SYNTHESIS CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF SOME METAL COMPLEXES OF QUINOLONE FAMILY OF ANTIBIOTICS

Islamabad

A dissertation submitted to the Department of Chemistry, Quaid-i-Azam University, Islamabad, in partial fulfillment of the requirements for the degree of

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In

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By

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CERTIFICATE

This is to certify that this Dissertation submitted by ***Mr. Muhammad Rizwan*** is accepted in its present form by the Department of Chemistry, Quaid-i-Azam University, Islamabad, as satisfying the dissertation requirement for the degree of ***Master of Philosophy*** in ***Inorganic/Analytical Chemistry***.

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DEDICATED

TO

MY PARENTS

&

ESPECIALLY TO MY FATHER (LATE)

THEIR LOVE GUIDANCE AND COURAGE ENLIGTENS MY PATH IN EVERY WALK OF MY LIFE

ACKNOWLEDGEMENT

By the grace of Allah, the Almighty, the creator of universe, who grants Hidayah to the mankind and peace and blessings be upon his prophet, Hazrat Muhammad ﷺ, who exhorted his followers to seek knowledge from cradle to grave, I've been able to complete this academic enterprise.

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(Muhammad Rizwan)

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ABSTRACT

Metal complexes of two very important antibiotics of flouroquinolone family i.e. Ciprofloxacin and Norfloxacin were made. All the metals from first transition series were used for complexation except Titanium, from second transition series Zirconium was used. All metal complexes were made in 2:1 ligands to metal ratio. Mixed ligand metal complexes using 2, 2′ Bipyridyl along with the drugs were also prepared using the same ratio of ligand to metal. Complexes were characterized using FT-IR spectroscopy and metal analysis. FT-IR analysis shows that the carboxylic acid peak which was appeared at 1708 cm^{-1} and 1731cm^{-1} respectively in spectrum of Ciprofloxacin and Norfloxacin were shifted towards lower wave number in spectrum of complexes i.e. bonding was done through carboxylic acid group. It also confirmed the purposed structure of complexes. Metal analyses by atomic absorption spectroscopy were also confirmed that the metal contents were found similar to that calculated theoretically. To check the effectiveness, the metal complexes are tested against four strains of microorganisms i.e. *Salmonella typhae, Bascillus subtilus , Staphlus coccus aurius , E.coli* in comparison with plane drugs (ligands) using "well diffusion" method All the complexes have shown comparable activity against these organisms, and few have shown greater activity than plane drugs

ABREVIATIONS

AAS	⇒	Atomic Absorption Spectroscopy
ALA	⇒	Alanin
ATP	⇒	Adenosine Triphosphate
Bpy	⇒	Bipiridyl
Cf.	⇒	Ciprofloxacin
DNA	⇒	Deoxyribonucleic Acid
DL	⇒	Deciliter
EDTA	⇒	Ethylene Diamine Tetra Acetate
FTIR	⇒	Foriur Transformed Infrared Spectroscopy
GTF	⇒	Glucose Tolerant Factor
Hb	⇒	Hemoglobin
HPLC	⇒	High Pressure Liquid Chromatography
MIC	⇒	Minimum Inhibitory Concentration
MPS	⇒	Mucopoly Sachride
NOR	⇒	Norfloxacin
Nf	⇒	Norfloxacin
Nm	⇒	Nano Meter
NSAID	⇒	Non Steroid Anti Inflammatory Drugs
PIP	⇒	Piperazine
RBC	⇒	Red Blood Cell
μg	⇒	Microgram
μm	⇒	Micrometer
ν	⇒	Wave Number
UV	⇒	Ultra Violet

CHAPTER #1

INTRODUCTION

INTRODUCTION

Many organic compound used in medicine do not have purely organic mode of action some of them are activated or bio transformed by metal ions. Metallic elements play crucial role in biological system.[1]

A characteristic of metal is that they easily loss electron from the familiar or metallic state to form positively charge ion which tend to be soluble in biological fluids. It is in this cationic form that metal play their role in biology.

The metal ions are electron deficient, the most biological molecule such as protein and DNA are electron rich. The attraction of these opposing charges leads to generate tendency for metal ions to bind and to interact with biological molecule. This same principal applies to the affinity of metal ions for many small molecules and ions crucial to life, such as O_2.Given this wide scope for the interaction of metal in biology, it is not surprising that natural evaluation has incorporated many metals into the essential biological functions [1, 2]

Metals perform a wide variety of tasks such as carrying oxygen throughout the body and scuttling electrons. Hemoglobin, an iron containing protein that binds to oxygen trough its iron atom, carries this vital molecule to body tissues.[3]

Metal ions such as Zinc provide the structural framework for the Zinc fingers that regulate the function of genes in the nuclei of cells.[3]

Similarly Calcium containing minerals are the basis of bones, the structural framework of the human body.

Zinc is a natural component of insulin, a substance crucial to the regulation of sugar metabolism.[3]
Chromium is said to be a potentiater of insulin and is known as glucose tolerant factor (GTF) [3]

Metal such as copper, zinc, iron and manganese are incorporated into catalytic proteins the metalloenzymes, which facilitate the chemical reactions needed for life.[2]

Since nature has made such extensive use of metal ions in biological systems, the following questions arises; "Can metal ion be incorporated into drugs? Are coordination compounds potential medicinal agents? Can coordination chemistry be used for medicinal purpose?" This area of scientific inquiry is termed as medicinal inorganic chemistry. [1]

Medicinal inorganic chemistry as discipline has only existed for about the last 30 years, since the discovery of cisplatin. Since then a lot of chemical compound have been developed and their mechanism of action is also elucidated. These compounds are effective in many ways. Many of them are in clinical use for treating different ailments. Chemotherapeutics, such as the anticancer agents, metal-mediated antibiotics also appear in medicinal inorganic chemistry.

Antibiotics is a class of natural or synthetic compounds that inhibit the growth or kill microorganisms in dilution .For example penicillin, ciprofloxacin etc.

Many antibiotics such as streptomycin, aspergillic acid, usnic acid, the tetracyclines, flouroquinolone, and other antibiotics are known to have chelating properties; presumably some antibiotics are delicately balanced, so as to be able to compete successfully with the metal binding agents of the bacteria while not disturbing the metal processing by the host. There is evidence that at least some bacteria have developed resistance to antibiotics through development of altered enzyme systems that can compete successfully with the antibiotic.[4] The action of antibiotic should not be simple competitive one. The chelating properties of the antibiotic may be used in metal transport across membrane s or attach the antibiotic to a specific site from which it can interfere with the growth of bacteria.

It has been seen that the metal complexes of antibiotics have good activity against certain disease and are used in cure of different diseases.

There are many evidences in the literature for the metal complexes of quinolnes and other antibiotics and found to be effective against different microorganisms in vitro and some are in vivo.

Hoffken et al.[5] reported that concurrent administration of magnesium-aluminum containing antacid with ciprofloxacin resulted in nearly complete loss of activity of drug in serum.. Antacids not only contain magnesium or aluminum but can also contain calcium and bismuth ions. Several authors have begun to study the reason of reduction in activity of quinolnes and gave the suggestion that multivalent cat ions should be avoided in patients receiving quinolnes antibiotics.

Xiao-Zeng You [6] have prepared complexes with quinolnes coordinated to magnesium, calcium, copper(I) and zinc by using hydrothermal methods. In some of these complexes different bonding mode was observed.

D. Rrehder et al.[7] have prepared a vanadium complex from water solution of $VOSO_4$ and ciprofloxacin the crystals were very unstable and could not be investigated further. The tentative formula for the complex was $[VO (cfH)_2] SO_4 .10 H_2O$.

The iron (III) complex with ciprofloxacin and nitrilotriacetate (nta) as ligand was isolated from water solution of ciprofloxacin and $Fe (NO_3)_3$ and nitrilotriacetate (Disodium salt) by S C Wallis et al.[8]

I. Turel et al [9] prepared two copper complexes with ciprofloxacin from water solution of ciprofloxacin and copper (II) salts. In the complex $[Cu (cfH)_2 Cl_2] .6 H_2O$ the copper atom is coordinating with four oxygen atoms of two ligands .In the second complex of copper ciprofloxacin $[Cu (cfH) (H_2O)_3] SO_4 .2H_2O$ only one molecule of quinolne is coordinated. Many mixed ligand complexes have also been prepared in recent years.

Two zinc Norfloxacin complexes,$[Zn (Nf)_2].4H_2O$ and $[Zn (H_2O)_2(nfH)_2] (NO_3)_2$ were isolated by Z.F.Chen et al [11] using hydrothermal synthesis. Ionic compounds

of quinolnes with different metals i.e. Magnesium, iron, bismuth, platinum, copper and zinc have also be isolated and characterized.

F.Gao. et al [12] have observed the biological activity of Iron (III), $[Fe(nfH)_2(H_2O)_2]$ $Cl_3.6H_2O$ and zinc(II)-$[Zn(nfH)_2]Cl_2.7H_2O$ in vitro against the Gram negative microorganisms E.coli and Bacillus dysenteria bacteria. The complexes showed stronger activity than Norfloxacin

I.Turel, et al [13] tested the two magnesium complexes, $[Mg\ (FCl)_2].H_2O$ and $[Mg(cf)_2(H_2O)_2].2H_2O$.against various Gram positive and Gram negative microorganisms. The results show that both complexes are significantly less active than parent quinolone drugs. It was also found that activity of quinolnes is reduced when the solutions are titrated with magnesium ions.

It was established that Cu (II) and Ni (II) are effective in induction of the cytotoxicity of some quinolone against leukemia cells in vitro, whereas Mg (II) as not effective .the different effects of metals on the quinolone cytotoxicity can be explained by their different modes of interaction with quinolone. The authors claim that the transition metals can form DNA intercalated agent with quinolone that can cause the cytotoxicity [14]

It was found that the vanadium ciprofloxacin complex is promising with respect to insulin-mimetic behavior and concomitant low toxicity in the physiological concentration range [14]

OBJECTIVES OF PRESENT STUDY

1. To synthesis the metal complexes of two very important antibiotics of flouroquinolone famiiy
    a) Ciprofloxacin
    b) Norfloxacin

    Metals used in this study are of first transition metal series i.e. Vanadium, Chromium, Manganese, Iron, Cobalt, Copper, Nickel, Zinc. and Zirconium from second transition metal series.

2. Characterization of these complexes using spectrometric method To check the biological activities (antibiotic potency) by Disc diffusion method on different strains of microorganism

CHAPTER #2

THEORETICAL ASPECTS

2.1 THEORY OF COMPLEXATION

2.1.1 COORDINATION COMPLEXES:

Complexes are compounds that contain a central atom or ion, usually a metal, surrounded by a cluster of ions or molecules. The complex tends to retain its identity even in solution, although partial dissociation may occur. It may be a cat ion or an anion or nonionic depending on the sum of the charges of the central atom and the surroundings ions and molecules.

The importance of metal complexes become clear when one realizes that chlorophyll, which is vital to photosynthesis in plants is a magnesium complex and that hemoglobin, which caries oxygen to animal cells is an iron complex.

In the formation complex, each molecule or atom (ligand) donates a pair of electron the empty orbital of the central atom, thus forming a semi covalent bond. For example in $[CoF_6]^{3-}$ complex each of the fluoride ion (F^{1-}) donates a lone pair of electrons to Co^{3+} cat ion

$Co\ (27) = [\ Ar\ ], 3d^6, 4S^2, 4P^0$

$Co^{3+}\ (24) = [Ar], 3d^6, 4S^0, 4P^0$

$[CoF_6]^{3-} = [Kr]$

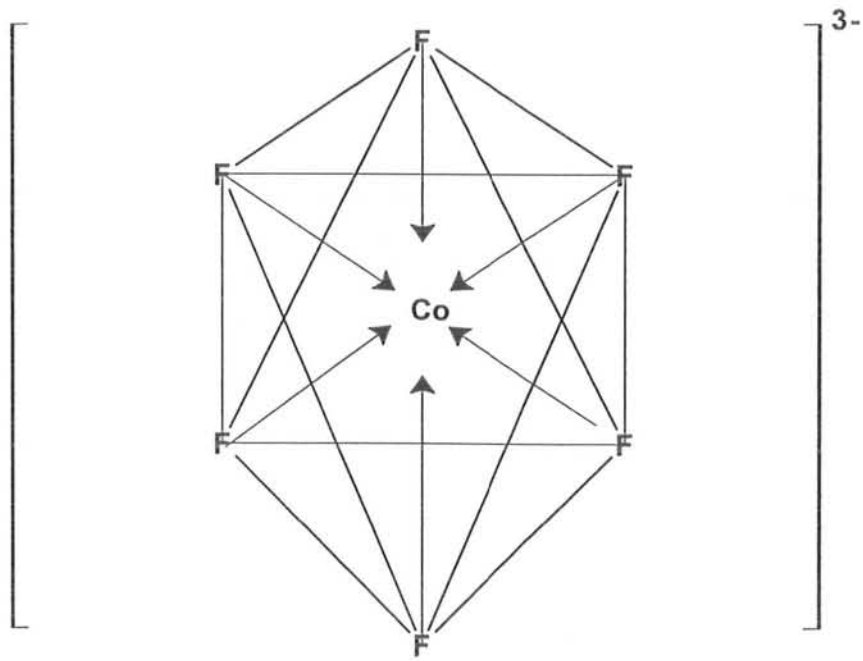


Fig 2.1 Structure of $[CoF_6]^{3-}$

In this complex union, the chemical characteristics, not only of metal but also of ligand are lost.

The total number of bonds, which the ligands form with the central atom in the complex, is called its "coordination number". Coordination number varies form 2 to 12 of which 4 and 6 are very common and important. Coordination number "4" has five principal symmetries i.e. Td, D4h, C4v, C3v and C2v and those with coordination number "6" have Oh and C4v symmetry.

2.1.2 STABILITY OF COMPLEXES:

Isolation of bioactive ingredients from plant matter via complexion depends on the stability of the complex, which the added metal from with bioactive component. Stability is the most important factor in selective complexation reaction.

So stability is the most important factor in the synthesis of metal complexes. A number of factors may be contributing towards the stability of complexes but mainly two factors are held responsible.

a) **Enthalpy Changes:** Greater the amount of heat evolved in a reaction more stable will be the reaction products.
b) **Entropy changes:** Greater the entropy changes, more stable will be the reaction product.

$$\Delta G = \Delta H - T \Delta S$$

In addition to these factors stability of complexes depends upon the following factors,

i.) Nature of ligand, its size, geometrical shape, basicity and localizability of its donor atom, chelate effect
ii.) Nature of metal ion, its size, oxidation state, Polarizability and symmetry of its bonding orbital,

By systematic variation of these factors, complex formation can be made selective and under optimum condition, even specific. Some important factors on which, stability depends are discussed below briefly.

Following factors are important w.r.t. Metals.

1) Size and Charge:

Because of the significant influence of electrostatic forces in these systems, the smaller the size and larger the charge of a metal ion the more stable are the metal complexes. The stability is favored by a charge to radius of the metal ion.

