TFMAN) with the metal ions in a molar ratio of (1:2) (metal:ligand). All of the coordination compounds were identified by elemental analysis, flame atomic absorption, FT-IR, UV-Vis, magnetic susceptibility and conductivity measurements, in addition to thermal analyses and mass spectra for some of the chelate complexes.The in vitro antibacterial activity of the synthesized compounds have been tested against gram positive and gram negative bacteria. The antibacterial activity assay results also showed that metal complexes have higher antibacterial activity compared to free ligand [72].

While the complexes of the 5-(4-aminoantipyrineazo)-2-naphthol with metal ions Cu (II) and Co (II) were prepared in methanol solution. Characterization of these complexes has been done on the basis of melting point, elemental analysis (C, H, N), molar conductance, UV-Visible spectroscopy, FT-IR, 1H NMR. Elemental analyses of the complexes suggested that the metal to ligand ratio was 1:2. The anti-bacterial activities against Gram-positive and Gram-negative bacteria (*Escherichia coli* and *Staphylococcus aureus*) were tested using disc diffusion method and appreciate activity was observed[73].However, inour research the ligands(DDPPL1),(HPPL2) and metal complexes with (Ni),(Cu) were prepared and characterized by melting point , UV-visible spectroscopy, FT-IR spectrophotometer. The antibacterial activity was tested using disc diffusion method against gram positive and gram negative bacteria. The metal complexes [Cu(DDPP)], [Ni(DDPP)], [Cu(HDPP)],[Ni(HDPP)] were also showed higher antibacterial activity than free ligands (DDPPL1),(HPPL2).

1-6-Nickel :

Is a chemical element with symbol Ni and atomic number 28. It is a silvery-white lustrous metal with a slight golden tinge. Nickel belongs to the transition metals and is hard and ductile. Pure nickel shows a significant chemical activity that can be observed when nickel is powdered to maximize the exposed surface area on which reactions can occur, but larger pieces of the metal are slow to react with air at ambient conditions due to the formation of a protective oxide surface. Even then, nickel is reactive enough .The fraction of global nickel production presently used for various applications is as follows: 46% for making nickel steels; 34% in nonferrous alloys and super alloys; 14% electroplating, and 6% into other uses. Nickel is used in many specific and recognizable industrial and consumer products, including stainless steel, alnico magnets, coinage, rechargeable batteries, electric guitar strings, microphone capsules, and special alloys. It is also used for plating and as a green tint in glass. Nickel is preeminently 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 [74,75].

1-7- copper

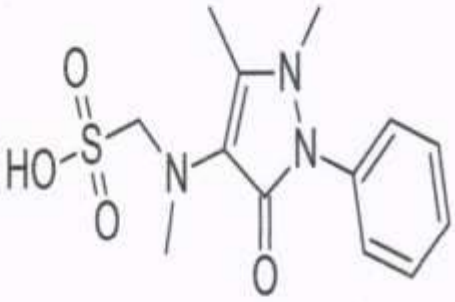
Is a chemical element with symbol Cu (from Latin: cuprum) and atomic number 29 . It is a soft , malleable and ductile metal with very high thermal and electrical conductivity. A freshly exposed surface of pure copper has a reddish-orange color. It is used as a

conductor of heat and electricity, as a building material, and as a constituent of various metal alloys [76].

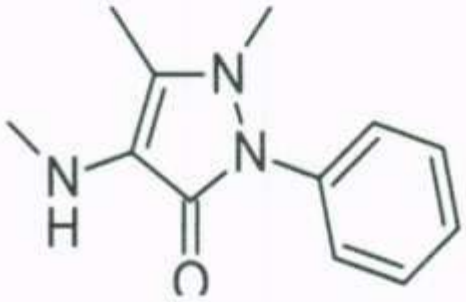
1-8-Pharmacology:-

Its precise mechanism of action is unknown, although it is believed that inhibiting brain and spinal cord prostaglandin (fat-like molecules that are involved in inflammation, pain and fever) synthesis might be involved [77]. Recently , researchers uncovered another potential mechanism involving metamizole . Fig(6). being a prodrug. In this proposal, not yet verified by other researchers, the metamizole itself breaks down into other chemicals that are the actual active agents . Despite this studies in animals have found that the first cannabinoid receptor is not involved in the analgesia induced by metamizole [78]. The result is a couple of cannabinoid and NSAID arachidonic acid conjugates[l] (although not in the strict chemical meaning of the word) of metamizole's breakdown products [79]. Although it seems to inhibit fevers caused by prostaglandins, especially prostaglandin E2 [80].

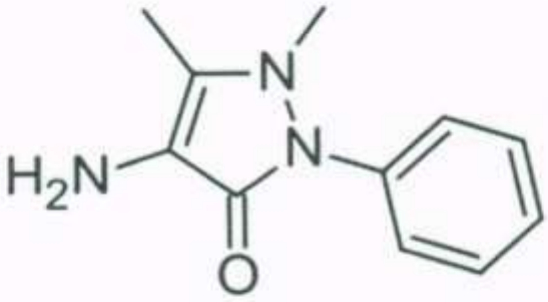
It appears to produce its therapeutic effects by means of its metabolites, especially **N**-methyl-4-aminoantipyrine (MAA) and 4-aminoantipyrine (AA) [81]. N-methyl-4-aminoantipyrine (MAA) have a bioavailability≈90%, plasma protein binding: 58% and excreted in the urine as 3±1% of the initial (oral) dose. Fig (7).4-aminoantipyrine(AA) have a bioavailability≈22.5%, plasma protein binding: 48% and excreted in the urine as 6±3% of the initial (oral) dose . Fig(8).



Fig(6):- show the derivatives structure of metamizole



Fig(7):-The structure of N-methyl-4-aminoantipyrine.



Fig(8):- The structure of 4-aminoantipyrine.

1-9-Toxicity of Pyrazolones:-

Pyrazolone intoxication accounts for most (52 percent) mild analgesic poisonings in West Germany. Severe and fatal intoxication with pyrazolones is, however, rare. In the German literature, only 50 cases have been described in the past 62 years; 80 to 90 percent of these were caused by aminopyrine, which was withdrawn from the West German market in 1978 and replaced by propyphenazone. Up to now, no fatal poisoning with propyphenazone

has bee reported. However, the signs and symptoms of severe intoxication are similar for both propyphenazone and aminopyrine.

The acute toxicity of dipyrone is slightly lower than that of propyphenazone, whereas phenylbutazone and oxyphenbutazone clearly cause less severe reactions. Characteristic symptoms include impaired consciousness progressing to coma, and convulsions. In addition, arrhythmia and cardiogenic shock may occur. Severe aminopyrine intoxication may also be complicated by sudden apnea . Liver damage may develop after a latent period of about 24 hours, especially after phenylbutazone and oxyphenbutazone poisoning. Therapy involves supportive measures as well as gastric emptying by emesis or lavage, installation of medical charcoal, and induction of diarrhea or gut lavage. Although exact clinicotoxicologic data on hemoperfusion are not available as yet, distribution volumes, plasma half-lives, and endogenous plasma clearances as well as results of in vitro trials all suggest the efficacy of this procedure. Hemoperfusion with uncoated amberlite XAD-4 resin is, therefore, recommended for patients with severe pyrazolone intoxication .(Okonek S, Reinecke HJ). 4-Aminoantipyrine is a metabolite of aminophenazone, an aromatic substance [82].

Since AAP is widely used for different applications,AAP as an aromatic pollutant exposes in the environment. The toxic effect of 4-aminoantipyrine on experimental animals has been reported[83]. AAP can reduce blood flow [84]. and 13,14-dihydro-15-keto prostaglandinF2 alpha concentration [85]. after it is infused into the blood .AAP can form stable complexes with heme [86].

and has an obvious denaturing effect on bovine hemoglobin [87]. yet the interaction mechanism between AAP and lysozyme has not been reported.

1-10-Azo compound and its biological activity:-

Many metal complexes have powerful antimicrobial activities and are already in common day-to-day use in medicinal field such as silver bandages for treatment of burns, zinc antiseptic creams, bismuth drugs for the treatment of ulcers and metal clusters as anti-HIV drugs. The potential for further development of metal-based drugs and treatments as an antimicrobial agent is enormous and also of great importance with the evolution of drug-resistant bacteria and threats from a range of viral diseases. The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. The most spectacular advances in medicinal chemistry have been made when heterocyclic compounds played an important role in regulating biological activities. The transition metal complexes of 4-aminoantipyrine and its derivatives have been extensively examined due to their wide applications in various fields like biological, analytical and therapeutical[88,89]. Further, they have been investigated due to their diverse biological properties as antifungal, antibacterial, analgesic, sedative, antipyretic and anti-inflammatory agents [90].