2) Crystal Field Effects:

The crystal field stabilization energy (CFSE) plays an important role in the stability of transition – metal complexes and appears to be responsible for natural order of stability of complexes of the first-row transition metals;

3) Polarizability of Metal and Ligands.

Metal ions are grouped as hard and soft acids while ligands are termed as hard and soft bases. The concept of hard and soft acids and bases developed by R J Pearson can be used to understand stability of complexes.

Metal ions having small radii, carrying high charge and do not have easily distortable valence shell electrons, are termed as hard acids e.g.: Co^{3+}, Fe^{3+} etc.

The soft acid group include metals which are large, have low charge and their valence shell electrons can easily be removed e.g., Cu, Pd, Hg.

It is believed that hard acids interact strongly with hard bases and vice versa. So complexes formed by interaction of hard acid with hard bases (low Polarizability, empty molecular orbital of high energy) are very stable.

Most of first row transition metal ions are hard acid and form stable complexes with hard base ligands containing O, N, F etc. as donor atom.

Metals are classified into two classes i.e. class 'a' and classes 'b' metal. The more electropositive metals, for example, Na, Ca, Al, the Lanthanides, Ti, and Fe, belong to class 'a' the less electropositive metal, for example Pt, Pd, Hg, Pb, and Rh, belong to class 'b' . Class "a" metal form their most stable complexes with ligand in which the donor atom is N, O or F; class `b' metals prefer ligands in which donor atom is one of the heavier elements in the N, O or F families. It is

believed that the stability of the complexes of the class "b" metals results from an important covalent contribution to metal-ligand bonds and from the transfer of electron density from the metal to ligand via bonding.

With reference to the role of the ligands in determining the stability of metal complexes the following factors are important:

1. Base Strength:

The greater the base strength of a ligand the greater is the tendency of the ligands to form stable complexes, which class "a" metals. The order of stability with various ligands is as follows:

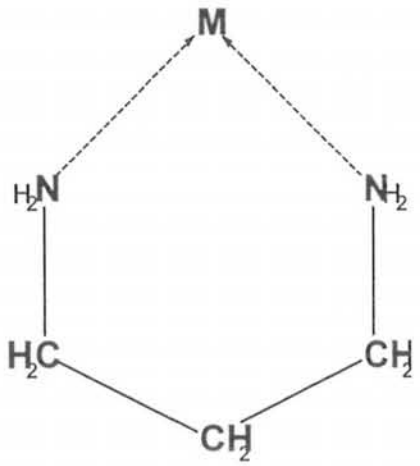
$F > Cl > Br > I$ and $NH_3 > H_2O$

2. Chelate Effect:

Complexes of ligands, which are capable of forming chelates, are more stable as compared to those, which occupy only single coordination position. Complexes such as $[Ni\ (en)_3]^{2+}$ or $[Ni\ (dien)_2]^{2+}$ are much more stable than $[Ni\ (NH_3)_6]^{2+}$. Stability of metal chelates increases with number of donor atoms in a ligand e.g. diethylenetriamine forms more stable complex with a metal ion, than bidentate ligand e.g. ethylenediamine .

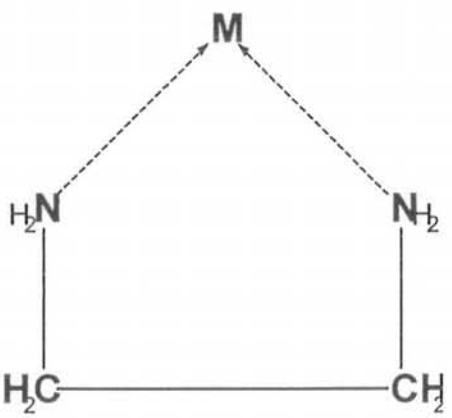
3. Chelate Ring Size:

The most stable metal Chelate contains saturated ligands. Greater the ring size of the chelate lesser it would be stable Ligand form five Memberd chelate ring or unsaturated ligand are more stable then that form six Memberd rings.



A

Fig 2.2 (A) Six Memberd Chelate Ring

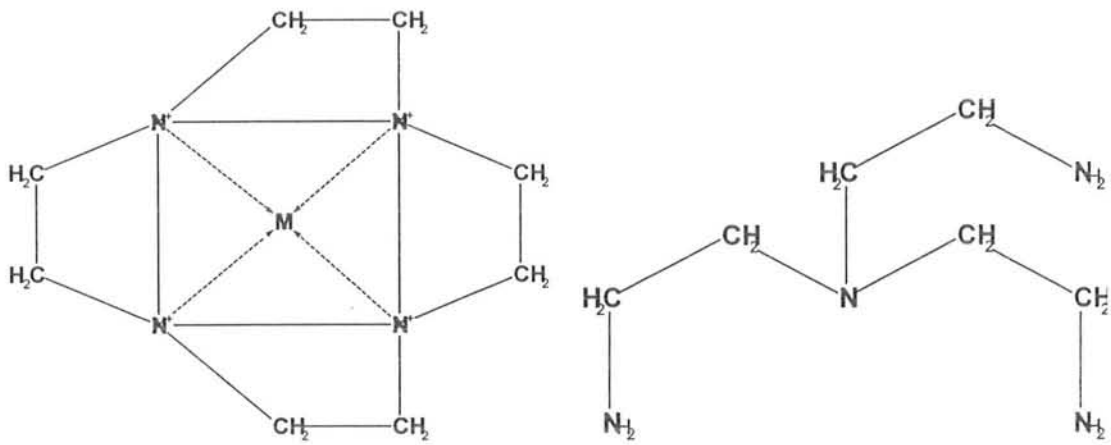


B

(B) Five Memberd Chelate Ring

4. Steric Strain:

Because of the steric factors, large bulky ligands form stable Metal complexes then do analogous smaller ligands for example $NH_2\ CH_2\ CH_2\ NH_2$ forms more stable complexes then $(CH_3)_2\ NCH_2\ CH_2N\ (CH_3)_2$. The strain is sometimes due to the geometry of the ligand coupled with the stereochemistry of the metal complexes. For example $H_2NCH_2\ CH_2NHCH_2CH_2NHCH_2CH_2HN_2$ can coordinate its four nitrogen at the corner of square, but $N\ (CH_2CH_2NH_2)_3$ cannot.



A B

Fig 2.3 (A) structure of $H_2NCH_2\ CH_2\ NHCH_2CH_2NHCH_2CH_2HN_2$

(B) Structure of $N\ (CH_2CH_2NH_2)_3$

5. Electronegativity of Donor Atoms

The stability of complexes depends on the electronegativity of donor atoms. Greater the electronegativity of donor atoms, stronger will be its binding with metal through electrostatic forces. The order of stability on the basis of electronegativity is

$$F > O > N > Cl > Br > I$$

6. Size of Ligands

Large size ligand with the same charge form less stable complex, than small size ligand for example 'F' forms stable complex than 'Cl' with same metal ion as shown

$[Fe\ (F)_6]^{4-}$ $K= 1 \times 10^6$

$[Fe\ (Cl)_6]^{4-}$ $K=2 \times 10^1$

From the very beginning man has engaged in a constant battle against diseases and with the increase of knowledge, weapons against disease have become sophisticated, Attention has always been focused on curing diseases. In some, instances chemical agents have been devised to aid the natural defense of body against invaders. A perfect therapeutic agent should be completely effective in the desired situation and have no side effect. In such agents organic molecules, metals and coordination complexes have widely tested for treatment of large number of dieses and some of them have been found quite effective. Nearly all the work had been done with organic compounds and their metal derivatives have hardly ever been studied in a systematic way until late 1960.

2.2.1 CHELATION THERAPY:

Chelation is a chemical term given to molecules that combine or complex with metals such as lead, mercury, copper, iron, calcium, etc. The term is taken from 'chela' the pincer like claws of crabs. Such agents hold metals making them ineffective by limiting their availability for metabolic activity. One such chelating agent that is widely used is a synthetic amino acid, ethylene diamine tetra-acetic acid, or EDTA that chelates toxic metals such as lead and mercury, as well as calcium and the other essential trace metals. As chelating agents the other amino acids (protein fragments), vitamins A, C and E, are also used in chelation therapy as antioxidants. Aspirin and other antinflamatory (NSAIDS) chelates convey a variety of benefits to multiple organ systems. Most notably by combining with Calcium as an anticoagulant improves the cardiovascular and macular degeneration. By reducing arterial plaque formation with an increased blood supply relieves patients from angina, claudication, and dizziness. [16]

The tetracyclines are potent chelating medications and as such have been found to act as anti-inflammatory, immunosuppressive, and anti-metallic enzymes as well as antimicrobial. When tetracyclines complex with copper they do the activated leucocytes produce active antioxidants and anti-inflammatory agents by

neutralizing the damaging oxygen free radicals. By combining with the copper, zinc, iron and other trace metal elements in enzymes such as collagenase, tetracyclines can inhibit the enzymatic destruction of tissues. [16]

The beneficial effects of tetracyclines and other chelating agents such as EDTA, aspirin, and vitamins C, E, or A, are attributed to their metal complex and their resulting multiple actions, including antimicrobial. The administration of multiple acting chelating drugs could enhance or inhibit their activity and effectiveness depending upon their relative affinity. For example EDTA with a greater affinity for Calcium than tetracyclines could have a greater anticoagulant action. On the other hand tetracycline's greater affinity for nucleic acids and lipids than most chelates results in a greater inhibition of protein synthesis and microbial growth. It is of interest to note that for many years the combination of Vitamin E and Selenium, a metal chelate, was a favorite arthritis medication used by veterinarians. [16]

2.2.2 METALS IN MEDICINE

Inorganic chemistry and more specifically the chemistry relating to therapeutic compounds used in medicine is becoming more important all the time. Science is exploring combinations of metal ions and ligands never thought possible before. If successes such as cis-platin anti-cancer drugs, bismuth anti-ulcer drugs, and vanadium insulin-mimetic over the last few years are anything to go by, more discoveries could be just around the corner. [17, 18]

Many metals in the diet are essential for maintaining good health and therefore provide a great deal of potential for research into diet supplementation.

Medical imaging using metals is becoming increasingly important as we continue to improve techniques and as patients demand less invasive procedures. As well as being safer for the patients and more useful for the clinicians. It also improves hospital efficiency by reducing recovery time. Although radioactivity can be

harmful to the human body, it is this very property that makes these metals useful as diagnostic tools.

2.2.3 CHRYSOTHERAPY:

Use of nobel metal compounds in chemotherapy is called crysotherapy. Gold salt have been used in the treatment of rheumatoid arthritis for over forty years .Gold is normally used in the form of complex e.g. sodium aurothiomalate, aurothioglucoseand less commonly aurothiosulphate . [17]

Jessop and Jhon confirmed an older observation that gold salts are more effective, if given early in the course of active rheumatoid disease. Most common complex Na_3 [Au $(S_2O_3)_2$] .$2H_2O$ has been in use as chrysotheraputic agent under the name of "Sanochrysin" (H.Mollgaard) in treatment of tuberculosis. [17]

Many metals other than Nobel metals also used as therapeutic agents.
Silver and its compounds have long been used as antimicrobial agents in medicine. Silver is active at low concentrations and has a low toxicity. The practice of instilling the eyes of infants with 1% $AgNO_3$ solution immediately after birth is still common in some countries for prevention of ophthalmia neonatorum. [20] Silver sulfadiazine, made from AgI and sulfadiazine , is used clinically as an antimicrobial and antifungal agent. It is an insoluble polymeric compound which releases AgI ions slowly, and is applied topically as a cream to prevent bacterial infections in cases of severe burns. .[21]

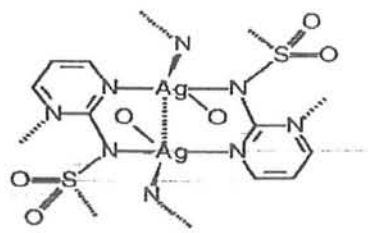


Fig 2.1 Structural Formula of Silver Sulfadiazine

Antimony has been used for medicinal purposes for many centuries. Complexes of Sb (III) are generally more toxic than those of Sb(V.) Two Sb(V) drugs, N-methylglucamine antimonite (Glucantime) and sodium stibogluconate

(Pentostam), are used clinically for the treatment of leishmaniasis, a disease caused by intracellular parasites. [22]

The iron chelate desferrioxamine is clinically approved for the treatment of malaria. Its activity may arise from disruption of Fe(III) metabolism within the digestive vacuole of malarial parasites. [23]

Copper aspirin complex is considered to be the good antinflamatory agent. The therapeutic potency and safety of the copper complexes of aspirin (acetyl salicylic acid) and salicylic acid is much better than for aspirin itself or for inorganic copper . These complexes are 5 to 8 times more effective than aspirin itself but less toxic. Aspirin causes or aggravates peptic ulcers; the copper complexes have a better ulcer healing effect than commonly used anti-inflammatory ulcer drugs. Harmful effects of aspirin and similar drugs apparently arise because they bind copper in the body and cause a localized copper deficiency in the tissues. [23]

2.2.4 METALS IN HUMAN BODY:

Metal perform a verity of task in biological system. Transition metals are also very important of the healthy body to function. These metals are presents in trace amounts in the body and grouped in trace essential elements in human body In this study the trace element i.e. the 3d element V, Cr, Mn, Fe, Ni, Cu, Co ,and Zn have been used for complexation with quinolone antibiotics. [3]

CHROMIUM

It is widely distributed in our body. Infant have high concentration than adult. Most grain and cereal contain significant quantities. Blood Serum level in normal healthy adult is 6- 20 μg/dl. [3]

- **BIOLOGICAL FUNCTIONS**
  - **Role in carbohydrate metabolism**

Chromium is true potentiater of insulin and is known as glucose tolerant factor (GTF) Experiment suggest that it act the first step i.e. cell transport.

- **Role in lipid transport**

It decrease serum cholesterol level and prevent atheromatous plaque formation in aorta.

- **Role in protein metabolism**

It improves amino acid incorporation mainly amino isobutiric acids glycerin, serine and methionin.

➢ CLINICAL SIGNIFICANT

- **Risk of cancer**

Chronic occupational exposure is associated with increase risk of lung cancer. Serum level decrease during pregnancy and acute infection.

NICKEL

➢ SOURCE AND DISTRIBUTION

Nickel occurs in trace amount in human and animal tissues. Nickel dose not accumulate with age in any of human tissues other than lungs. Dietary nickel is poorly absorbed from intestine to the extent of about 1-10%.It is excreted mainly in feces.

➢ BLOOD LEVEL

Blood contain 1.1 –3.6μg per liter of blood.

➢ REQURMENT

The minimum daily requirement of nickel is approximately 20μg per adult.

➢ FUNCTION

- **Role in enzyme action.**

Nickel activates several enzyme systems via arginase corboxylase,trypsin and acetyl coA synthatase.

- **Role in growth and reproduction**

Nickel is required in trace amount for growth and reproduction.

➢ Deficiency Manifestation

Its deficiency causes impaired growth, reproduction and anemia. Ultra structural changes have been observed.