The biological activity of these compounds has been attributed to its scavenging activity against reactive oxygen and nitrogen species (ROS and RNS), as well as to the inhibition of neutrophil's oxidative burst. Indeed, aminopyrinewas demonstrated to be a highly efficient

scavenger of the ROS hydroxyl radical (HO_) [90] hypochlorous acid (HOCl) [90–92]. peroxyl radical [90]. and singlet oxygen [$1O_2$] [93].and of the RNS nitric oxide (_NO) and peroxynitrite (ONOO) [94]. as well as to effectively prevent the phorbol-12-myristate-13-acetate-induced neutrophil oxidative burst[90] . Antipyrine was shown to be the most effective pyrazolone in scavenging ROO_, though it was inefficient against the other ROS and RNS mentioned above, or to the neutrophil's oxidative burst [90,93,94] . Properties of 4-amino antipyrine to coordinate with metal is varied by condensing it with aldehydes, ketone, thiosemicarbazides and carbazides. Metal complexes of 4-amino antipyrine and biological behavior involving the amino group of 4-aminoantipyrine has been studied exhaustively, when compared to the work carried out on the chemistry of transition metal complexes and biological behavior involving the amino group of 4-amino antipyrine [95,96] . The investigations of structure and bonding of Schiff base complexes help understand the complexes.Metal ions present systems. These structures can be modified through condensation with aldehydes, ketones [97,98].

Azo-dyes are the most important and versatile class of synthetic organic compounds, with an enormous variety of applications [99]. These can be obtained easily and inexpensively using a wide variety of diazo and coupling components. They have high dyeing and good fastness properties and wide applications in areas such as dyeing of textile fibers, plastics, leather, paper and bio-medical studies [100].

In recent, years heterocyclic based azo dyes, have found great success due to their higher tinctorial strength, brighter dyeing and excellent light, washing and sublimation fastness, and chromophoric strength in relation to diazo dyes based uniquely on azobenzene

derivatives [101]. Heterocyclic azo dyes have wide applications as high level-dying agents in the dyestuff industries [102]. The increasing usage of these dyes in electronic industry, such as colorimetric sensors, nonlinear optical (NLO) devices and liquid crystalline displays (LCDs) have been investigated as potential sensitizers for photodynamic therapy (PDT) and has attracted much attention [103]. Before the middle-nineteenth century, all of the dyes used to dye fabric were extracted from natural sources. In 1856, William Perkin, synthesized the first dye that was used commercially. This dye is known as Perkin's mauve or mauveine. The first commercially important azo dye was Bismarck Brown, first manufactured in 1865. By the 1970s, over 60% of the synthetic dyes were azo dyes [104]. Azo compounds constitute one of the largest classes of industrially synthesized organic compounds. They are important in dye, drugs and cosmetics [105]. and show a variety of interesting biological activities including antibacterial and pesticidal activities. Compounds of naphthalene have been reported to show a variety of biological activities including antimicrobial activities, Heteocyclic amines have been used extensively in the preparation of disperse dyes. These dyes show outstanding discharge-ability on polyester. Disperse dyes before 1950 were mostly amino anthraqunone derivatives. Through these dyes are bright in color they have limitations of poor discharge-ability and are sensitive to the oxides of nitrogen. Dyes contain heterocyclic ring as 2- amino -5-nitrothiazole have been reported to have extinction coefficient , this type of azo dyes are classified as donor – acceptor chromogen [106]. Compounds containing one or more azo groups (N=N- linked to two carbon atoms) have a variety of uses. Aliphatic azo compounds, like azo*bis*isobutyronitrile (AIBN), can be as radical

initiators in polymerization of alkenes to make plastics. Aromatic azo compounds are used as acid-base indicators, biological stains, and commercial colorants for clothing, plastics, cosmetics, and food beverages. Many azo-dyes, such as methyl red, methyl orange, and congo red, can be used as acid-base indicators due to their ability to function as weak acids or bases. Color changes are caused by changes in extent of delocalization of electrons: more delocalization shifts the maximum absorption to longer wavelengths and makes the light absorbed redder, while less delocalization shifts the maximum absorption to shorter wavelengths. Color changes can also be due to geometrical isomerism of the azo group. UV radiation can cause a *trans* azo group to become *cis*.

This can lead to photochromism, a light-induced reversible color change. Some azo dyes with this property (and which can revert slowly to the *trans* isomer in the dark) are used in sunglasses and car sunroofs. Many azo dyes, like Sudan red and scarlet red, can be used as biological dyes because they are fatsoluble and can be absorbed into fat cell tissues on microscope slides. Azo dyes form 60-70% of all synthetic dyes used as commercial colorants. Azo dyes have several advantages over other commercial dyes including their wide color range, good color fastness and ability to absorb light. They can also be synthesized cheaply because the starting materials are readily available, inexpensive compounds; most of the chemistry is completed at or below room temperature; and the environmental impact is low due to the use of water as a solvent in all of the reactions. Cost advantages tend to compensate for the lower resistance to bleaching and lower brilliance of azo dyes compared to anthraquinones, the second most used dye class [107-108]. Biological importance of azo

compounds is well known for their use as antineoplastics, antidiabetics, antiseptics, anti-inflammatory, and other useful chemotherapeutic agents [109-112]. Azo compounds are known to be involved in a number of biological reactions such as inhibition of DNA, Evans blue and Congo Red are being studied as HIV inhibitors of viral replications. This effect is believed to be caused by binding of azo dyes to both protease and reverse transcriptase of this virus [113]. Biological activity of azo colorants mostly results from the specific pathway of Their metabolism. An enzyme-mediated reduction of the azo bond occurs *in vivo* [114,115]. In mammalian organisms it has found in liver [116]. in digestive tract bacteria [117-119]. and in skin bacteria such as *Staphylococcus* aureus [120].The result of this reaction is cleavage of the azo bond and the release of the corresponding aromatic amines originating from the azo dye [121]. The products can be more or less toxic than the parent molecules, so this process can decrease or increase any toxic or carcinogenic effects of the dyes [122-124]. Nevertheless, in most cases the products, in particular benzidine-based derivatives, demonstrate an increased toxicity [116,119,120]. For example, the azo dye Direct Black 38 is metabolized to the mutagen benzidine by human intestinal microflora[119]. Several independent research groups showed in 70-ies that benzidine-based dyes increased the risk of cancer of the bladder and other organs in humans [124]. It was proved that benzidine was the major factor responsible for mutagenesis [125]. Further studies also confirmed the mutagenicity of its analogs [126,127]. Despite the above described negative features of azo dyes, there are numerous biological activities which make them medically attractive compounds. One of the positive pharmaceutical application

of azo dyes and their specific azo reduction *in vivo* is polymeric azo compound for site specific drug delivery in the colon diseases such as colitis and irritable bowel syndrome [128]. Reductive degradation and subsequent splitting of the azo bond occur in colon, and therefore they are highly site-specific [129]. what gives an opportunity to prepare a targeted therapy.

A-The staphylococci

are gram-positive spherical cells, usually arranged in grape-like irregular clusters. *S.aureus* case several infections including:

- Localized skin infections, Deep, localized infections, Acute endocarditis, Septicemia.
- Pneumonia:
- Nosocomial infections, Progression to septicemia
- Toxinoses such as Toxic shock syndrome, Scalded skin syndrome and Staphylococcal gastroenteritis.

B-Streptococci

are gram-positive, nonmotile, and catalase-negative. Clinically important genera include Streptococcus and Enterococcus. Diseases caused by this group of organisms include acute infections of the throat and skin caused by Group A streptococci (*Streptococcus pyogenes*); female genital tract colonization, resulting in neonatal sepsis caused by Group B streptococci *(Streptococcus agalactiae*); pneumonia, otitis media, and meningitis caused by *Streptococcus pneumoniae*; and endocarditis caused by the viridans group of streptococci.

C-*Escherichia coli*

is part of the normal flora of the colon in humans and other animals, but can be pathogenic both within and outside of the GI tract. At least five types of intestinal infections that differ in pathogenic mechanisms have been identified [Figure 12.3]. Enterotoxigenic (ETEC), enteropathogenic (EPEC),enterohemorrhagic (EH BC), enteroinvasive (EIEC), and enteroaggregative (EAEC). *E.coli* all are basically the same organism, differing only by the acquisition of specific pathogenic traits. extraintestinal disease such as: Urinary tract infections (UTI), neonatal meningitis and nosocornial (hospital-acquired) infections.

D-*Salmonella spp.*

Members of the genus *Salmonella* can cause a variety of diseases, including gastroenteritis and enteric (typhoid) fever. *Salmonella* classification has undergone numerous revisions; currently, all strains are grouped in a single species: *S. enterica*, which has approximately 2500 different serotypes, or serovars including the clinically significant serotypes *typhimurium and typhi*.

E-*Yersinia Enterocotitica.*

The genus *Yersinia* includes three species of medical importance: *Y. enterocolitica* and *Y. pseudotuberculosis*, both potential pathogens of the GI tract that are discussed in this chapter, and *Y pestis*, the etiologic agent of bubonic plague, *Y. enterocolitica* and *Y. pseudotuberculosis* are both motile when grown at 25A°C but not at 37A°C.

F-*Klebsiella spp.*

Klebsiellae are large, nonmotile bacilli that possess a luxurious capsule They are Lactose fermentor. K. pneumoniae and K. oxytoca cause necrotizing lobar pneumonia in individuals compromised by alcoholism, diabetes, or chronic obstructive pulmonary disease. K. pneumoniae also causes urinary tract infections and bacteremia, particularly in hospitalized patients.

G-*Proteus spp.*

Members of these genera are agents of urinary tract and other extraintestinal infections. Proteus species are relatively common causes of uncomplicated as well as nosocomial UTIs. Other extraintestinal infections, such as wound infections, pneumonias, and septicemias, are associated with compromised patients. Proteus organisms produce urease, which catalyzes the hydrolysis of urea to ammonia. The resulting alkaline environment promotes the precipitation of struvite stone containing insoluble phosphates of magnesium and phosphate.

H-Vibrios

Members of the genus Vibrio are short, curved, rod-shaped organisms.

Vibrios are closely related to the family Enterobacteriaceae. They are rapidly motile by means of a single polar flagellum. O and H antigens are both present, but only O antigens are useful in distinguishing strains of vibrios that cause epidemics. Vibrios are facultative anaerobes. The growth of many Vibrio strains either

requires or is stimulated by NaCl. Pathogenic vibrios include: 1) *V. cholerae*, serogroup O1 strains that are associated with epidemic;2) non-O1 *V.cholera* and related strains that cause sporadic cases of choleralike and other illnesses; and 3) *V. parahaemolyticus* and other halophilic vibrios, which cause gastroenteritis and extraintestinal infections. *V. cholerae* is transmitted by contaminated water and food. There are no known animal reservoirs, or animal or arthropod vectors. Among humans, long-term carriage is considered uncommon [130].

Despite primarily affecting developing countries, cholera remains a serious public health problem for some developed countries[131].

In 1965 – 1966 the Eltor biotype was transmitted from Asia and Middle East to Iraq and Iran through trading in the seventh pandemic [132].the presence of cholera in Iraq was due to its warm climate[133-134-135].

WHO has received notification from the National IHR Focal Point of Iraq of additional laboratory-confirmed cases of cholera.

As of 8 October 2015, a total of 1,263 laboratory-confirmed cases of *Vibrio cholerae* O1 Inaba were reported. These cases were reported from at least 15 governorates of the country – These are Babylon (469 cases), Baghdad (304 cases), Qadisiyyah (146 cases), Muthanna (155 cases), Basra (61 cases), Wassit (41 cases), Karbala (33 case), Najaf (32 cases), Thi-qar (6 cases), Maysan (6 cases), Diyala (2 cases), Duhok (2 cases), Erbil (2 cases), Kirkuk (2 cases), Salah al-din (1 case) and Suleimaniyah (1 case) [136].

Later, on 22 November 2015, a total of 2,810 laboratory-confirmed cases of *Vibrio cholerae*01 Inaba had been confirmed at the Central Public Health Laboratory in Baghdad, and only 2 deaths related to cholera were reported. These cases were reported from 17 Governorates of the country, namely Baghdad (940 cases), Babylon (675 cases), Qadisiyyah (442 cases), Muthanna (287 cases), Karbala (157 cases), Basra (102 cases), Wassit (68 cases), Najaf (46 cases), Thyqar (20 cases), Missan (21 cases), Dahuk (16 cases), Kirkuk (19 cases), Erbil (10 cases) Diyala (3 cases), Salaheddine (2 cases) Sulaimanneya (1 case) and Ninewa (1 case) [137].

1-11- Aim

In this research we report the synthesis and characterization of Azo compounds and metal complexes derived from 4-aminoantipyrine and study the biological activity against gram positive and gram negative bacterial species including *Streptococcus spp* and *S. aureus* (Gram positive bacteria) and *E.coli* , *K. pneumonia* , *proteus spp* *S. typhi*, *Acinetobacter spp* , *V.cholera* , and *Yersinia spp* (Gram negative bacteria). Cholera is endemic disease in Iraq, therefore we test the bioactivity of Azo chemical reagents on *V.cholerae*.

Chapter2

Experimental

Chapter 2

2-Experimental

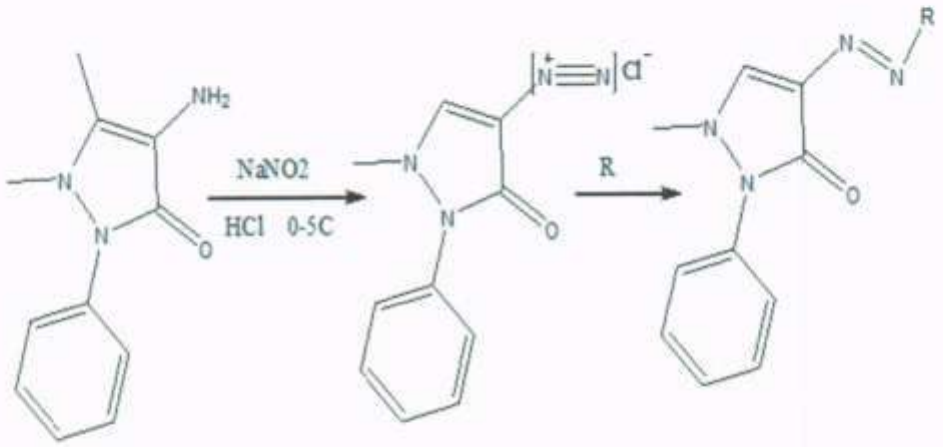
2-1-Materials:

4-Aminoantipyrine, methanol, resorcinol and B-naphthol and solvents were of reagent-grade quality without purification , melting point were taken on SAMP10 apparatus (Steurat ,UK), Infrared spectra were recorded on bruker optics tensor II FT-IR spectrometer (Germany).The electronic spectra of the ligands and complexe were recorded on UV-Visible Shimadzu(Japan) spectrophotometer.

2-2-Method:

2-2-1- Synthesis of Azo ligands:

The azo dyes reagents were prepared by dissolving (0.11g,0.001 mol of resorcinol or (0.5g,0.001 mol) B-naphthol in 100 ml of (2M) sodium bicarbonate and cold it (0-0.5C°) then the resulting solution added slowly to a solution of diazonium salt was prepared from (0.25g) sodium nitrite dissolved in 5 ml distilled water added with keeping temperature between (0-0.5C°) to (0.255g 4-aminoantipyrin , in 10 ml 2M hydrochloric acid HCL. Then converting the prepared dye from sodium salt into hydrogen form by adding of dilute HCl . The dye was purified by recrystallization from methanol.



L1=R=Resercinol
L2=R=B-naphthol

2-2-2-Collection of specimens and bacterial identification:

nine samples collected from patients with different infections including (UTI, stool, Burn Infections, Otitis Media, Skin infections) Those patients did not receive any antibiotic treatment earlier, the samples were transported as quickly possible to the laboratory

Then the samples had been inoculated on the diagnostic culture media then identified by biochemical differentiation tests . The samples were cloned three successive times nutrient agar and stored on a nutrient agar slant at 4 $^{\circ}C$ [138-139-140].

2-2-3-In Vitro Antibacterial activity testing using Agar well diffusion assay NCCLS:

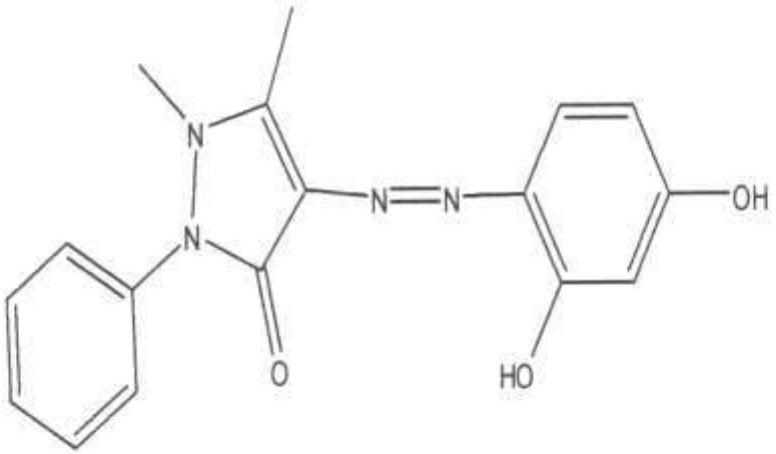
Loop full growths from bacterial isolates were inoculated into nutrient broth incubated at 37°C for 18 hours. The bacterial suspensions were diluted normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5 x 108 CFU/ ml. Cotton swab was dipped and streak into adjustment suspension the entire Mueller Hinton agar (for all tested bacteria) surface of plates and the plates were left for one (5-15) minutes at room temperature to dry. Media were cut into four wells (5mm diameter) by cork borer and add (20μ) of the TEST agent dilutions (The plates were performed in triplicates). All plates of the TESTED organisms were then allowed to incubate at 37°C for overnight. After (24 h) of incubation, each tested agent dilution was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter [141].