➢ Toxicity and clinical significant

Nickel is relatively non toxic but prolong exposure result in respiratory tract neoplasia, and dermitoses observed in occupational worker.

MANGANESE

➢ SOURCE AND DISTRIBUTION

Manganese is also an essential trace element required by the body The average diet provides approximately 3-4 mg of Manganese. Which is obtained principally from cereal vegetable fruits nut, and tea From animal sources liver and kidney are rich sources. Average amount in our body is 15- 18 mg, mainly found in liver and kidney.[3]

➢ BLOOD LEVEL

Blood contained about 4-100 μg per 100 ml. It present mainly in RBC in combination with several porphrine and is transported in the plasma in combination with β globulin called transemaganine.

➢ FUNCTION

- **Role in enzymes**

Act as cofactor or as an activator of many enzyme like arginase isocitrate dehydrogenase choline estrase lipoprotienlipase enolase,lucine aminopeptidases inintestinne phosphotransferase Zn 5-oxsoprolinases of kidneyand small intestine and many other .

- **Role in animal reproduction**

In animal its deficiency produces sterility, in cattle disturbance of eostrus cycle, and degeneration of testes and inability to feed offspring in rat.

- **Role in bone formation**

Mn play s role in synthesis or deposition of mucopolysachride It deficiency causes significant lowering in content of chondoriten SO_4.Abnormal bone formation due to Mn deficiency may lead to peruses.

- **Role in carbohydrate metabolism**

It is reported to influence carbohydrate metabolism by affecting peripheral utilization and their conversion to MPS.

- **Role in porpharine synthesis**

Some porpharine of RBC contained Mn^{2+}. .Mn also help in porphrine synthesis by participation in α-ALA synthatase activity Hb synthesis appear to be depressed in Mn deficient rat. Mn also act as cofactor of hydrolase and decarboxylase enzymes Mn also participate in glycoprotieneand protieoglycane synthesis.

- **Manganese Toxicity**

Inhalation poisons produces psychotic and Parkinsonism like symptoms

IRON

➢ SOURCE AND DISTRIBUTION

Organ meat such as liver ,spleen, kidney, redmarow, redmusslle, meat, are rich source of iron. It is also found in shellfish, green leafy vegetable, jiggery are rich source of iron. Banana apple and certain other fruit also contained iron.

Iron present inhuman body of about70 Kg weight, amount 3-4 gms. It present in different enzymes and proteins. The prosthetic group heme, which is constituted of certain protein and enzyme, contains Iron.

➢ FUNCTION

- **Transferrin**

It is non heme protein. Apotranferrine is the apoenzyme and Iron is its prosthetic group. It can bind to two atom of Iron in ferric state exist in the plasma as β-globulin. It is true carrier of Iron.

- **Cytochrome**

Cytochrome systemis chiefly present in mitochondria and exist as lipoprotein complex .They are heme containing enzyme and iron containing protein in which iron atom oscillate between Fe^{3+}- Fe^{2+} during oxidation reduction 3 Peroxidases It is typically oxidize enzyme also found in milk and erythrocyte ,leukocyte. Its prosthetic groups protoheme which is loosely bounded to apoprotein e.g. glutahione peroxidase catalyse the destruction of H_2O_2 by reduce ed glutathione ,protecting membrane lipid and Hb against oxidation.

- **Catalase**

It is heme containing enzyme. It contains 4 heme groups. It is found in blood, bone marrow, mucus membrane kidney and liver

- **Ferritin**

It is non heme iron containing protein, Apoferritin is the apoprotein .It can take up to 4300 atoms to form ferritin This is storage protein of iron found in blood ,liver and spleen Iron is in +3 oxidation state.

➢ Dietary Requirement

Adult----------10 mg / day

Pregnant woman----------40 mg / day

Lactating woman ----------20 mg / day

Children ----------10-15 mg / day

COBALT

➢ Source and requirement

Cobalt form an integral part of vitamin B_{12}. normal average diet contain about 5-8 μg of cobalt .(1-2 μg of vitamin B_{12} approximately 0.045-0.09μg of cobalt.

➢ FUNCTION

- **Role in the formation of cobamide enzyme**

In the formation of cobamide enzyme (Adenosyl co enzyme) Cobalt of B_{12} under go successive reduction.

$B_{12\,\alpha}$ ----------red color Co^{+++}

↓

B_{12} γ----------Orange colored Co^{++}

↓

B_{12} γ----------Gray green.

This last one reacts with ATP, form adenosylcoenzyme.

- **Bone marrow function**

Cobalt is required to maintain normal bone marrow function and for development and maturation of RBC. A deficiency of Co result in decreased B12 supply and produces nutritional anemia. While on the other hand, excess causes over production of RBC causing polycythemia. Cobalt may act as co factor enzyme like glycyl-glycine dipeptidases of intestinal juices.

2.3 Ligands

Ligands are the species which donate a pair of electrons to the central metal ion to form coordination complexes. These may be elements like N , O ,F etc or they may be organic compounds that contain these elements in their structures. Coordination complexes formed by the organic molecules with metals are most important species form the therapeutic point of view.

Most of the drugs are organic in nature and have certain donating atoms like O,F,N in their structures . So these can also act as potential ligands for formation of metal complexes.

In the present study we have used the drugs "Ciprofloxacin" and "Norfloxacin". Both of these two drugs belong to quinolone family of antibiotics.

The main features of the ligands are following

2.3.1 CIPROFLOXACIN [24,32]

Name: Ciprofloxacin

Trade products

1. Ciprox (Wilson)
2. Cipro (Bayer)
3. Medicip Tab (Vision)
4. Olax Inj (Vision)

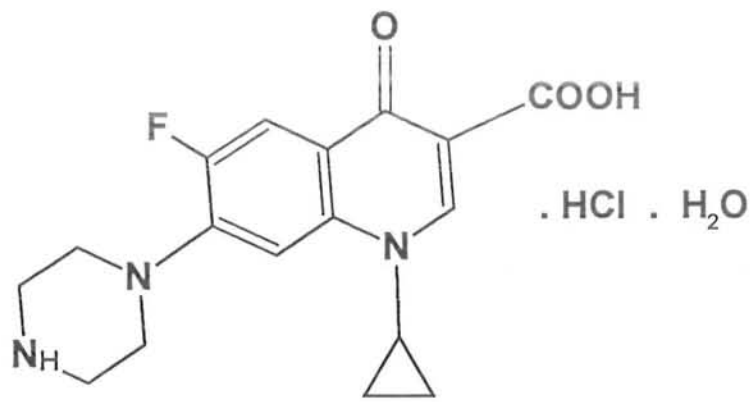
Empirical Formula:

$C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$

Chemical Name

1-cyclopropyl-6-fluoro-1, 4- dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

Structural Formula



Formula Weight: 385.8.

Appearance: It is off-white powder.

Solubility: It is soluble in water, DMSO and acetic acid. It is insoluble in ethanol, THF and acetonitrile

Melting point / Decomposition point 252 C^{O} . It decomposed on its melting point

INDICATIONS AND USAGE:

Ciprofloxacin is indicated for the treatment of infections caused by susceptible strains of the designated microorganisms in the conditions and patient populations listed below.

Adult Patients:

- Urinary Tract Infections
- Acute Uncomplicated Cystitis in females
- Chronic Bacterial Prostatitis
- Lower Respiratory Tract Infections
- Acute Sinusitis
- Skin and Skin Structure Infections
- Bone and Joint Infections
- Complicated Intra-Abdominal Infections
- Infectious Diarrhea
- Typhoid Fever (Enteric Fever)
- Uncomplicated cervical and urethral gonorrhea

Pediatric patients (1 to 17 years of age):

Complicated Urinary Tract Infections and Pyelonephritis ,

Adult and Pediatric Patients:

Inhalational anthrax (post-exposure):

MICROBIOLOGICAL APPLICATIONS:

Ciprofloxacin has been shown to be active against most strains of the following microorganisms, both *in vitro* and in clinical infections.

Aerobic gram-positive microorganisms:

- *Enterococcus faecalis* (Many strains are only moderately susceptible.)
- *Staphylococcus aureus* (methicillin-susceptible strains only)
- *Staphylococcus epidermidis* (methicillin-susceptible strains only)
- *Staphylococcus saprophyticus*
- *Streptococcus pneumoniae* (penicillin-susceptible strains only)
- *Streptococcus pyogenes*

Aerobic gram-negative microorganisms

- *Campylobacter jejuni*
- *Proteus mirabilis*
- *Citrobacter diversus*
- *Proteus vulgaris*
- *Citrobacter freundii*
- *Providencia rettgeri*
- *Enterobacter cloacae*
- *Providencia stuartii*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Haemophilus influenzae*
- *Salmonella typhi*
- *Haemophilus parainfluenzae*
- *Serratia marcescens*
- *Klebsiella pneumoniae*
- *Shigella boydii*
- *Moraxella catarrhalis*
- *Shigella dysenteriae*
- *Morganella morganii*
- *Shigella flexneri*

Ciprofloxacin exhibits *in vitro* minimum inhibitory concentrations (MICs) of 1 µg/mL or less against most (≥90%) strains of the following microorganisms; however, the safety and effectiveness of ciprofloxacin in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials.

Aerobic gram-positive microorganisms

- *Staphylococcus haemolyticus*
- *Staphylococcus hominis*
- *Streptococcus pneumoniae* (penicillin-resistant strains only)

Aerobic gram-negative microorganisms

- *Acinetobacter Iwoffi*
- *Pasteurella multocida*
- *Aeromonas hydrophila*
- *Salmonella enteritidis*
- *Edwardsiella tarda*
- *Vibrio cholerae*
- *Enterobacter aerogenes*
- *Vibrio parahaemolyticus*
- *Klebsiella oxytoca*
- *Vibrio vulnificus*
- *Legionella pneumophila*
- *Yersinia enterocolitica*

SIDE EFFECTS:

The following side effects have been observed:

- **Effects on gastro-intestinal tract**:
  Nausea, diarrhea, vomiting digestive disorders.
- **Effects on Nervous system**:
  Dizziness, headache ,tiredness ,insomnia , agitation, trembling, very rarely peripheral paralrgesia sweating , convulsions ,anxiety states, nightmares, confusion, depression, visual disturbance,
- **Hypersensitivity reaction:**
  Skin reaction i.e. rashes
  Pruritus, Drug fever
- **Effects on Cardiovascular system:**
  Vary rarely tachycardia, hot flushes, migraine, fainting

2.3.2 NORFLOXACIN[33-34]

Name: Norfloxacin

Trade products

1. NOROXIN Tab 400 mg (MSD Pharma)
2. NICAFLEX Tab 400mg (Arco pharma)
3. NOLACIN Tab 400mg (Novartis)
4. NEW SADAL tab 400 mg (Neutro Pharma)
5. NORACIN Tab 400 mg (Bosch Pharma)
6. FLOXCIN (Hilton pharma).
7. BACTINAR (Remington Pharma)
8. URID Tab 400 mg (Bloom pharma)

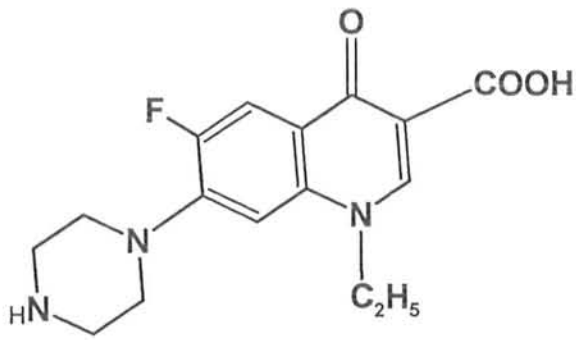
Empirical Formula:

$C_{16}H_{18}FN_3O_3$

Chemical Name

1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid.

Structural Formula



Formula Weight: 319.33 g /mol

Appearance: Norfloxacin is a white to pale yellow crystalline powder

Solubility: It is freely soluble in glacial acetic acid, and very slightly soluble in ethanol, methanol and water.

Melting point 221 C°

INDICATIONS AND USAGE

Norfloxacin is indicated for the treatment of adults with the following infections caused by susceptible strains microorganisms:

- Used In Urinary Tract Infection and Polynephritis,
- Cystitis, Pyelitis, Cystopyelitis,
- In Typhoid Fever.
- Respiratory Tract Infection.
- Eyes Infection
- Bacterial Infection of Gastrointestinal and Bilary Tract.
- Skin and Soft Tissue
- Bone and Joints.
- Sexually Transmitted Diseases Including Gonorrhea

MICROBIOLOGICAL APPLICATIONS:

Norfloxacin has *in vitro* activity against a broad range of gram-positive and gram-negative aerobic bacteria. The fluorine atom at the 6 position provides increased potency against gram-negative organisms, and the piperazine moiety at the 7 position is responsible for antipseudomonal activity. Norfloxacin inhibits bacterial deoxyribonucleic acid synthesis and is bactericidal. At the molecular level, three specific events are attributed to Norfloxacin in *E. coli* cells:

1) Inhibition of the ATP-dependent DNA supercoiling reaction catalyzed by DNA gyrase,
2) Inhibition of the relaxation of supercoiled DNA,
3) Promotion of double-stranded DNA breakage.

Resistance to Norfloxacin due to spontaneous mutation *in vitro* is a rare occurrence (range: 10–9 to 10-12 cells). Resistant organisms have emerged during therapy with Norfloxacin in less than 1% of patients treated. Organisms in which development of resistance is greatest are the following:

- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*
- *Acinetobacter* spp.
- *Enterococcus* spp.

There is generally no cross-resistance between Norfloxacin and other classes of antibacterial agents. Therefore, Norfloxacin may demonstrate activity against

indicated organisms resistant to some other antimicrobial agents including the aminoglycosides, penicillins, cephalosporins, tetracyclines, macrolides, and sulfonamides, including combinations of sulfamethoxazole and trimethoprim. Antagonism has been demonstrated *in vitro* between Norfloxacin and nitrofurantoin. Norfloxacin has been shown to be active against most strains of the following microorganisms both *in vitro* and in clinical infections

Gram-positive aerobes:

- *Enterococcus faecalis*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus saprophyticus*
- *Streptococcus agalactiae*
- **Gram-negative aerobes:**
- *Citrobacter freundii*
- *Enterobacter aerogenes*
- *Enterobacter cloacae*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Neisseria gonorrhoeae*
- *Proteus mirabilis*
- *Proteus vulgaris*
- *Pseudomonas aeruginosa*
- *Serratia marcescens*

The following *in vitro* data are available, but their clinical significance is unknown. Norfloxacin exhibits *in vitro* minimal inhibitory concentrations (MIC's) of $\leq$ 4 μg/mL against most ($\geq$90%) strains of the following microorganisms; however, the safety and effectiveness of Norfloxacin in treating clinical infections due to these microorganisms have not been established in adequate and well-controlled clinical trials.