Chapter3

Results & Disscusion

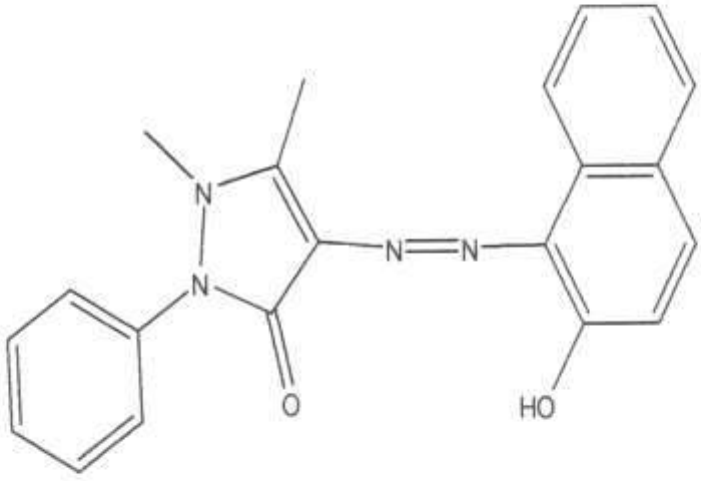
3- Results & Disscusion

The synthesized ligands and complexes were characterized by melting points , IR-spectroscopy and UV-Visible spectroscopy .The physical data of the ligands and their complexes were showed in the tab(1).



4-((2,4-dihydroxyphenyl)diazenyl)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one

Fig(21): The structure of the L1.



4-((2-hydroxynaphthalen-1-yl)diazenyl)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one

Fig(22): The structure of the L2.

Tab(1): The physical properties of the ligands and their complexes

Compound	Chemical formula	Molecular weight g/mole	color	Melting point C^0	*Yield %*
L1 (DDPP)	$C_{17}H_{16}N_4O_3$	324.33	Red	256-263	62
L2 (HDPP)	$C_{12}H_{18}N_4O_2$	358.39	brown	185-195	61.5
[Cu(DDPP)]	$C_{34}H_{30}C_4N_8O_6$	710.20	Black	218-224	70.4
[Ni (DDPP)]	$C_{34}H_{30}N_8NiO_6$	705.35	Light brown	>300	73.2
[Cu(HDPP)]	$C_{42}H_{34}CuN_8O_4$	778.32	black	227-240	71.3
[Ni(HDPP)]	$C_{42}H_{34}N_8NiO_4$	773.46	dark red	95-105	69.8

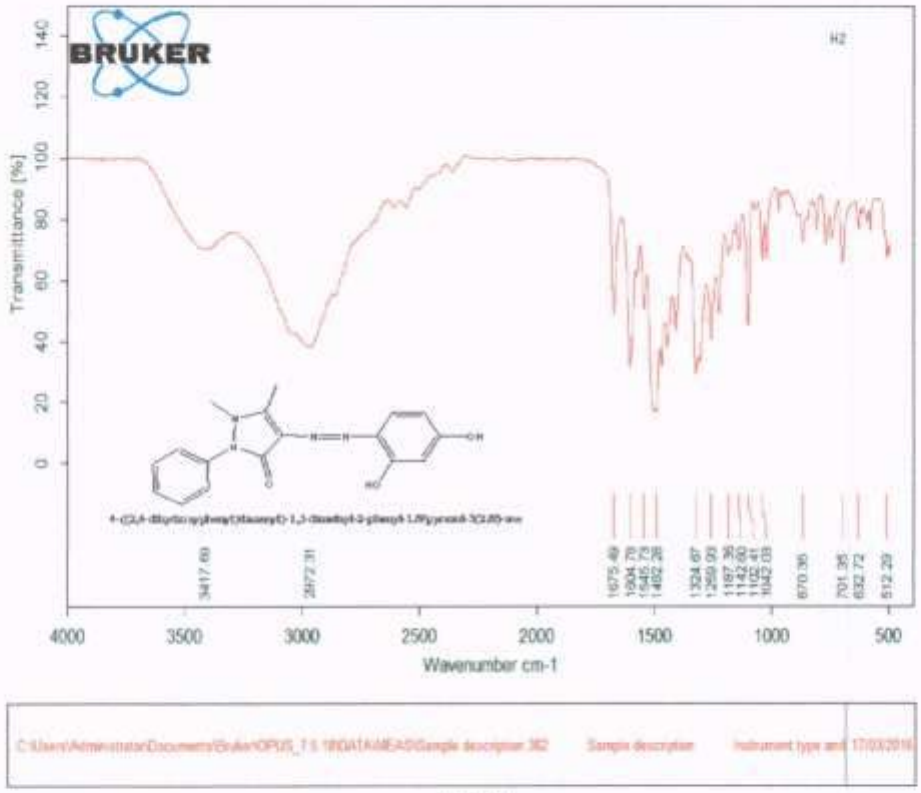
3-1-Infrared spectral studies:-

The most important absorption bands in the spectrum of 4-aminoantipyrine due to stretching vibrations of (N-H) bond symmetrical and asymmetrical(3300-3500), ν(C=O) and aromatic ν (C=C),while in the $DDPPL_1$ and $HDPPL_2$ the (N-H) absorption bands were disappeared , but with appearing new the absorption bands at (3417,3183) cm^{-1} were attributed to (O-H) bond stretching vibrations that related to $DDPPL_1$ and $HDPPL_2$ [142]. the absorption bands at (1675,1645-1633)cm^{-1} were related to

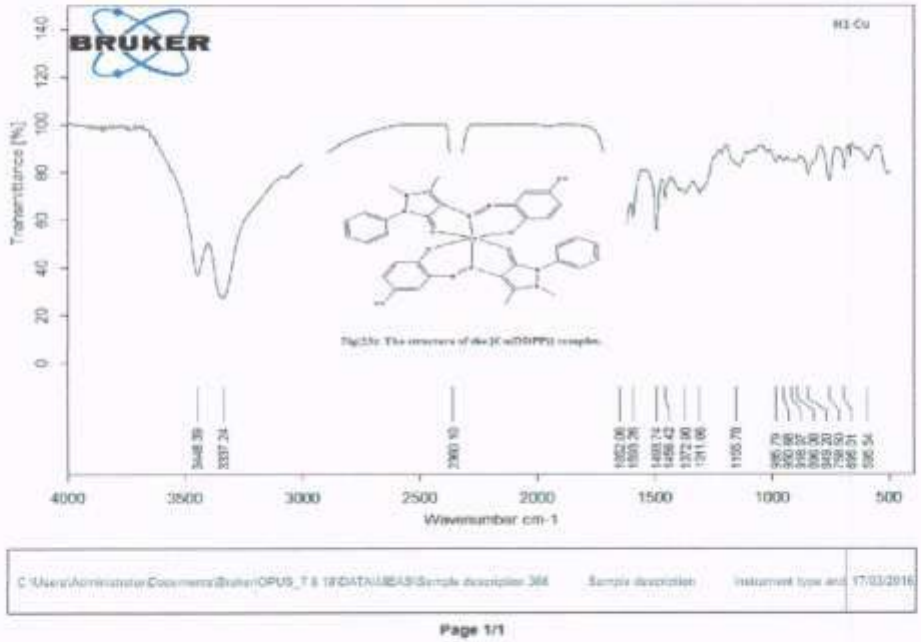
stretching vibration of (N=N) bond for $DDPPL_1$ and $HDPPL_2$ respectively, [143], this demonstrated that azo compound was formed, these bands were shifted to lower frequencies in the all metal complexes, this mean that (N=N) bond involving in coordination with metal ion. .The absorption bands at (3448,3355)cm^{-1} for $DDPPL_1$ with Cu and Ni complexes respectively, were attributed to (OH) that not involved in the coordination with metal ion ,while there is no (O-H) bond stretching vibration for $HDPPL_2$ with Cu and Ni complexes ,this is an indication that (O-H) bond involved in the coordination with metal ion[144]. The observed bands at (1604,1633)cm^{-1} for $DDPPL_1$ and $HDPPL_2$, respectively, were attributed to ν (C=O)where shifted to lower frequencies , when its compared with (C=O) bond stretching vibration in the spectrum of 4-aminoantipyrine alone ,this demonstrated that the Ligand was formed, also these bands were shifted to lower frequencies in the metal complexes of $DDPPL_1$ and $HDPPL_2$,mean involved in coordination with metals. [145]. The observed bands at (1187,1172)cm^{-1} attributed to ν(C-O) for $DDPPL_1$ and $HDPPL_2$, respectively, while shifting to lower frequencies in the metal complexes[146].