Gram-negative aerobes:

- *Citrobacter diversus*
- *Edwardsiella tarda*
- *Enterobacter agglomerans*

- *Haemophilus ducreyi*
- *Klebsiella oxytoca*
- *Morganella morganii*
- *Providencia alcalifaciens*
- *Providencia rettgeri*
- *Providencia stuartii*
- *Pseudomonas fluorescens*
- *Pseudomonas stutzeri*

SIDE EFFECTS

Hypersensitivity Reactions

Hypersensitivity reactions have been reported including anaphylactoid reactions, angioedema, dyspnea, vasculitis, urticaria, arthritis, arthralgia and myalgia

Skin

Toxic epidermal necrolysis, Stevens-Johnson syndrome and erythema multiforme, exfoliative dermatitis, photosensitivity

Gastrointestinal

Pseudomembranous colitis, hepatitis, jaundice including cholestatic jaundice and elevated liver function tests, pancreatitis (rare), stomatitis. The onset of pseudomembranous colitis symptoms may occur during or after antibacterial treatment.

Renal

Interstitial nephritis, renal failure

Nervous System/Psychiatric

Peripheral neuropathy, Guillain-Barré syndrome, ataxia, paresthesia; psychic disturbances including psychotic reactions and confusion

Musculoskeletal

Tendinitis, tendon rupture; exacerbation of myasthenia gravis

Hematological

Neutropenia, leukopenia, hemolytic anemia, sometimes associated with glucose-6-phosphate dehydrogenase deficiency; thrombocytopenia

Special Senses

Transient hearing loss (rare), tinnitus, diplopia, dysgeusia

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CHAPTER #3

CHARACTERIZATION

3.1 SPECTROPHOTOMETRY

Spectroscopy is the study of interactions between matter and electromagnetic radiation. It is the use of the absorption, emission, or scattering of electromagnetic radiation by matter to qualitatively or quantitatively study the matter or to study physical processes. The matter can be atoms, molecules, atomic or molecular ions, or solids. The interaction of radiation with matter can cause redirection of the radiation and/or transitions between the energy levels of the atoms or molecules. Different types of spectroscopy are concerned with:

- Various regions of the electromagnetic spectrum (e.g. X-rays, UV light, infrared radiation)
- Properties of the matter with which the interactions occur (e.g. molecular vibration, electron transitions)

The physical interactions involved (i.e. scattering, absorption or emission of radiation).

- **Absorption**: A transition from a lower level to a higher level with transfer of energy from the radiation field to an absorber, atom, molecule, or solid.
- **Emission**: A transition from a higher level to a lower level with transfer of energy from the emitter to the radiation field. If no radiation is emitted, the transition from higher to lower energy levels is called nonradiative decay.
- **Scattering**: Redirection of light due to its interaction with matter. Scattering might or might not occur with a transfer of energy, i.e., the scattered radiation might or might not have a slightly different wavelength compared to the light incident on the sample.

3.1.1 INFRA RED SPECTROSCOPY [41]

INTRODUCTION

The term "infra red" covers the range of the electromagnetic spectrum between 0.78 μm and 1000 μm. In the context of infra red spectroscopy, wavelength is measured in "wave numbers", which have the units' cm^{-1}.

Wave number = 1 / wavelength in centimeters

It is useful to divide the infra red region into three sections; *near*, *mid* and *far* infra red;

Region	Wavelength range (μm)	Wavenumber range (cm^{-1})
Near	0.78 - 2.5	12800 - 4000
Middle	2.5 - 50	4000 - 200
Far	50 -1000	200 – 10

Table 3.1 The IR Region Of Electromagnetic Spectrum

The most useful I.R. region lies between 4000 - 670cm^{-1}.

THEORY OF INFRA RED ABSORPTION:

IR radiation does not have enough energy to induce electronic transitions as seen with UV. Absorption of IR is restricted to compounds with small energy differences in the possible vibrational and rotational states.

For a molecule to absorb IR, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. The alternating electrical field of the radiation (electromagnetic radiation consists of an oscillating electrical field and an oscillating magnetic field, perpendicular to each other) interacts with fluctuations in the dipole moment of the molecule. If the frequency

of the radiation matches the vibrational frequency of the molecule then radiation will be absorbed, causing a change in the amplitude of molecular vibration.

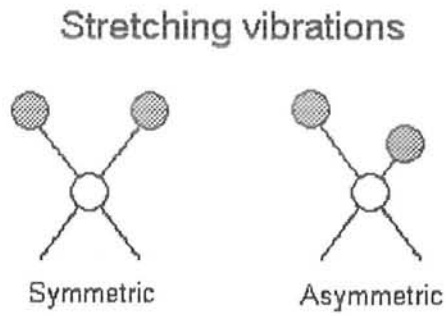
MOLECULAR ROTATIONS:

Rotational levels are quantized, and absorption of IR by gases yields line spectra. However, in liquids or solids, these lines broaden into a continuum due to molecular collisions and other interactions.

MOLECULAR VIBRATIONS:

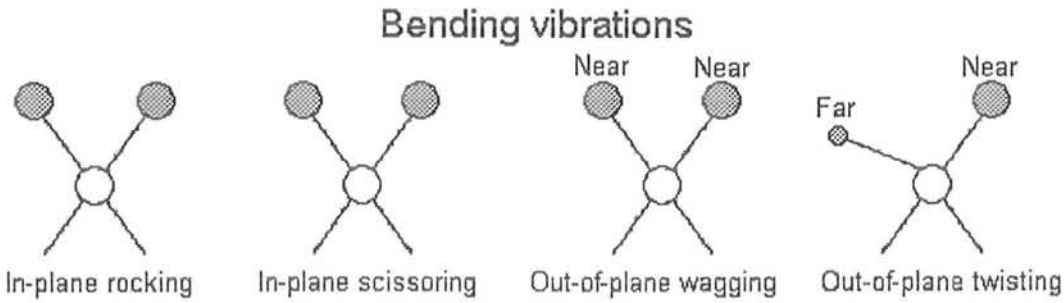
The positions of atoms in a molecule are not fixed; they are subject to a number of different vibrations. Vibrations fall into the two main categories of *stretching* and bending.

Stretching: Change in inter-atomic distance along bond axis



Bending: Change in angle between two bonds. There are four types of bend:

- Rocking
- Scissoring
- Wagging
- Twisting



VIBRATIONAL COUPLING:

In addition to the vibrations mentioned above, interaction between vibrations can occur (coupling) if the vibrating bonds are joined to a single, central atom. Vibrational coupling is influenced by a number of factors;

- Strong coupling of stretching vibrations occurs when there is a common atom between the two vibrating bonds
- Coupling of bending vibrations occurs when there is a common bond between vibrating groups
- Coupling between a stretching vibration and a bending vibration occurs if the stretching bond is one side of an angle varied by bending vibration
- Coupling is greatest when the coupled groups have approximately equal energies
- No coupling is seen between groups separated by two or more bonds

Major parts of an infra red spectrometer is given in the schematic diagram below

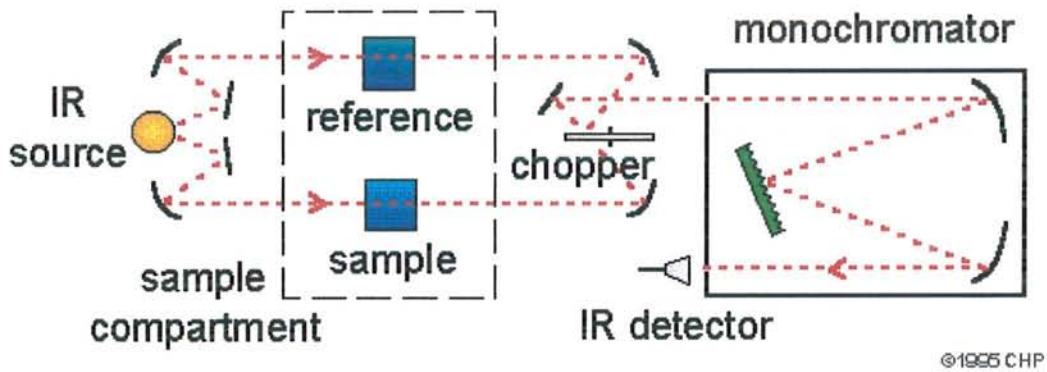


Fig. 3.1 Schematic Diagram of Diagram of IR Spectrometer

3.1.2 ATOMIC ABSORPTION SPECTROSCOPY

Dr. Alan Walsh of the CSIRO Division of Chemical Physics developed this method in the 1950's, and it is now a universally popular analytical technique.

Atomic absorption spectroscopy may be defined as a method for determining the concentration of an element in a sample by measuring the intensity of external radiation absorbed by atoms produced from a sample at a wavelength characteristic for that element. Soil scientists have used spectrochemical methods to determine elemental contents of soil digests, soil extracts and plant digests for many years. Early research related elemental soil content with measurement of essentials plant nutrients to determine their soil chemistry and to make appropriate fertility recommendations. Flame emission spectrometry was successfully used to make analytical determinations of K, Ca and Mg. The basic principles of flame atomic absorption were discovered since 1860, but it was until 1960 that the analytical potential of atomic absorption was widespread used and extended to micronutrients and nonessential elements that can affect crop growth and animal health. In the last 20 years, with the introduction and development of furnace atomic absorption, the use of these systems has greatly increased the knowledge to determine trace levels in the soil environment.

It exploits the narrowness of atomic absorption lines to avoid the necessity to separate a complex mixture prior to the analysis of its components. Of course, the conversion of a sample to its atomic constituents in AAS means that it is only a method of elemental analysis, and, for various practical reasons, it is essentially suitable for analysis only of metals. Nonetheless, a very large number of elements can be analyzed for at trace levels, and AAS has a very wide range of applications

PRINCIPLE OF AAS:

The sample solution is aspirated into flame and sample and the sample element is converted to atomic vapors [36] The flame then contains atoms of that element. Some are thermally excited by the flame, but most remain in the ground state. These ground state atoms can absorb radiation given off by a special source made from that element.

The wavelengths of radiation given off by the source are the same as those absorbed by the atoms in flame. Atomic absorption spectrophotometry is identical in principle to absorption spectrophotometry. The absorption follows Beer's law. That is absorption is directly proportional to path length in the flame and to the concentration of atomic vapor in the flame. Both of these variables are difficult to determine, but the path length can be held constant and the concentration of atomic vapor is directly proportional to the concentration of the analyte in the solution being aspirated. The procedure used is to prepare calibration curve of concentration in the solution versus absorbance. [37, 38]

The major disadvantage of making measurements by atomic absorption is that a different source is required for each element.

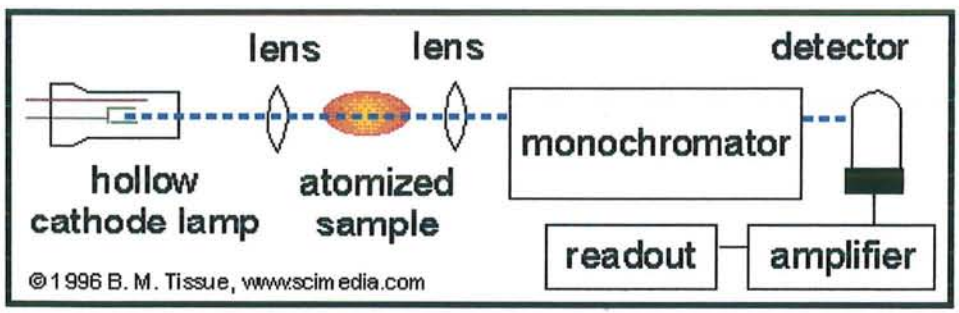


Fig 3.2 Schematic Diagram of Atomic Absorption Spectrophotometer

3.2 ANTIBIOTIC SUSCEPTIBILITY TEST

INTRODUCTION:

When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition".

PRINCIPLE

Antiseptics, disinfectants and antibiotics are used in different ways to combat microbial growth. Antiseptics are used on living tissue to remove pathogens. Disinfectants are similar in use but are used on inanimate objects. Antibiotics are substances produced by living organisms, such as Penicillium or Bacillus, that kill or inhibit the growth of other organisms, primarily bacteria. Many antibiotics are chemically altered to reduce toxicity, increase solubility, or give them some other desirable characteristic that they lack in their natural form. Other substances have been developed from plants or dyes and are used like antibiotics. A better term for these substances is antimicrobials, but the term antibiotic is widely used to mean all types of antimicrobial chemotherapy. Many conditions can affect a disc diffusion susceptibility test. When performing these tests certain things are held constant so only the size of the zone of inhibition is variable. Conditions that must be constant from test to test include the agar used, the amount of organism used, the concentration of chemical used, and incubation conditions (time, temperature, and atmosphere). The amount of organism used is standardized using a turbidity standard. This may be a visual approximation using a McFarland standard 0.5 or turbidity may be determined by using a spectrophotometer (optical density of 1.0 at 600 nm). For antibiotic susceptibility testing the antibiotic concentrations are predetermined and commercially available. Each test method has a prescribed media to be used and incubation is to be at 35-37C ° in ambient air for

18-24 hours. The disc diffusion method for antibiotic susceptibility testing is the Kirby- Bauer method. The depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. There is also a zone of intermediate resistance indicating that some inhibition occurs using this antimicrobial but it may not be sufficient inhibition to eradicate the organism from the body. The standardized methods for antiseptic and disinfectant testing are more rigorous and more difficult to reproduce in a student laboratory. Two common tests are the Phenol Coefficient Test (a comparison of the effect of the chemical and phenol on several organisms) and the Use Dilution Test (testing the chemical under actual conditions of use). A disc diffusion test can be used to approximate the Use Dilution Test. The chemical under consideration is used to saturate a filter paper disc. This disc is then used to introduce the chemical to the agar for testing. The actual zone sizes have not been standardized as in the Kirby-Bauer method, but a comparison of zone sizes for the same chemical among organisms will provide a n approximate effectiveness of the chemical.

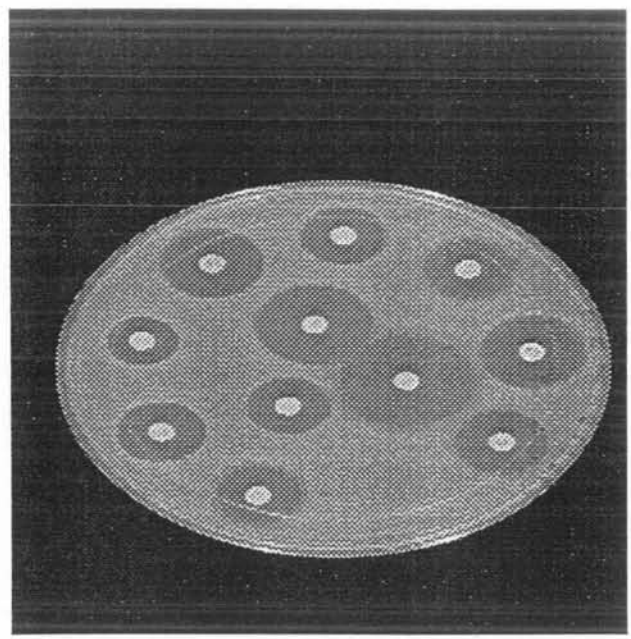


Fig. 3.3 Zone Of Inhibition of Antibiotic in a Cultured Media by Disc Diffusion Technique

CHAPTER #4

EXPERIMENTAL

4.1 MATERIALS AND METHODS

Ciprofloxacin & Norfloxacin in pure powder form (99.9 %) was obtained from local drug manufacturing company. Identification and purification was rechecked using UV spectrophotometery at wavelength of 273nm

All the reagents used were analytical grade reagents and obtained from Merck and BDH company .