(2):-Characteristic IR absorption bands of the ligand and it's complexes in cm^{-1}

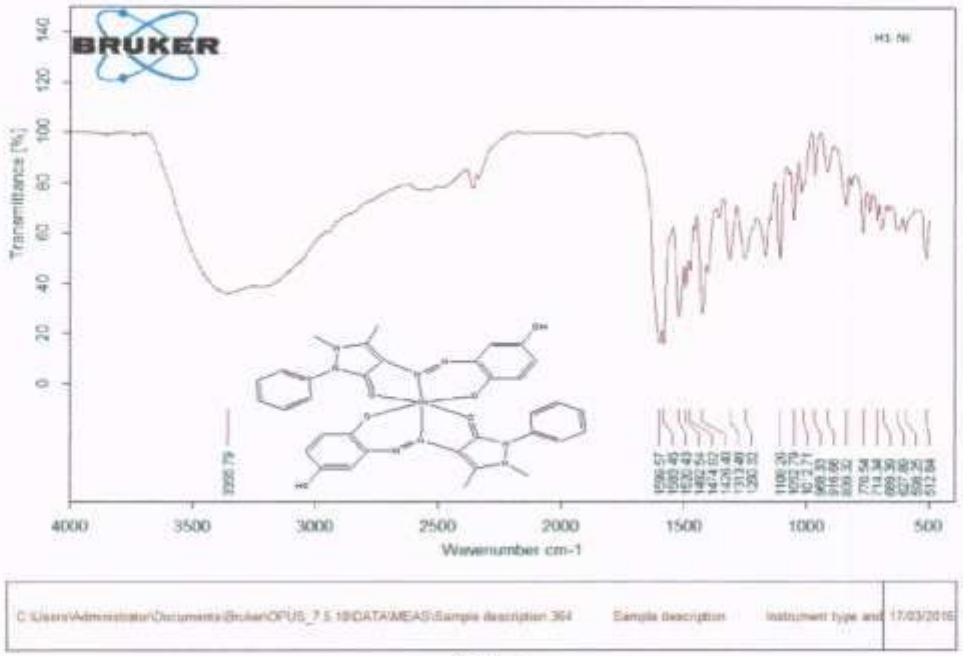
	L1	L2	L1(Cu)	L1(Ni)	L2(Cu)	L2(Ni)
υ (N=N)	1675	1633-1645	1652	1599	1627	1611
υ (C=O)	1604	1633	1593	1583	1600	1587
υ (O-H)	3417	3183	3448	3355	--------	---------
υ (C-O)	1187	1172	1155	1108	1121-1142	1144
υ(C=C)	1545	1453	1493	1492	1439	1417



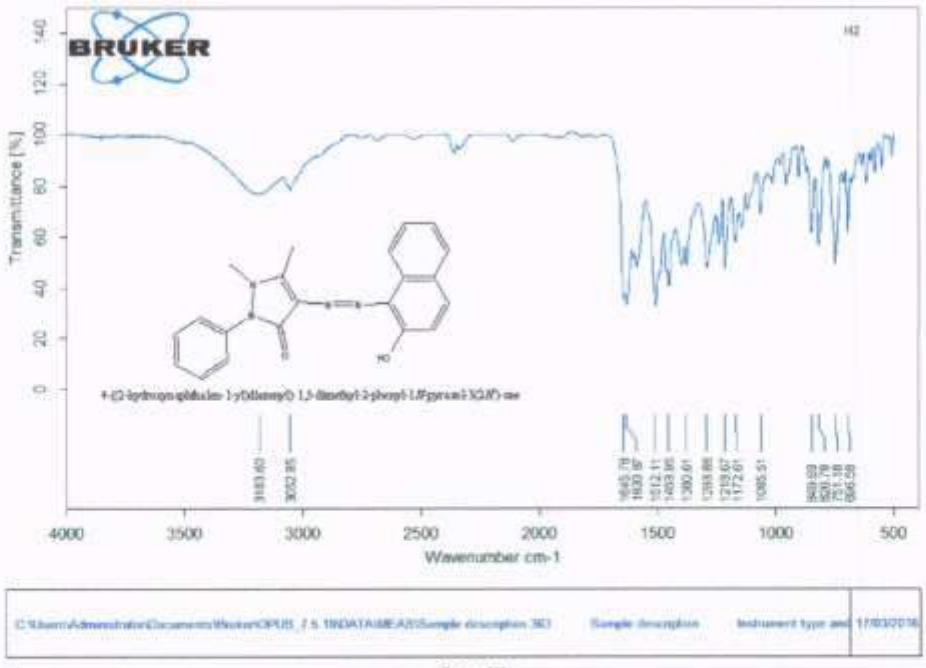
Fig(9): the IR- spectra of the L1.



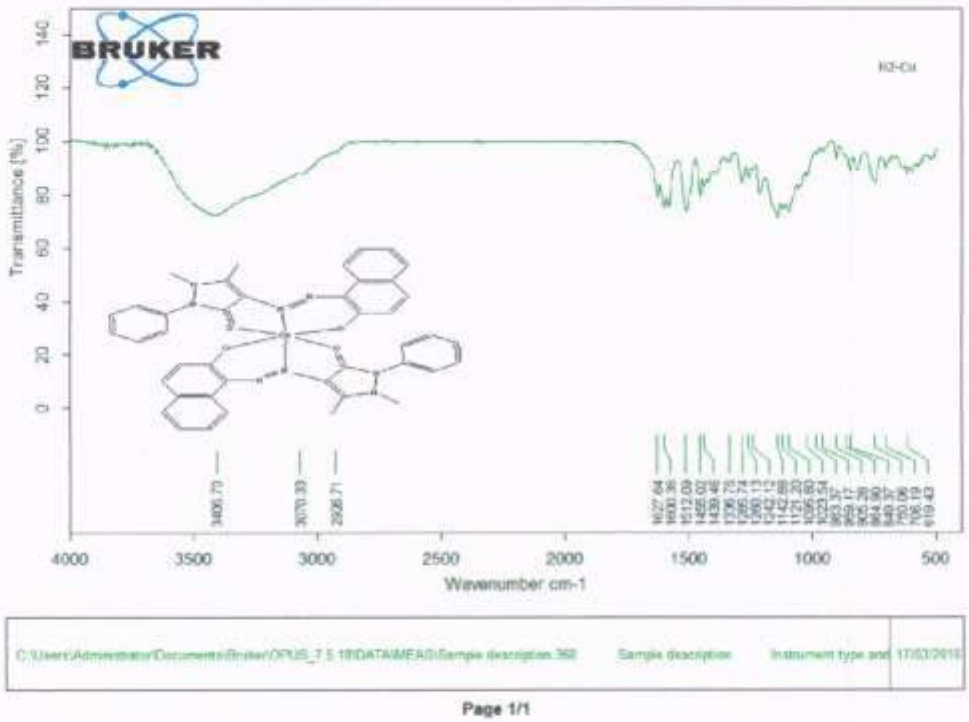
Fig(10):IR-spectrum of the L1 with Cu complex.



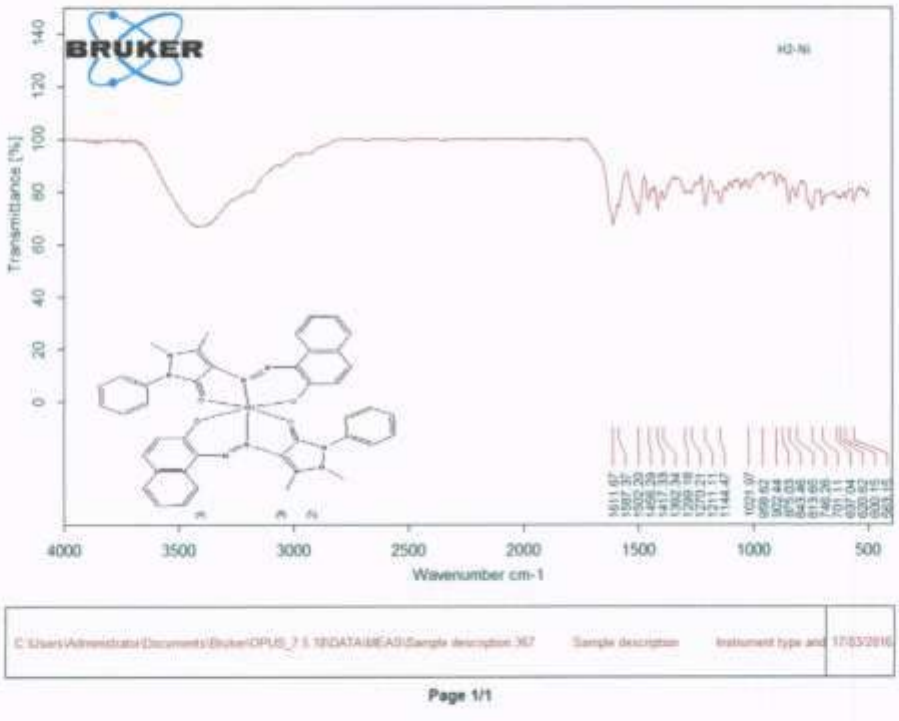
Fig(11): IR-spectrum of the L1 with Ni complex.



Fig(12): IR-spectrum of the L2.