IR analysis was performed on Bio Rad FTIR . and spectra was obtained using the software "Bio Rad"

Metal analysis was carried out on ANALYTIC JENA model "AAS VARIO 6" atomic absorption spectrometer at Explosives Factory Lab P O F Wah Cantt. Wet digestion method was used for the preparation of sample solutions. Calibration curve method was used for analysis.

4.2 SYNTHESIS OF NORFLOXACIN METAL COMPLEX

4.2.1 SYNTHESIS OF COPPER COMPLEX WITH NORFLOXACIN

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring To this solution 0.5 m mol $Cu(NO_3)_2.6H_2O$ salt (89mg) dissolved in 50 ml of methanol separately. Then added in above solution of Norfloxacin .The color of solution changed immediately to light green due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.2 SYNTHESIS OF NICKEL COMPLEX WITH NORFLOXACIN

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring To this solution 0.5 m mol $Ni(NO_3)_2.6H_2O$ salt (145mg) dissolved in 50 ml of methanol separately. Then added in above solution of

Norfloxacin .The color of solution changed immediately to parrot green due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.3 SYNTHESIS OF ZINC COMPLEX WITH NORFLOXACIN

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring To this solution 0.5 m mol Zn $(NO_3)_2.6H_2O$ salt (148mg) dissolved in 50 ml of methanol separately. Then added in above solution of Norfloxacin The color of solution changed immediately to white due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.4 SYNTHESIS OF IRON COMPLEX WITH NORFLOXACIN.

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring To this solution 0.5 m mol $FeCl_3$.$6H_2O$ salt (198mg) dissolved in 50 ml of methanol separately. Then added in above solution of Norfloxacin. The color of solution changed immediately to reddish yellow due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.5 SYNTHESIS OF CHROMIUM COMPLEX WITH NORFLOXACIN

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring To this solution 0.5 m mol $CrCl_3$.$6H_2O$ salt (133mg) dissolved in 50 ml of methanol separately. Then added in above solution of Norfloxacin. The color of solution changed immediately to dark green due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.6 SYNTHESIS OF MANGNESE COMPLEX WITH NORFLOXACIN

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring To this solution 0.5 m mol $MnCl_2 .2H_2O$ salt (80mg) dissolved in 50 ml of methanol separately. Then added in above solution of Norfloxacin. The color of solution changed immediately to yellow due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.7 SYNTHESIS OF COBALT COMPLEX WITH NORFLOXACIN

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring To this solution 0.5 m mol $CoCl_2 .6H_2O$ salt (136mg) dissolved in 50 ml of methanol separately. Then added in above solution of Norfloxacin. The color of solution changed immediately to pink due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.8 SYNTHESIS OF ZIRCONIUM COMPLEX WITH NORFLAXACIN:

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring To this solution 0.5 m mol $Zr(NO_3)_2 .5H_2O$ salt (152mg) dissolved in 50 ml of methanol separately. Then added in above solution of Norfloxacin. The color of solution changed immediately to yellow due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.9 SYNTHESIS OF COPPER COMPLEX WITH NORFLOXACIN AND 2, 2′ BIPYRIDYL

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring 0.5 m mol $Cu(NO_3)_2.6H_2O$ salt (89mg) dissolved in 10 ml of methanol separately. 0.5 m mol (80mg) 2, 2′ bipyridyl dissolved in 10 ml methanol Then mix all these three solutions by constant stirring and heating The

color of solution changed immediately to green due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature.

4.2.10 SYNTHESIS OF ZIRCONIUM COMPLEX WITH NORFLOXACIN AND 2, 2′ BIPYRIDYL

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring 0.5 m mol $Zr(NO_3)_2 .5H_2O$ salt (152mg) dissolved in 10 ml of methanol separately. 0.5 m mol (80mg) 2, 2′ bipyridyl dissolved in 10 ml methanol .Then mix all these three solutions by constant stirring and heating The color of solution changed immediately to light yellow due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.2.11 SYNTHESIS OF MANGANESE COMPLEX WITH NORFLOXACIN AND 2, 2′ BIPYRIDYL

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring 0.5 m mol $MnCl_2 .2H_2O$ salt (80mg) dissolved in 10 ml of methanol separately. 0.5 m mol (80mg) 2, 2′ bipyridyl dissolved in 10 ml methanol .Then mix all these three solutions by constant stirring and heating The color of solution changed immediately to light yellow due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature.

4.2.12 SYNTHESIS OF IRON COMPLEX WITH NORFLOXACIN AND 2, 2′ BIPYRIDYL

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring 0.5 m mol $FeCl_3 .6H_2O$ salt (198mg) dissolved in 10 ml of methanol separately. 0.5 m mol (80mg) 2, 2′ bipyridyl dissolved in 10 ml methanol .then mix all these three solutions during constant stirring and heating The color of solution changed immediately to reddish yellow due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature.

4.2.13 SYNTHESIS OF COBALT COMPLEX WITH NORFLOXACIN AND 2, 2′ BIPYRIDYL

1 m mol of Norfloxacin (319 mg) dissolved in HPLC grade 50 ml of methanol by continues heating and stirring 0.5 m mol $CoCl_2.6H_2O$ salt (136mg) dissolved in 10 ml of methanol separately. 0.5 m mol (80mg) 2, 2′ bipyridyl dissolved in 10 ml methanol .then mix all these three solutions during constant stirring and heating the color of solution changed immediately to pink due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature.

4.3 SYNTHESIS OF CIPROFLOXACIN METAL COMPLEX

4.3.1 SYNTHESIS OF IRON COMPLEX WITH CIPROFLOXACIN

1 m mol of ciprofloxacin (365 mg) dissolved in 50 ml of distilled water by continues heating and stirring To this solution 0.5 m mol $FeCl_3 .6H_2O$ salt (198mg) dissolved. The color of solution changed immediately to reddish yellow due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.3.2 SYNTHESIS OF CHROMIUM COMPLEX WITH CIPROFLOXACIN

1 m mol of ciprofloxacin (365 mg) dissolved 50 ml of distilled water by continues heating and stirring To this solution 0.5 m mol $CrCl_3 .6H_2O$ salt (133mg) dissolved . The color of solution changed immediately to dark green due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.3.3 SYNTHESIS OF VANADYL COMPLEX WITH CIPROFLOXACIN

0.5m mol.V_2O_5 (91mg) dissolved in 0.5 molar NaOH solution by continues stirring and heating. 1 m mol of ciprofloxacin (365 mg) dissolved in above solution. The color of solution changed immediately to light yellow due to formation of complex. Product was obtained in powder form by evaporation of solvent at room temperature

4.3.4 SYNTHESIS OF COPPER COMPLEX WITH CIPROFLOXACIN AND 2, 2′ BIPYRIDYL

Bpy (0.5 m mol) (80mg) dissolved in ethanol (10mL) was added to an aqueous solution (10 mL) containing $Cu(NO_3)_2 \cdot 6H_2O$ (0.5 m mol). (89mg) To this mixture was then added a solution prepared by dissolving ciprofloxacin .HCl (1mmol) (365mg) in water (100 mL) containing NaOH (2 m mol); the pH was adjusted to

7.0 to 8.0 with HNO_3 and NaOH. The resulting blue solution was slowly evaporated at room temperature, and blue powder was formed finally after a few days

4.3.5 SYNTHESIS OF NICKEL COMPLEX WITH CIPROFLOXACIN AND 2, 2 BIPYRIDYL

Bpy (0.5 mmol) (80mg) dissolved in ethanol (10mL) was added to an aqueous solution (10 mL) containing 0.5 m mol $Ni(NO_3)_2.6H_2O$ salt (145mg) To this mixture was then added a solution prepared by dissolving ciprofloxacin HCl (1mmol) (365mg) in water (100 mL) containing NaOH (2 mmol); the pH was adjusted to 7.0 to 8.0 with HNO_3 and NaOH. The resulting blue solution was slowly evaporated at room temperature, and parrot green powder was formed finally after a few days.

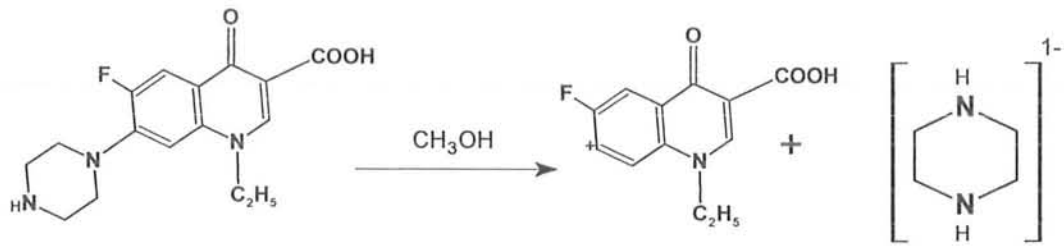
CHAPTER #5

RESULTS & DISCUSION

Ciprofloxacin and Norfloxacin are the most important members of flouroquinolone family of antibiotics. This group of medicine is used worldwide as broad spectrum antibiotics. The metal complexes of ciprofloxacin and Norfloxacin have been made using 1:2 ratios of metal and medicine.Mixed ligand metal complexes using 2,2′bipyridyl and Ciprofloxacin or Norfloxacin are also prepared Metals of first transition metal series are used and characterized using FT-IR spectrophotometry and Atomic absorption spectrophotometry. The biological activity of complex compounds has also been observed against different strains of bacteria.

POSSIBLE SYNTHETIC REACTION

During the reaction, the piperazine ring on Norfloxacin librated in the methanol solution,. Figure shows the possible synthetic reactions taking place. Further experimental research is required to confirm the intermediate products [42]



Norfloxacin **Intermediate** **Pip**

Two molecules of Norfloxacin combine with metal through coordinate covalent binds and structure is formed which is shown in the fig 5.1

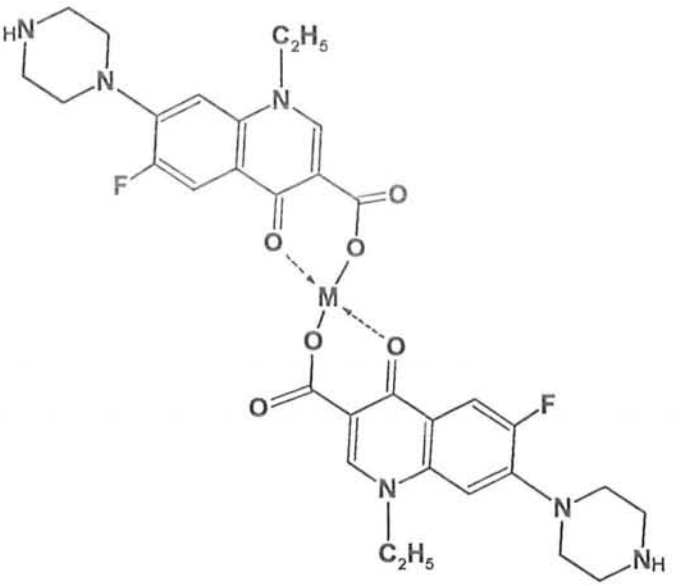


Fig. 5.1 Proposed Structure Of Metal Complexes Of Norfloxacin In 1:2 Ratio

Similar kind of structure is also purposed for ciprofloxacin metal complexes because there are similar kinds of groups are present in Ciprofloxacin.

In 2, 2′ bipyridyl complexes bpy ligand replaces the pip species and a structure is formed which is given in the fig 5.2

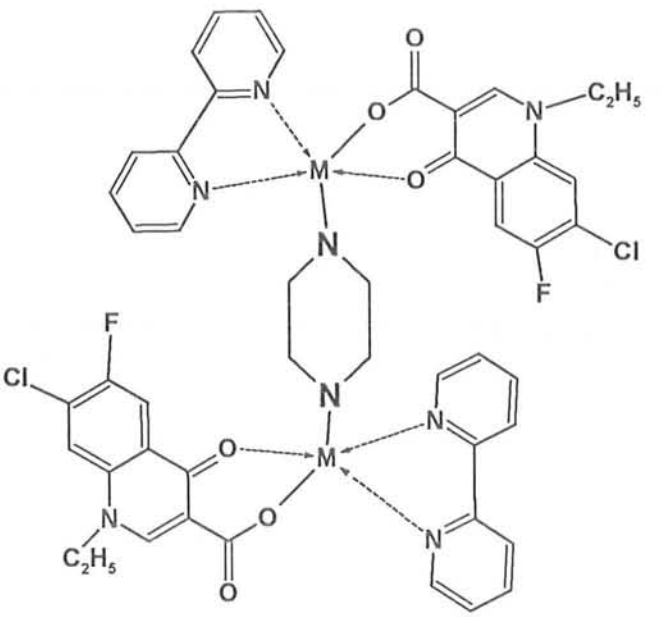


Fig. 5.2 Proposed Structure Of Metal Complexes Of Norfloxacin and 2, 2′ Bipyridyl

For characterization IR spectroscopy was used. Most important region of IR spectra was interpreted and tabulated in the Tables No 6.1 and 6.2 .A representative IR spectrum is explained as follows:

The most important region in IR spectra of flouroquinolone is 1800 to1300 cm^{-1}. The band responsible for carboxylic group of Norfloxacin appears at 1731 cm-1.and 1250cm^{-1} but it disappears in the spectrum of metal complexes, which indicate that carboxylic acid is definitely taking part in complex formation. The disappearance of 1731cm^{-1} due to carboxylic acid (C=O) in complex suggest that the coordination of carboxylic oxygen after protonation [44]

If the carbonyl group is taking part in the bonding to the metal we would expect shift of starching to lower wavelength it is not possible to distinguish the carbonyl stretching corresponding to carboxylic group and carbonyl group. Peak at 1620 $cm^{-1}$is assigned for carbonyl group of ketoene in the spectrum of Norfloxacin. In spectrum of Zn Norfloxacin a band appears near to this band at 1630 cm^{-1}.this peak is either for ketone group or for the carboxylic group bonded to metal atom. [44, 45]

If this band is due to ketone group the antisymmetric and symmetric modes of the carboxylic group would account for the bands at 1567 $cm^{-1}$and 1489 cm^{-1}respectively. However, if the ketone group participated in the bonding to the metal we would expect a shift of its stretching band towards lower wave numbers, thus corresponding to the band recorded at 1567 cm^{-1}, and the bands at 1630cm^{-1} and 1489 cm^{-1}would correspond to the carboxylic group.

The above discussion supports the purposed structures. The other metal complexes have shown similar types of shifts. The bipyridyl complexes have also similar kind of behavior.

Ciprofloxacin metal complexes have also sown the similar kind of IR spectra .this is because the groups which are involved in the bonding with metal are similar to that of Norfloxacin.

Metal analysis of the complexes was done by using atomic absorption spectroscopy and results are tabulated in the table no 6.3. The results are similar to that of calculated on the bases of proposed structure and it supports the purposed structure

The antibiotic potency test against different micrograms was carried out for metal complexes and zone of inhibition was measured. It is compared with the starting materials (Ciprofloxacin, Norfloxacin)

The microbiological analysis shows that Norfloxacin complexes have almost equal inhibitory effect. The giving dose contains less amount of active Norfloxacin which is a good sign in sense that its side effect would be reduced.

Comparatively the zone of inhibition of Zr Norfloxacin Bpy complex and Co Norfloxacin (20mm) is similar to the Norfloxacin (20mm) against *E.coli.*

Zone of inhibition of Cr Norfloxacin complex (30mm) is greater than the Norfloxacin (25mm) against *Staphlus coccus aurius*

Zone of inhibition of Co Norfloxacin bpy (19mm) is greater than the Norfloxacin (18mm)against. *Salmonella typhae*

Zone of inhibition of Co Norfloxacin complex (20mm) is similar to the Norfloxacin (20mm)against *Bascillus subtilus*

Zone of inhibition of Cu ciprofloxacin bpy complex (20mm), Cr ciprofloxacin (20mm) and Vanadyl ciprofloxacin is similar to ciprofloxacin (22mm) against *Staphlus coccus aurius*

Zone of inhibition of Cr ciprofloxacin complex (19mm) is similar to ciprofloxacin (19mm). against *Bascillus subtilus.*

Zone of inhibition of Cr ciprofloxacin complex (21mm), greater than ciprofloxacin (19mm) against . *Salmonella typhae.*

CONCLUSION:

The metal complexes of ciprofloxacin and Norfloxacin were made. The proposed structures of these complexes have supported with IR analysis and atomic absorption analysis. Complexes have shown good antibacterial properties against many microorganisms. This was an attempt to prepare new potential medicinal compounds to cope with the problem of antibiotic resistance. It also gives new dimension s to the development of antibiotic.

CHAPTER #6

TABLES & GRAPHS

Table No 6.1

FT-IR Data of Norfloxacin Metal Complexes

Sr No	*Complexes*	*COO-* ν *(C=O)* cm^{-1}	*COO⁻* ν *(C-O)* cm^{-1}	ν_a *(COO-)* cm^{-1}	ν_s *(COO-)* cm^{-1}	*Ring C=O* ν *(C=O)r* cm^{-1}
1	**Norfloxacin (ligand)**	1731	1250	_	_	1620
2	**Fe Nor**			1630	1464	1516
3	**Zr Nor**			1632	1489	1527
4	**Mn Nor**			1631	1493	1574
5	**Mn Nor Bpy**			1613	1489	1572
6	**Cr Nor**			1631	1479	1520
7	**Cu Nor Bpy**			1612	1480	1572
8	**Ni Nor Bpy**			1620	1490	1580
9	**Zn Nor**			1630	1489	1567
10	**Fe Nor Bpy**			1630	1466	1528
11	**Co Nor Bpy**			1626	1491	1566

Table No 6.2

FT-IR Data of Ciprofloxacin Metal Complexes

Sr No	*Complexes*	*COO- ν (C=O) cm^{-1}*	*COO- ν (C-O) cm^{-1}*	*ν_a (COO-) cm^{-1}*	*ν_s (COO-) cm^{-1}*	*Ring C=O $\nu (C=O)_r$ cm^{-1}*
1	Ciprofloxacin (ligand)	1708	1269	–	–	1625
2	Fe Cip			1634	1474	1567
3	VO Cip			1628	1487	1580
4	Mn Cip			1631	1493	1576
5	Cr Cip			1631	1474	1518
6	Cu Cip Bpy			1624	1481	1582
7	Ni Cip Bpy			1626	1483	1576

Table No 6.3

Metal Contents in Prepared Complexes by AAS

Serial No	*Sample code*	*% Metal calculated*	*% Metal found*
1	Cr Cip	7.30	7.09
2	Cr Nor	7.53	6.45
3	Mn Nor	7.92	6.94
4	Mn Nor Bpy	11.35	10.55
5	Fe Cip	7.77	6.65
6	Ni Nor Bpy	12.03	11.66
7	Zn Nor	9.29	8.45
8	Fe Nor Bpy	11.51	9.44
9	Cu Nor Bpy	12.90	11.95

Table No 6.4

Biological Activities of Norfloxacin Metal Complexes against Various Strains of Microorganisms

Sr No	Complex compounds	Microorganisms (Zone Of Inhibition in mm)			
		Staphlus cocus aurius	*Bascillus subtilus*	*Salomonala typhea*	*E .colli*
1	Norfloxacin	25	20	18	20
2	Fe Norfloxacin	23	20	17	19
3	Co Norfloxacin	25	18	18	20
4	Ni Norfloxacin	20	18	17	18
5	Cu Norfloxacin	21	19	18	18
6	Cr Norfloxacin	30	18	16	16
7	Zn Norfloxacin	22	16	18	18
8	Mn Norfloxacin	22	18	18	18
9	Zr Norfloxacin	20	17	18	19
10	Co Norfloxacin Bipyridyl	22	17	19	18
11	Mn Norfloxacin Bipyridyl	21	17	18	18
12	Zr Norfloxacin Bipyridyl	21	17	19	20
13	Cu Norfloxacin Bipyridyl	20	17	18	17
14	Fe Norfloxacin Bipyridyl	20	18	16	16

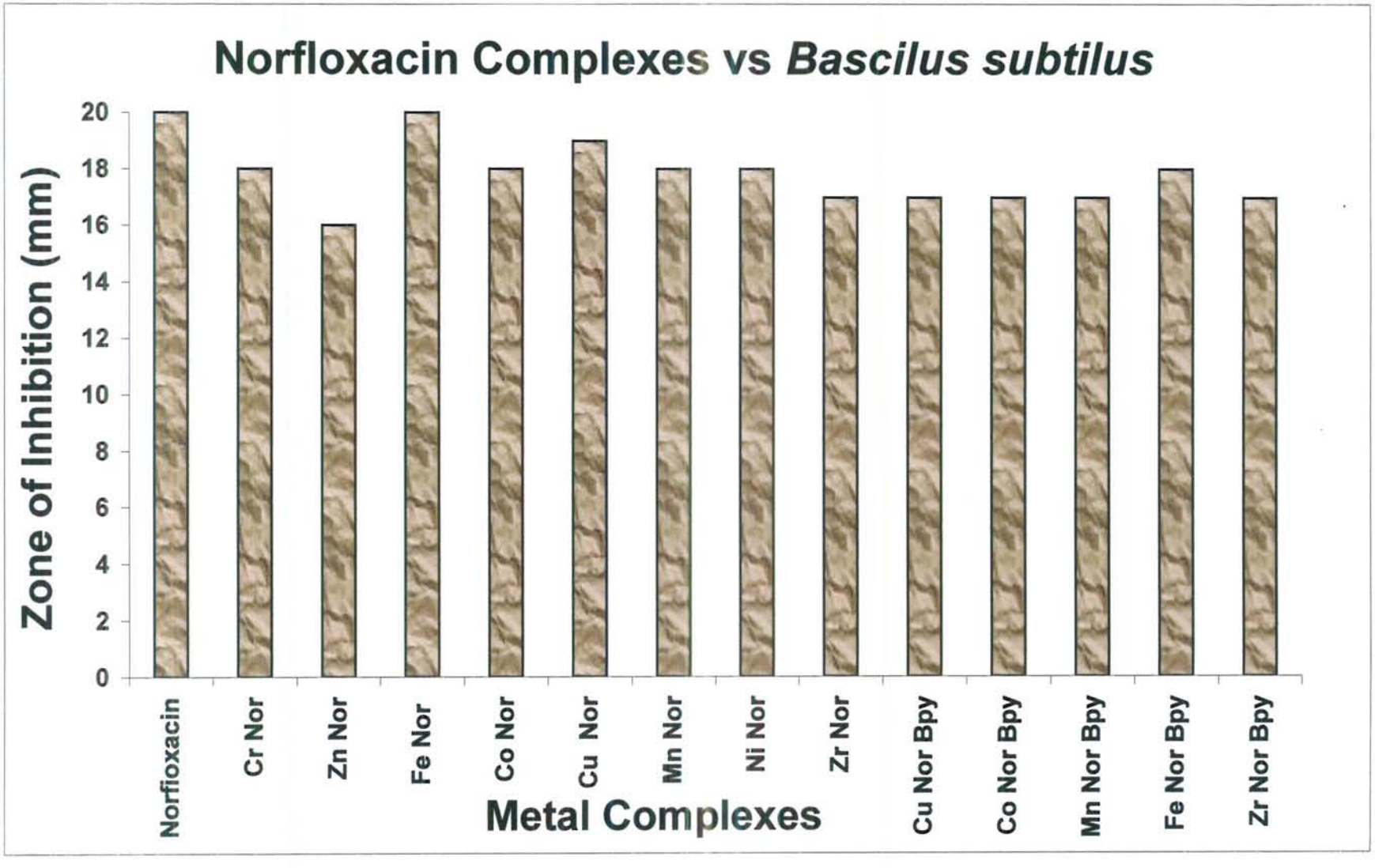
Table No 6.5

Biological Activities of Ciprofloxacin Metal Complexes against Various Strains of Microorganisms

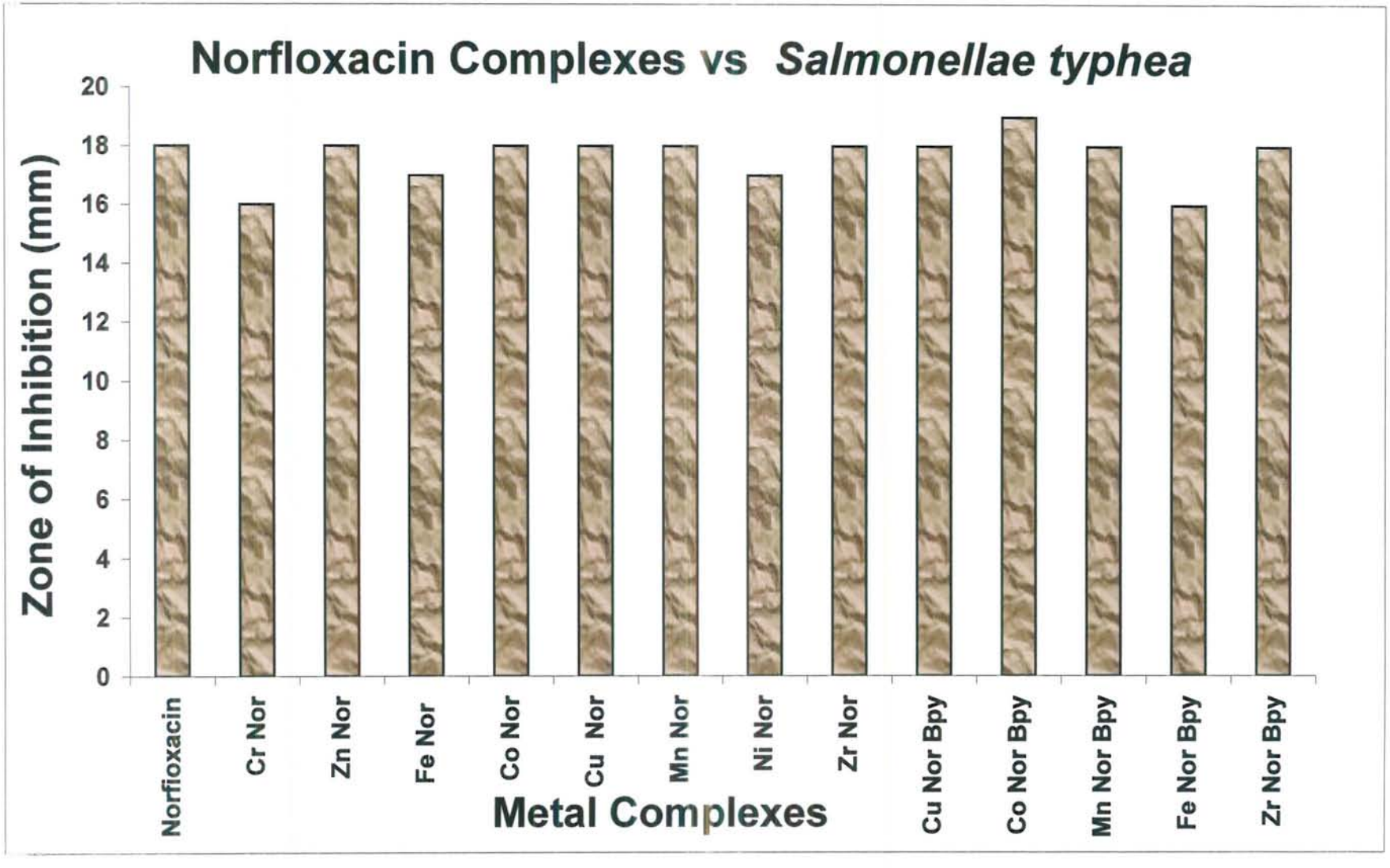
Sr No	Complex compounds	Microorganisms (Zone of inhibition in mm)			
		Staphlus cocus aurius	*Bascillus subtilus*	*Salomonala typhea*	*E .colli*
1	Ciprofloxacin	20	19	19	26
2	Fe ciprofloxacin	19	18	19	24
3	VO ciprofloxacin	20	18	20	20
4	Cr ciprofloxacin	20	19	21	21
5	Cu ciprofloxacin Bipyridyl	20	18	20	22
6	Ni ciprofloxacin Bipyridyl	16	17	16	16