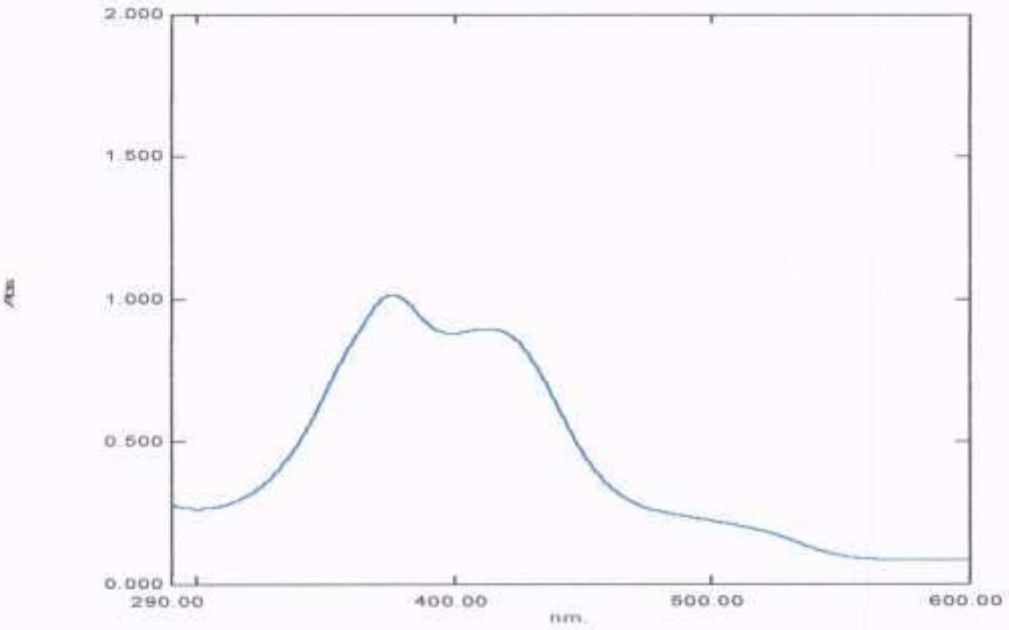
Fig(13): IR-spectrum of the L2 with Cu complex.



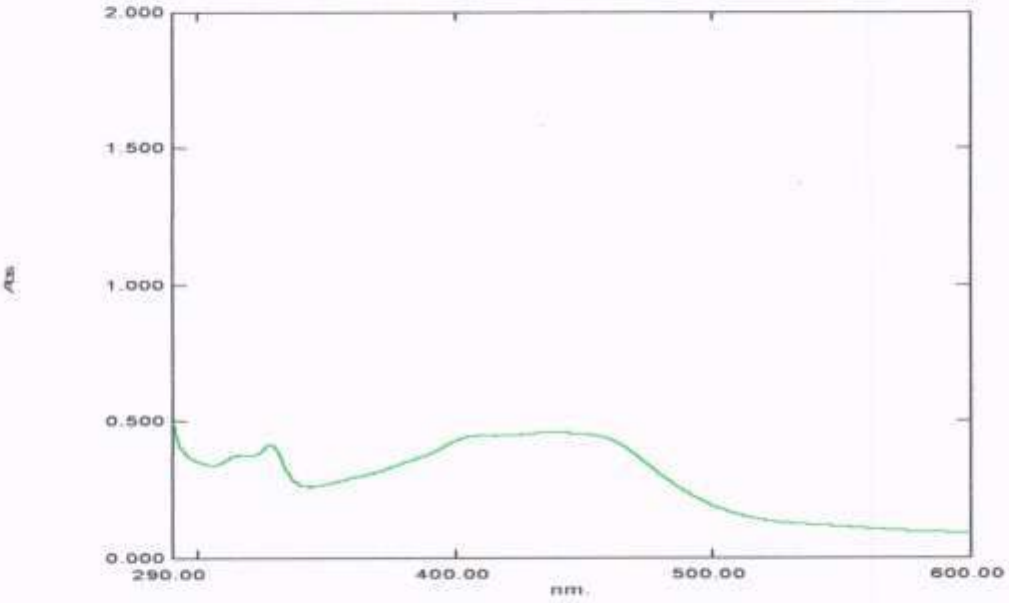
Fig(14): IR-spectrum of L2 with Ni complex.

3-2-UV-Visible studies:

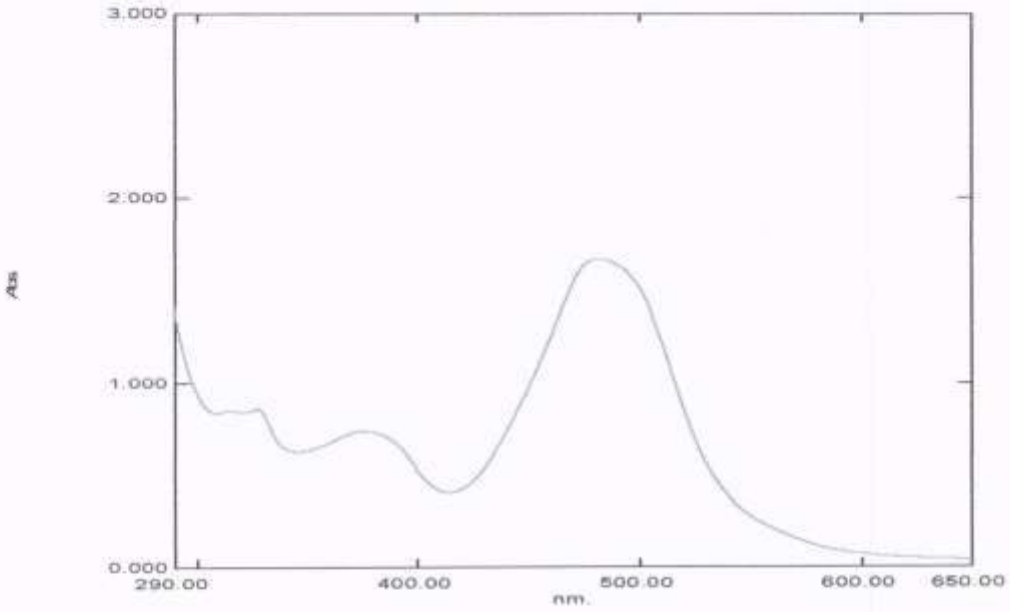
The UV-Vis spectra were recorded in methanol for the ligand and its metal complexes with a Shimadzu 1800 Spectrophotometer using 1 cm quartz cuvettes from (1100-200) nm range. The UV-Vis spectrum of the free ligands were exhibited two absorption peaks ,the first peak at the visible region (413 , 436) nm for($DDPPL_1$) , was attributed to $n \rightarrow \pi^*$ electronic transition , while the second peak at ultraviolet region (376,328)nm for ($DDPPL_1$) and ($HDPPL_2$),respectively, was attributed to $\pi \rightarrow \pi^*$ electronic transition . The electronic spectra of the all metal complexes were exhibited two absorption peaks the first absorption peaks were shifted to sorter wavelengths due to complexation (375) nm for $DDPPL_1$ with Cu and Ni respectively,375,359,for $HDPPL_2$ with Cu and Ni, respectively)nm and attributed to $n \rightarrow \pi^*$ electronic transition, the second peaks (325 , 237) nm for $DDPPL_1$ with Cu and Ni, 375 ,359 for $HDPPL_2$ with Cu and Ni, respectively, was shifted to sorter wavelengths) nm were attributed to $\pi \rightarrow \pi^*$ electronic transition, with appearing new absorption peak at (481, 452) nm for $DDPPL_1$ with Cu and Ni , respectively, (485 , 481) nm for $HDPPL_2$ with Cu and Ni)nm indicated that the ligands were coordinated with metals ion .



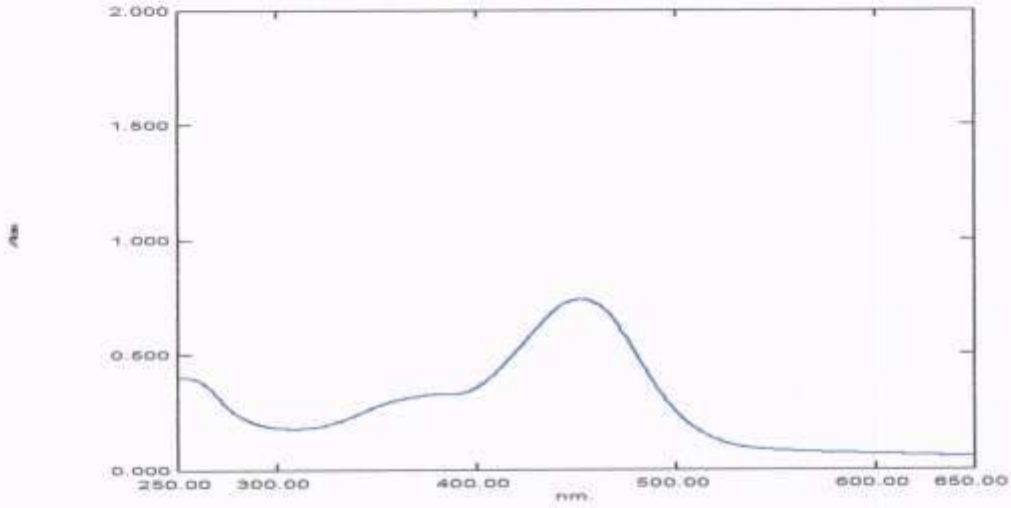
Fig(15): Show the wave length of L1.