Graph 6.1

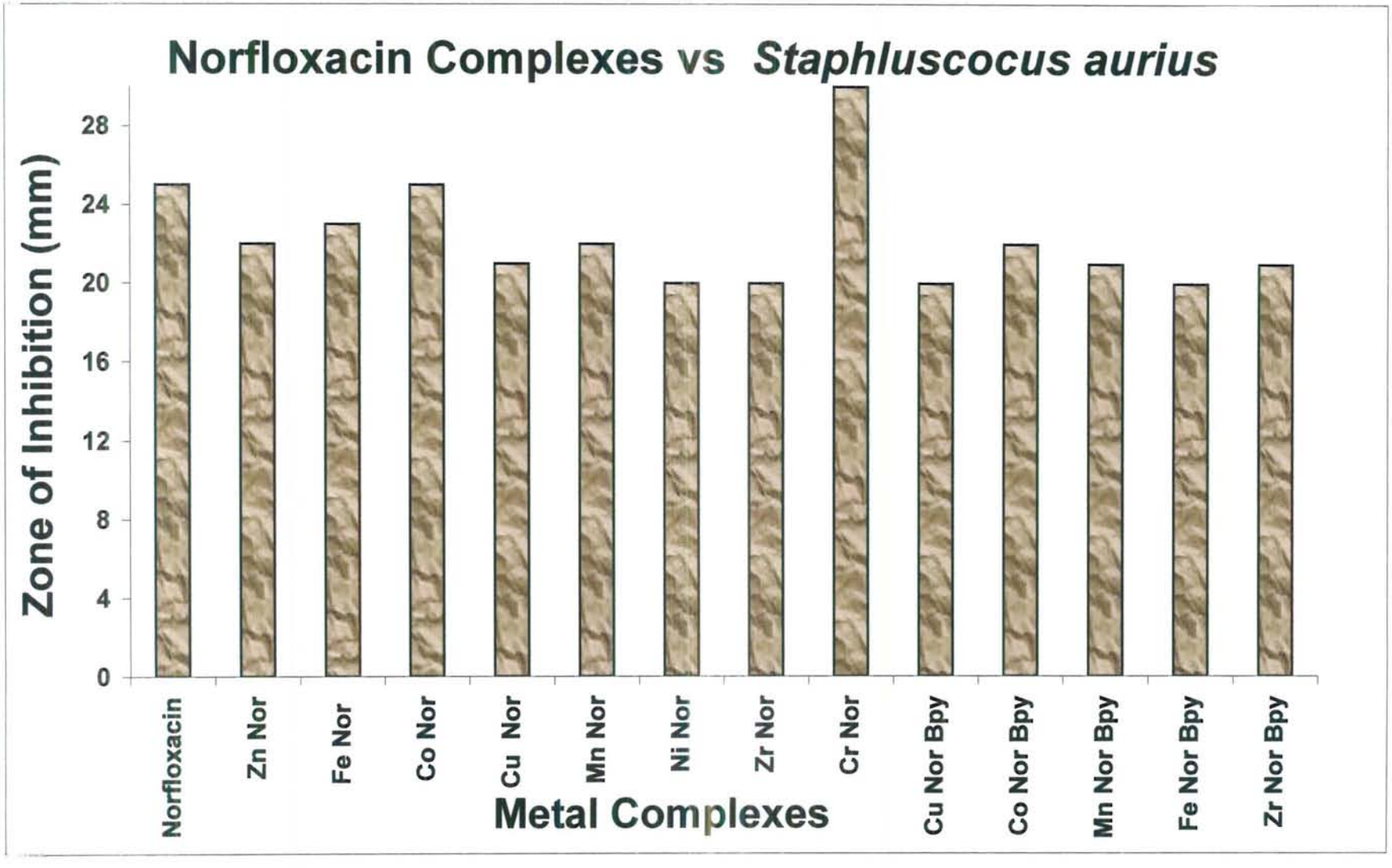
Norfloxacin Metal Complexes Vs *Bascilus subtilus*



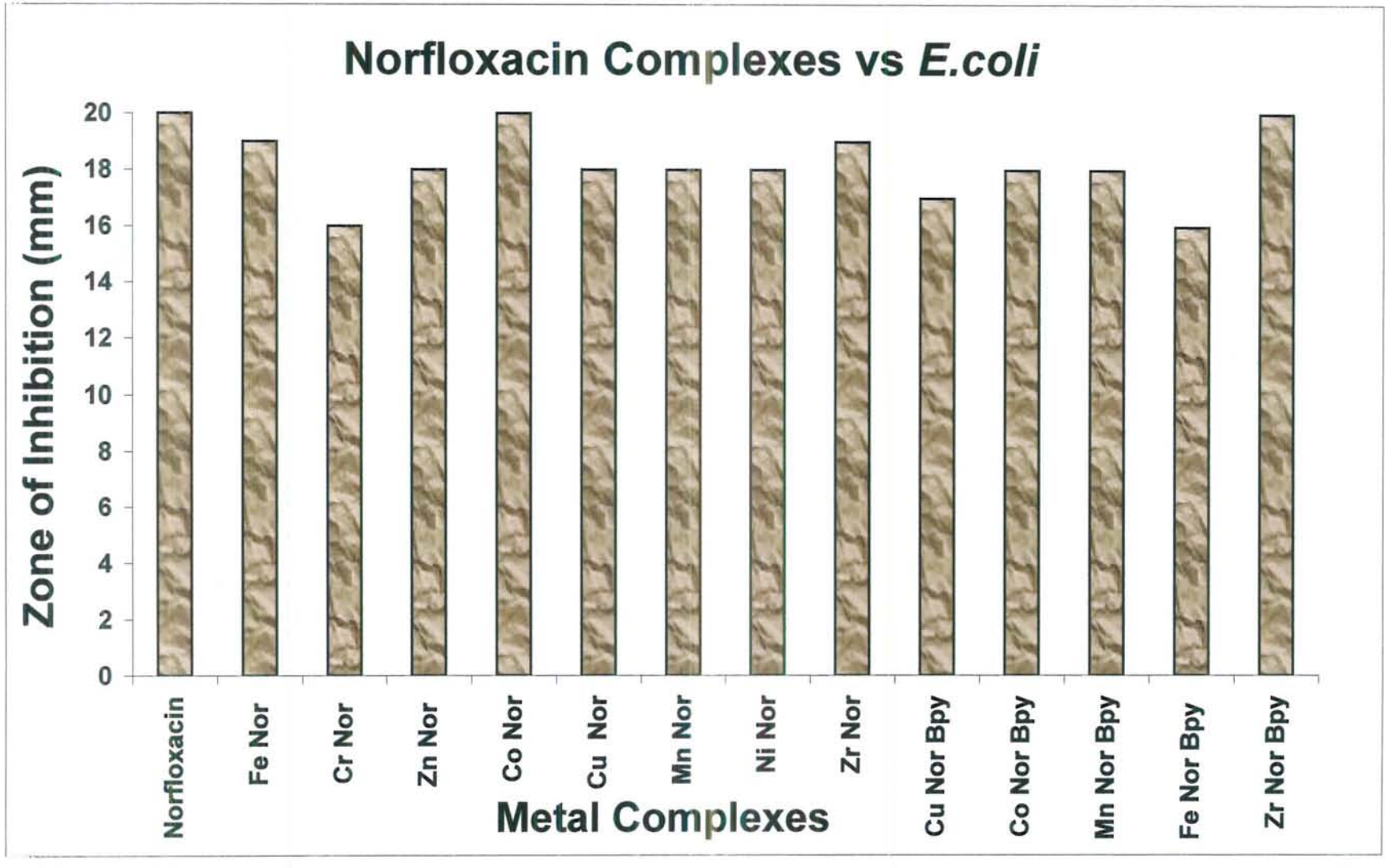
Graph 6.2
Norfloxacin Metal Complexes Vs *Salmonellae typhea*