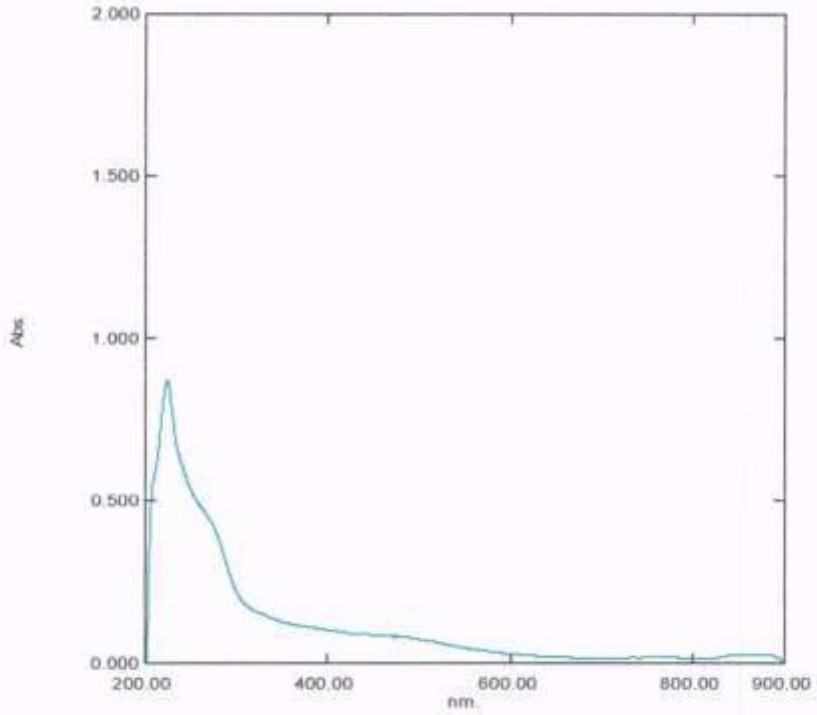
Fig(16): Show the wavelength of L2.



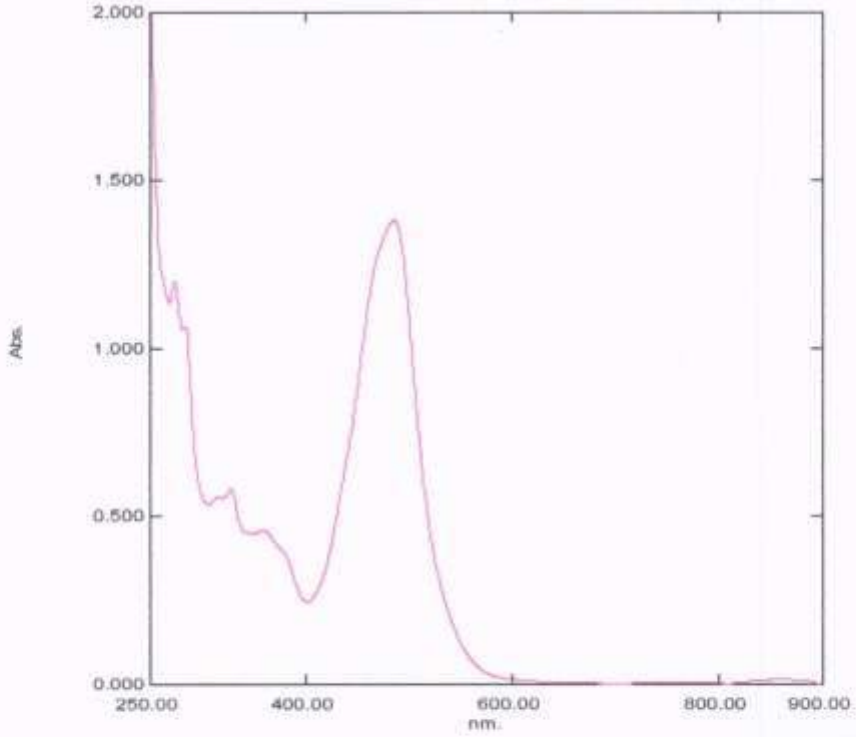
Fig(17): Show the wavelength of L1with Cu.



Fig(18): Show the wavelength of L1 with Ni.



Fig(19) :show the wavelength of L2 with Cu.



Fig(20): Show the wavelength L2 Ni.

3-3-The biological activity:

In this research antibacterial bio effects of the ligands and its complexes were tested against nine bacterial samples namely, *Streptococcus spp* and *S. aureus* (Gram positive bacteria) and *E.coli* , *K. pneumonia* , *proteus spp* *S. typhi*, *Acinetobacter spp* , *V.cholera* , and *Yersinia* ***enterocotitica*** (Gram negative bacteria) by agar well diffusion method using nutrient agar medium for antibacterial activity.The diameter of inhibition zones were measured and expressed in millimeters (mm). All of tested compounds exhibited remarkable antibacterial activity against tested bacteria. A comparative study of the antibacterial activity values of the ligand and their complexes indicate that the metal complexes exhibited higher antibacterial activity compared to the free ligand. This is probably due to the greater lipophilic nature of the metal complexes. The increased activity of the metal chelates can be explained on the basis of Overtone,s concept of cell permeability. **Charles Ernest Overton** (1865–1933) was a British physiologist and biologist , now regarded as a pioneer of the theory of the cell membranes. [147]. He studied the permeability of a range of biological materials to around 500 chemical compounds.[148]. Hans Meyer and Ernest Overton independently noticed that the chemicals which act as general anesthetics are also those soluble in both water and oil. They interpreted this as meaning that to pass the cell membrane a molecule must be at least sparingly soluble in oil.[149]. Meyer and Overton established a simple rule to predict membrane permeabilities[150,151].This rule does not account for transport processes, mediated by membrane carriers, channels or pumps, which were not known at that time, and it ignores in homogeneities, such as rafts, [152]. which may exist in the biological membrane. The rule works fine for all molecules that merely cross the lipid matrix by simple diffusion.

The lipid membrane that surrounds the cell favours the passage of lipid soluble materials due to which liposolubility is an important factor which controls the antibacterial activity [153]. The mechanism of action of antibacterial drug can be discussed in general under four headings , (1) inhibition of cell wall , (2) inhibition of cell membrane function (3) , Inhibition of protein synthesis and (4) inhibition of nucleic acid synthesis [154].The biological activity of ligand and its metal complexes were showed in the tab(3).In the best of our knowledge, there are very few studies related to investigation the biological activity of Azo compounds on pathogenic bacteria. In study done performed in the College of Science/Al-Kufa University in 2013 [155]. they found that 4-aminoantipyrine(Azo) gave highest activity against *P.aeroginosa* and *E.coli* had lowest sensitivity, while *S.aureus* didn't affected by this compounds. In other research in Egypt [156]. A series of copper (II) complexes of azo ligands tested to detect the antimicrobial activity of Azo Complexes on Some affected bacteria, they tested the bioactivity on some pathogenic microorganism, Such as bacteria(*S. aureus* , *E. coli* and *K. pneumoniae*). The tested complexes have good antibacterial activity against *S.aureus* and *E.coli*.

Our results not in agreement with the results of these studies and that may be due to various factors such as:-

1- Differences in chemical preparation methods of Azo compounds and using diverse metals as ligands may affect the results .
2- The possibility of using different concentrations of the ligands and its complexes in the studying the biological activity.

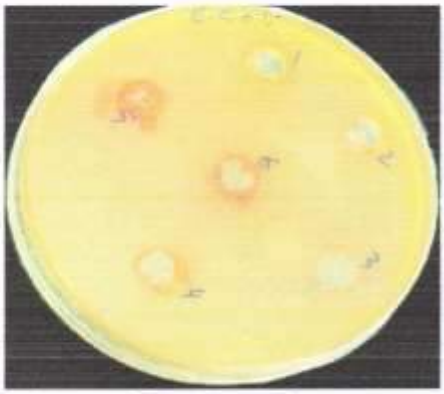
3- Abusing of antimicrobial drugs without any understanding from patients, so it may increase the chances of transferring the resistances between the bacterial species in different ways especially in nosocomial infection.

Table-3-Antibacterial activity of AZO compounds on Bacterial isolates - Inhibition Zone in (mm) at concentration of ($1x10^{-5}$)mm.

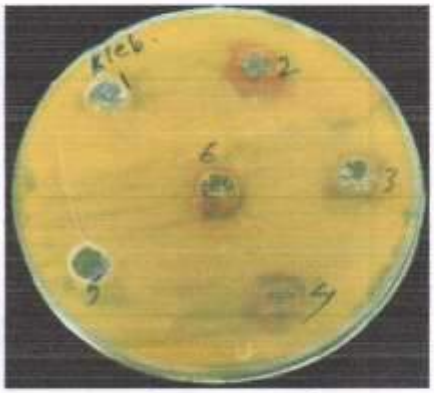
N	Bacteria	1	2	3	4	5	6
1.	*S. aureus*	15	18	18	20	23	25
2.	*Streptococcus spp.*	15	15	17	19	20	20
3.	*E. coli*	15	16	20	25	25	27
4.	*K. pneumonia*	9	9	11	17	17	19
5.	*Proteus spp.*	12	13	14	17	20	20
6.	*S. typhi*	15	18	20	20	21	26
7.	*Acinetobacter spp.*	7	8	8	11	11	12
8.	*V. cholera*	20	20	25	25	25	26
9.	*Yersinia enterocotitica .*	12	12	15	15	16	19

1

2



3



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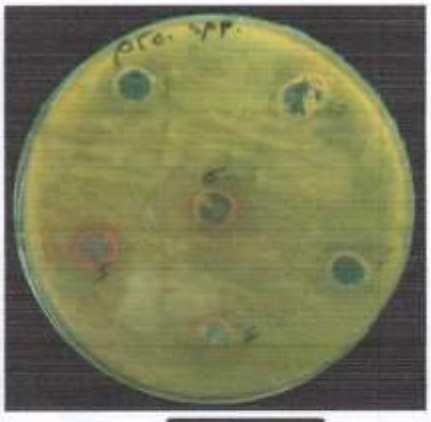

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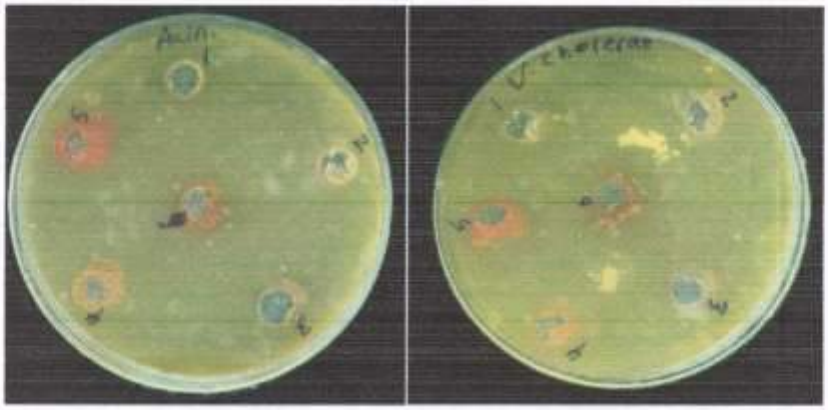


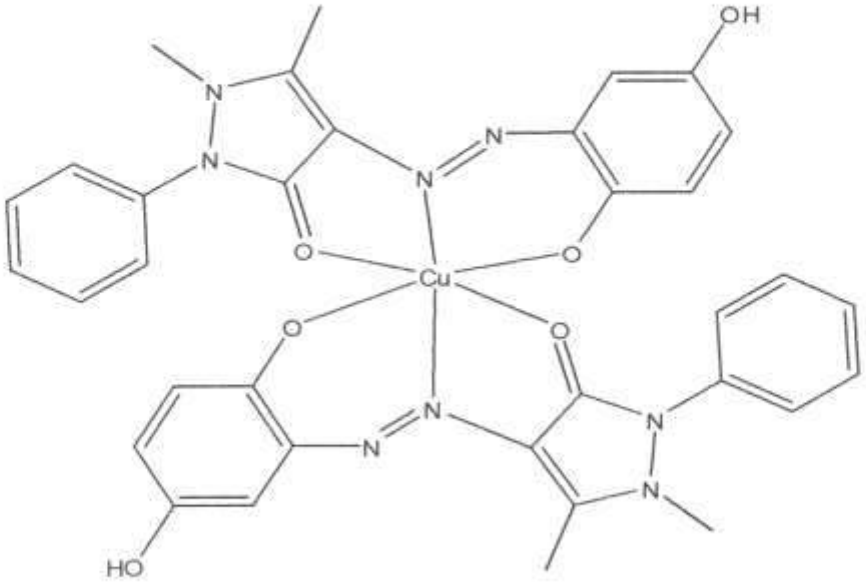
7 8

9

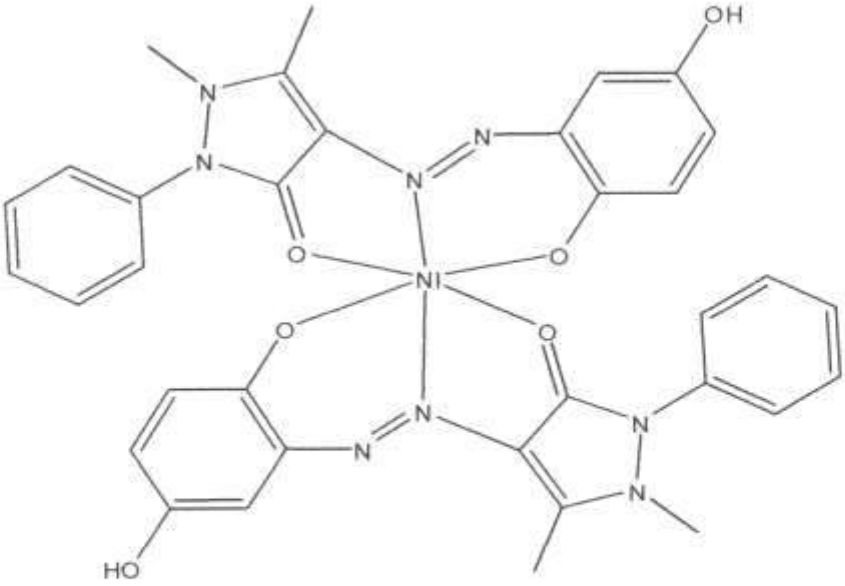
Well agar diffusion test of bacterial isolates

1- $DDPPL_1$
2- $HDPPL_2$
3- DDPPL1+Cu
4- DDPPL1+Ni
5- HDPPL2+Cu
6- HDPPL2+Ni

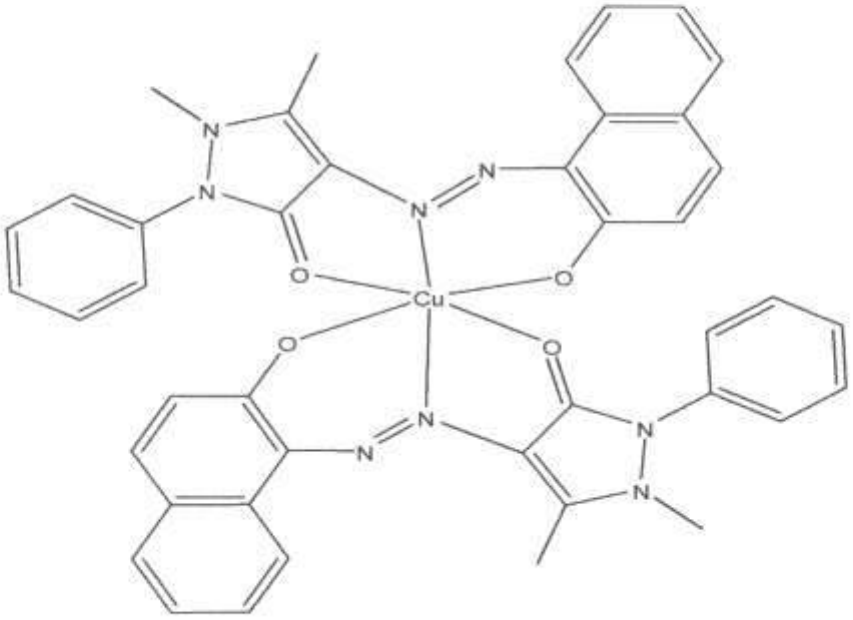
3-4- The proposed structures of complexes



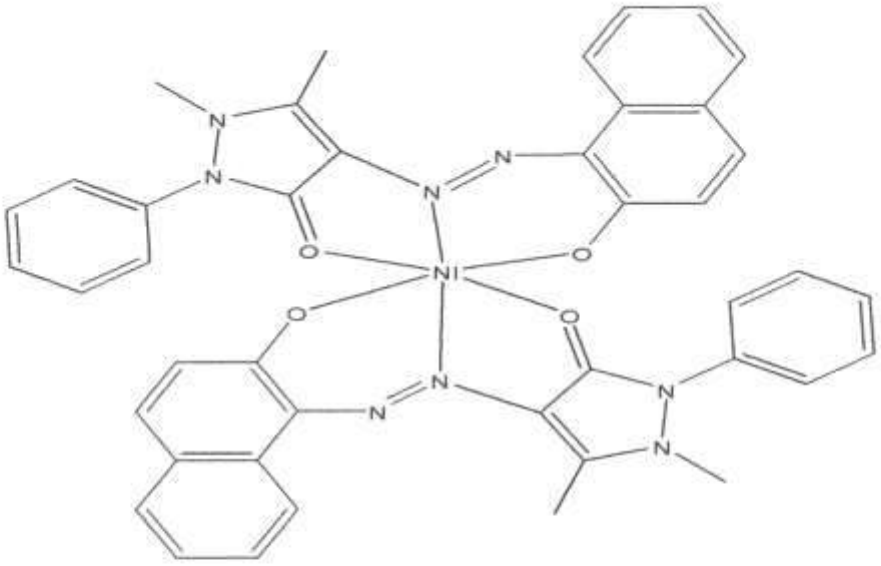
Fig(23): The structure of the [Cu(DDPP)] complex.



Fig(24): The structure of the[Ni(DDPP)] complex.



Fig(25): The structure of the[Cu(HDPP)] complex.



Fig(26):- The structure of the [Ni(HDPP)] complex .

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