Graph 6.3
Norfloxacin Metal Complexes Vs *Staphluscocus aurius*

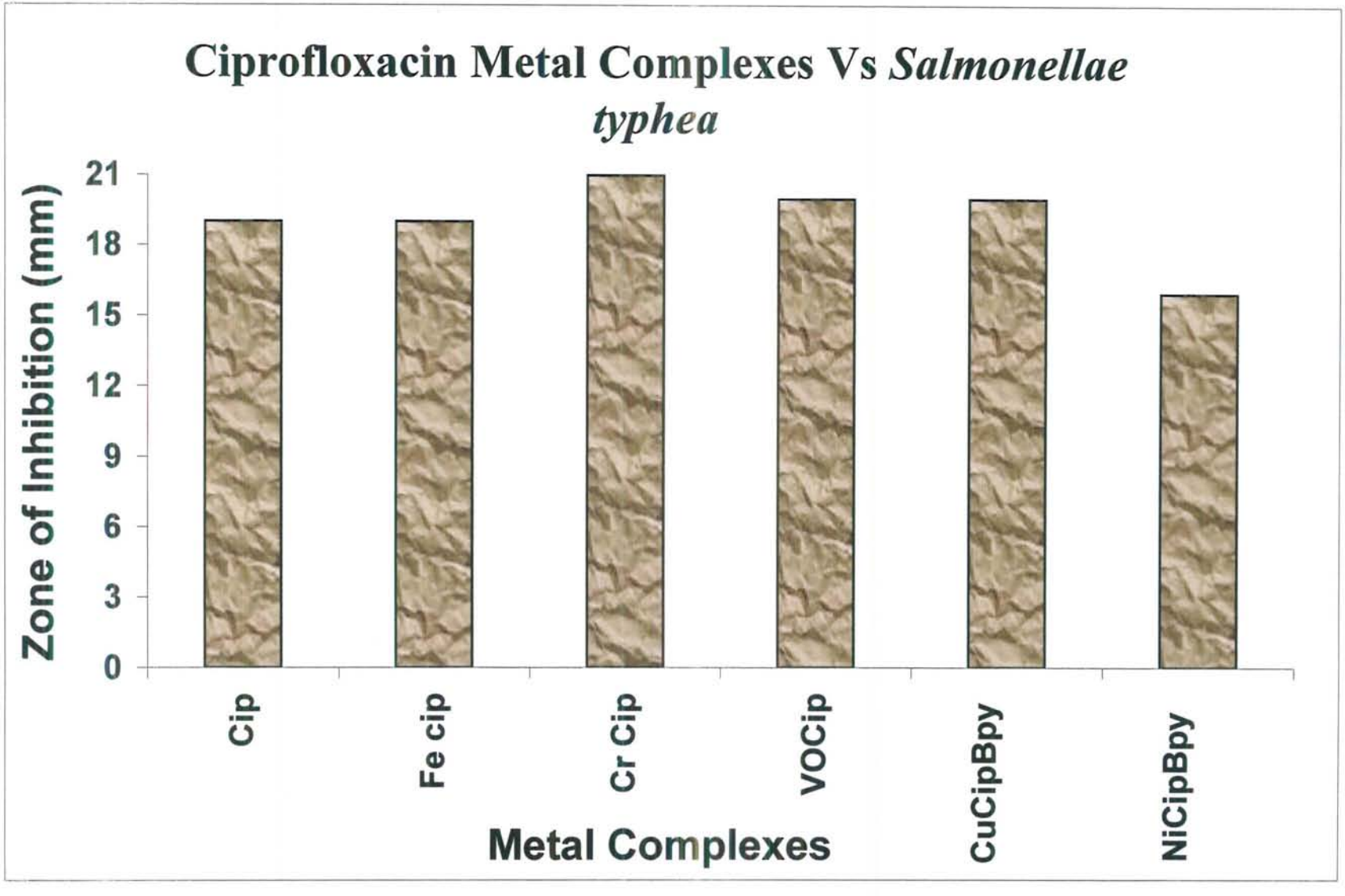


Graph 6.4
Norfloxacin Metal Complexes Vs *E Coli*

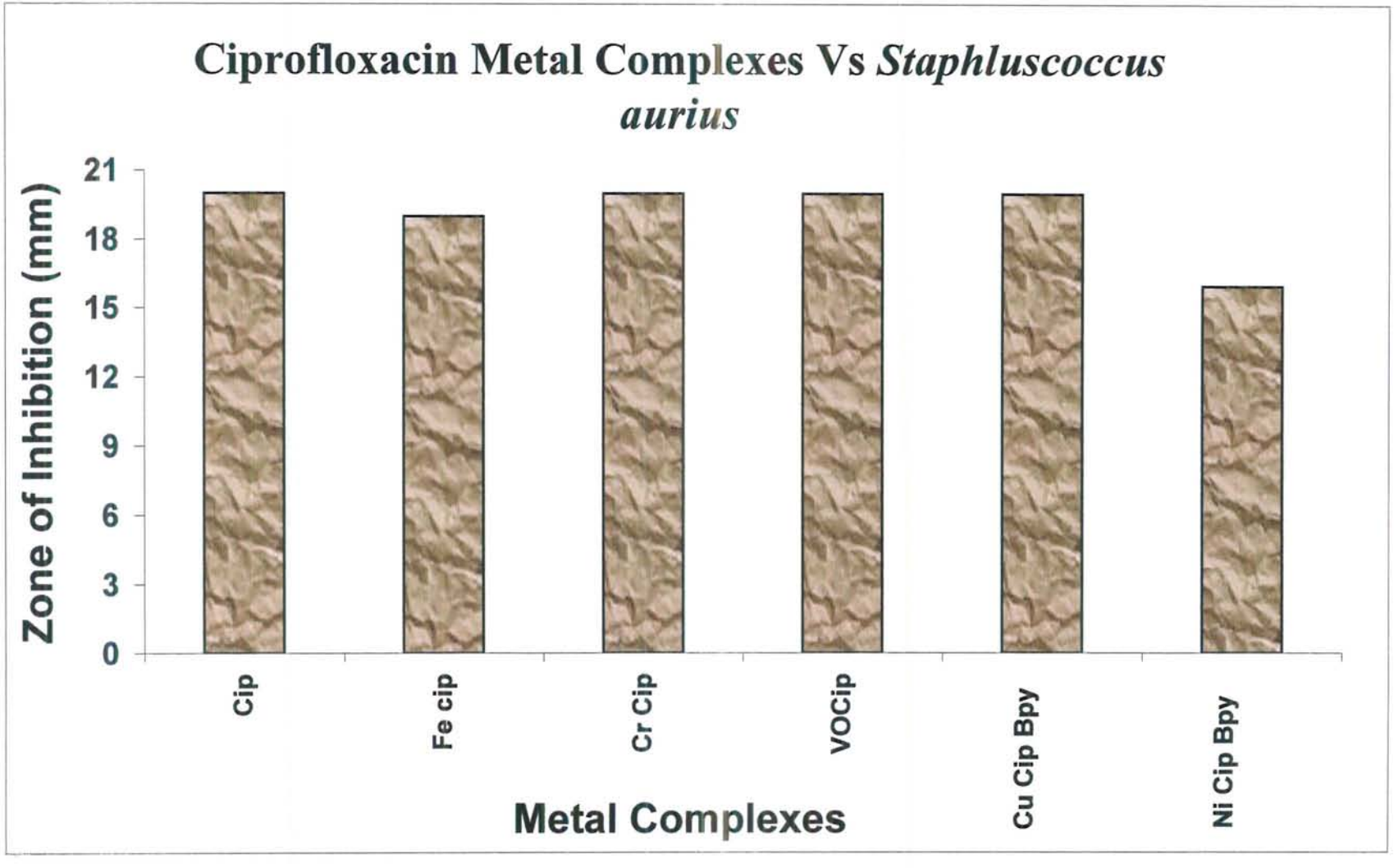


Graph 6.5

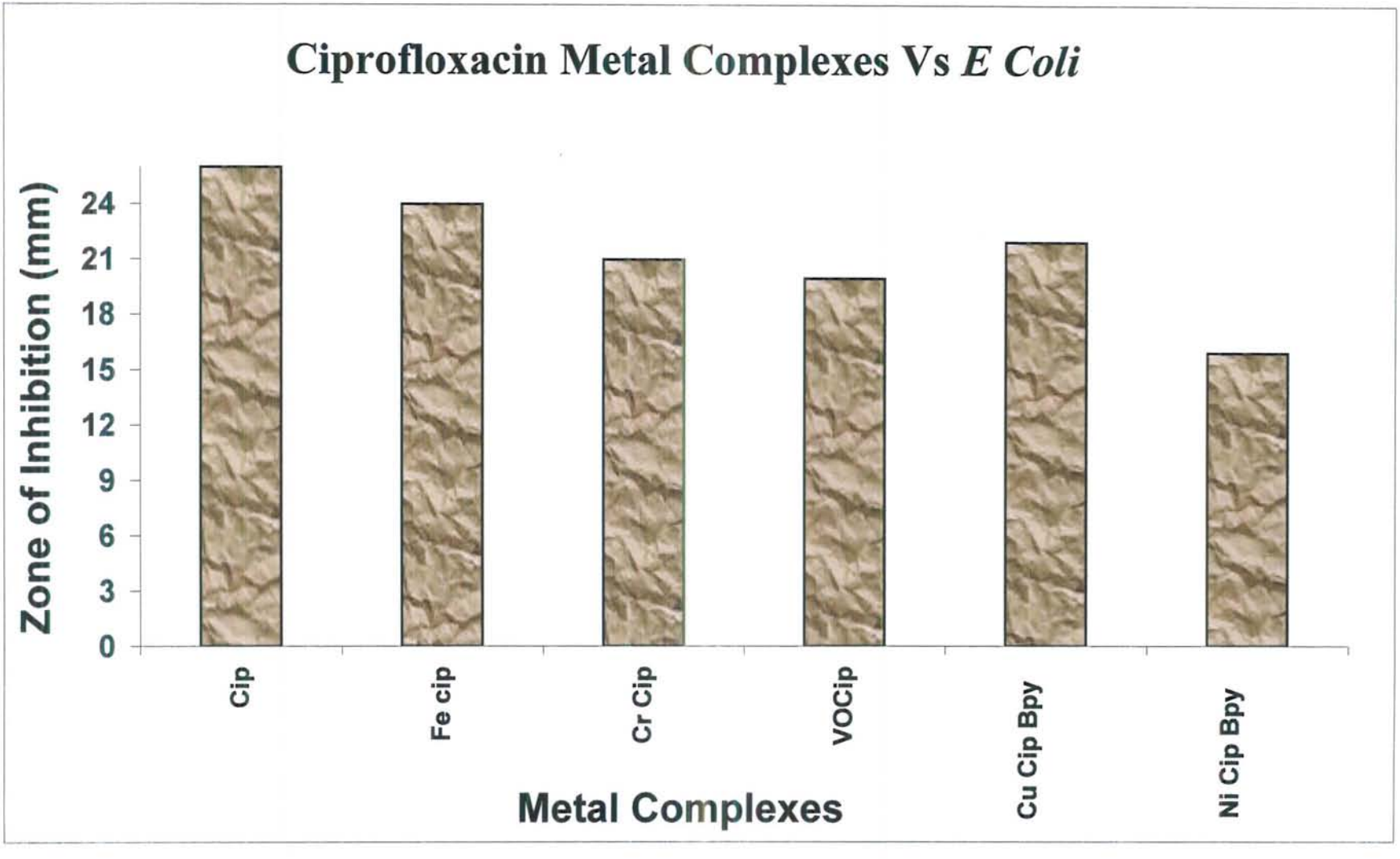
Ciprofloxacin Metal Complexes Vs *Salmonellae Typhea*



Graph 6.6
Ciprofloxacin Metal Complexes Vs *Staphluscoccus aurius*



Graph 6.7
Ciprofloxacin Metal Complexes Vs *E Coli*



Graph 6.8

Ciprofloxacin Metal Complexes Vs *Bascilus subtilus*

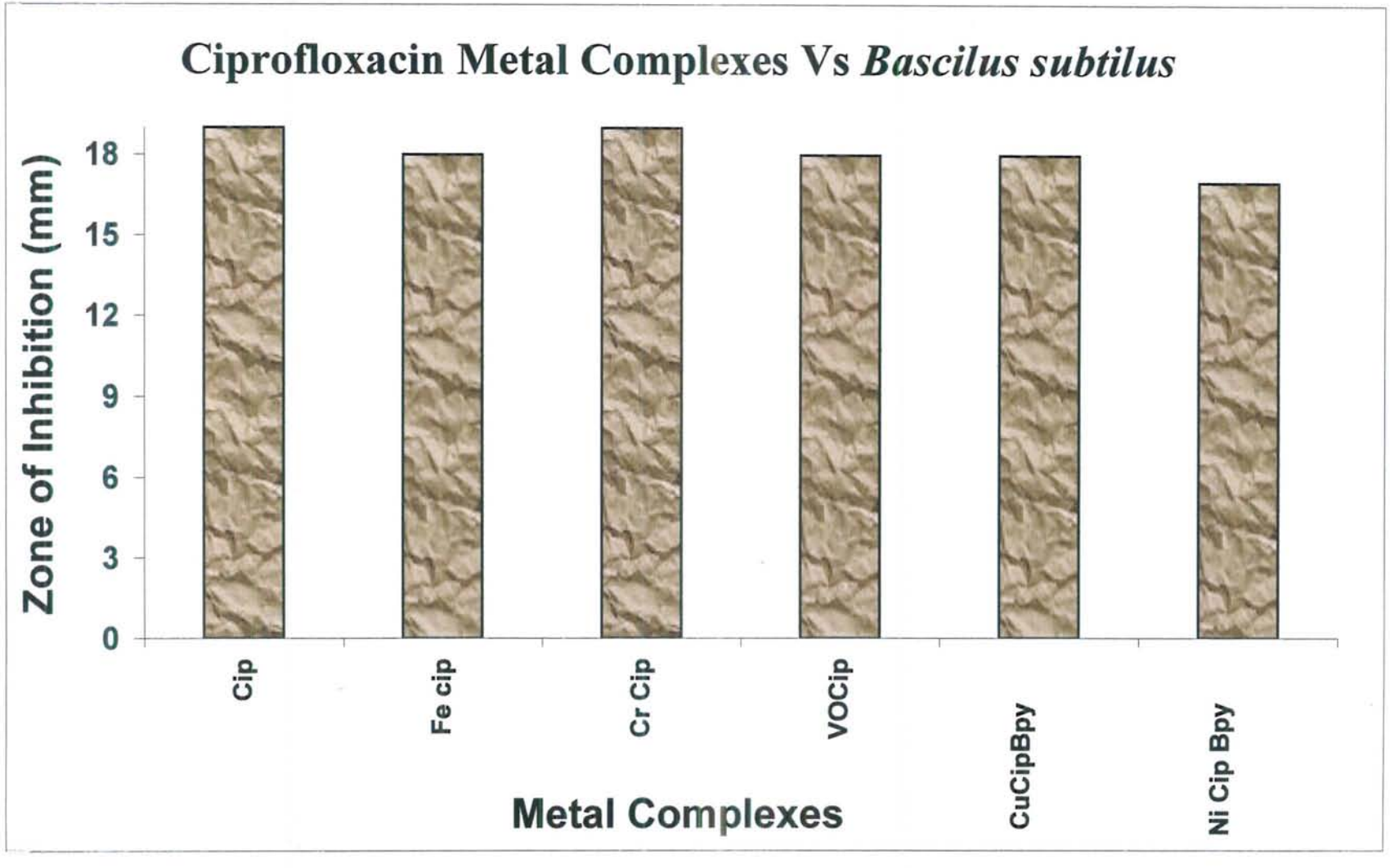


Fig 6.1
FT-IR Spectrum of Ciprofloxacin

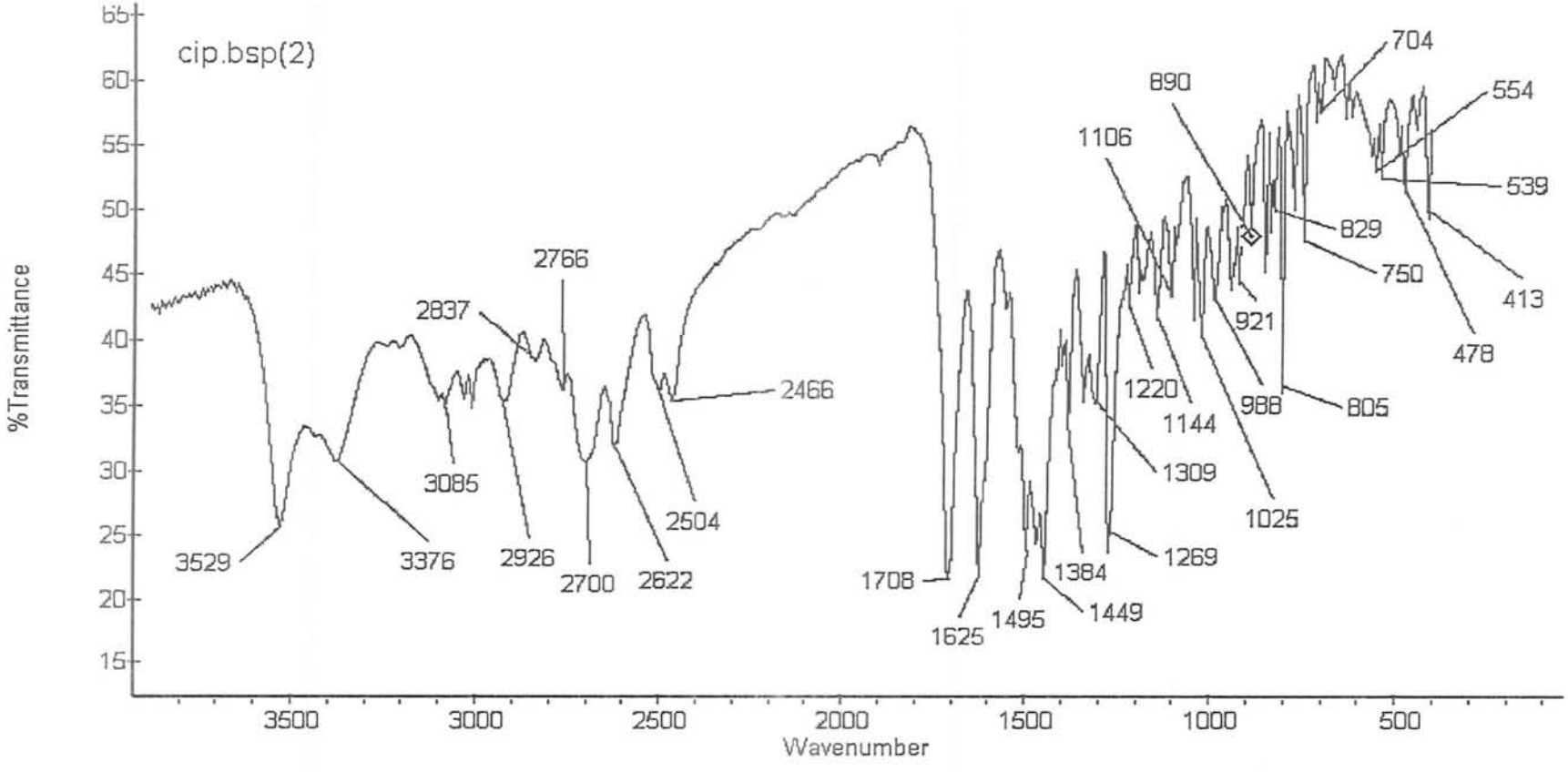


Fig 6.2
FT-IR Spectrum of Iron Ciprofloxacin

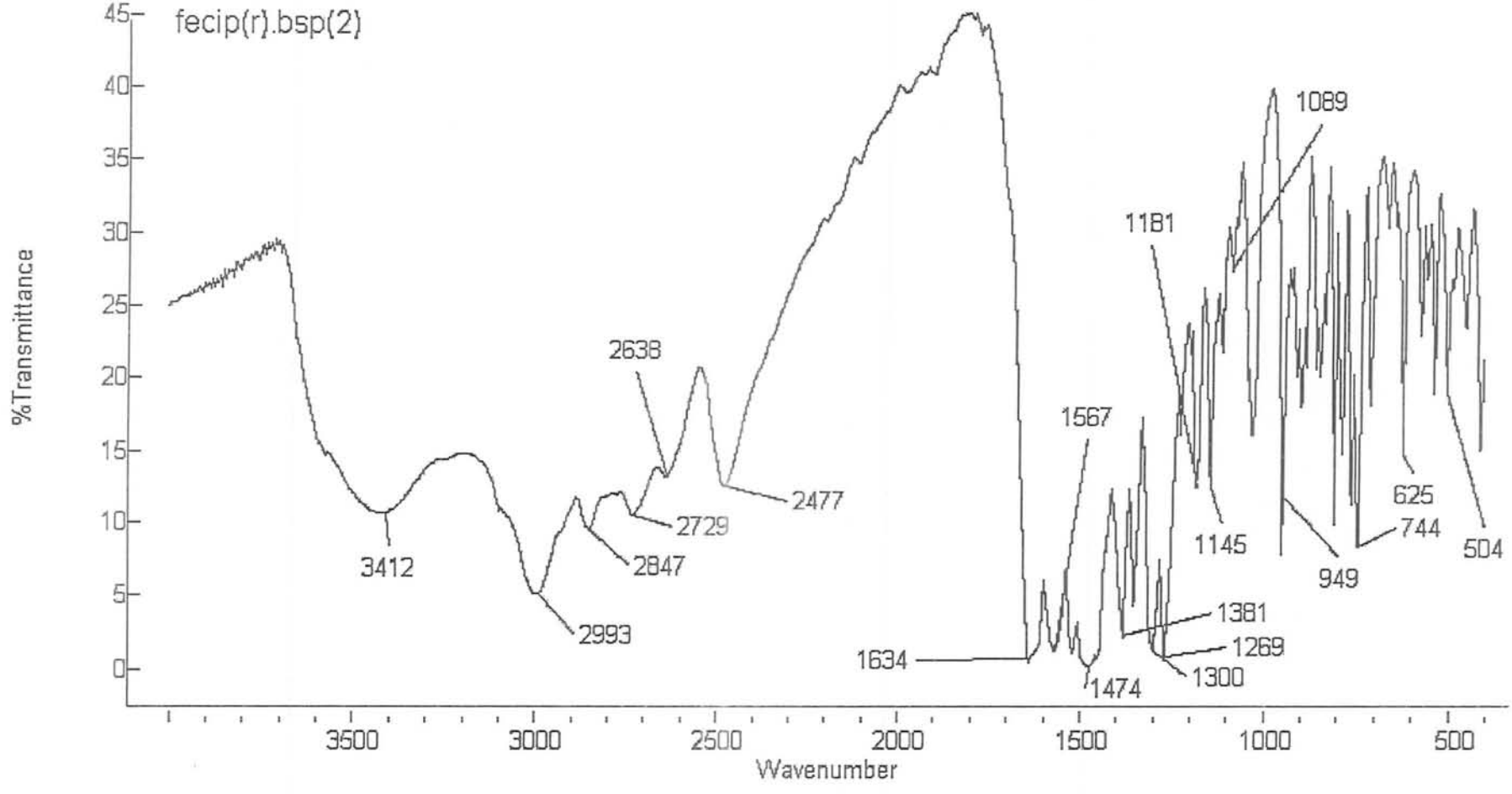


Fig 6.3
FT-IR Spectrum of Vanadyle Ciprofloxacin

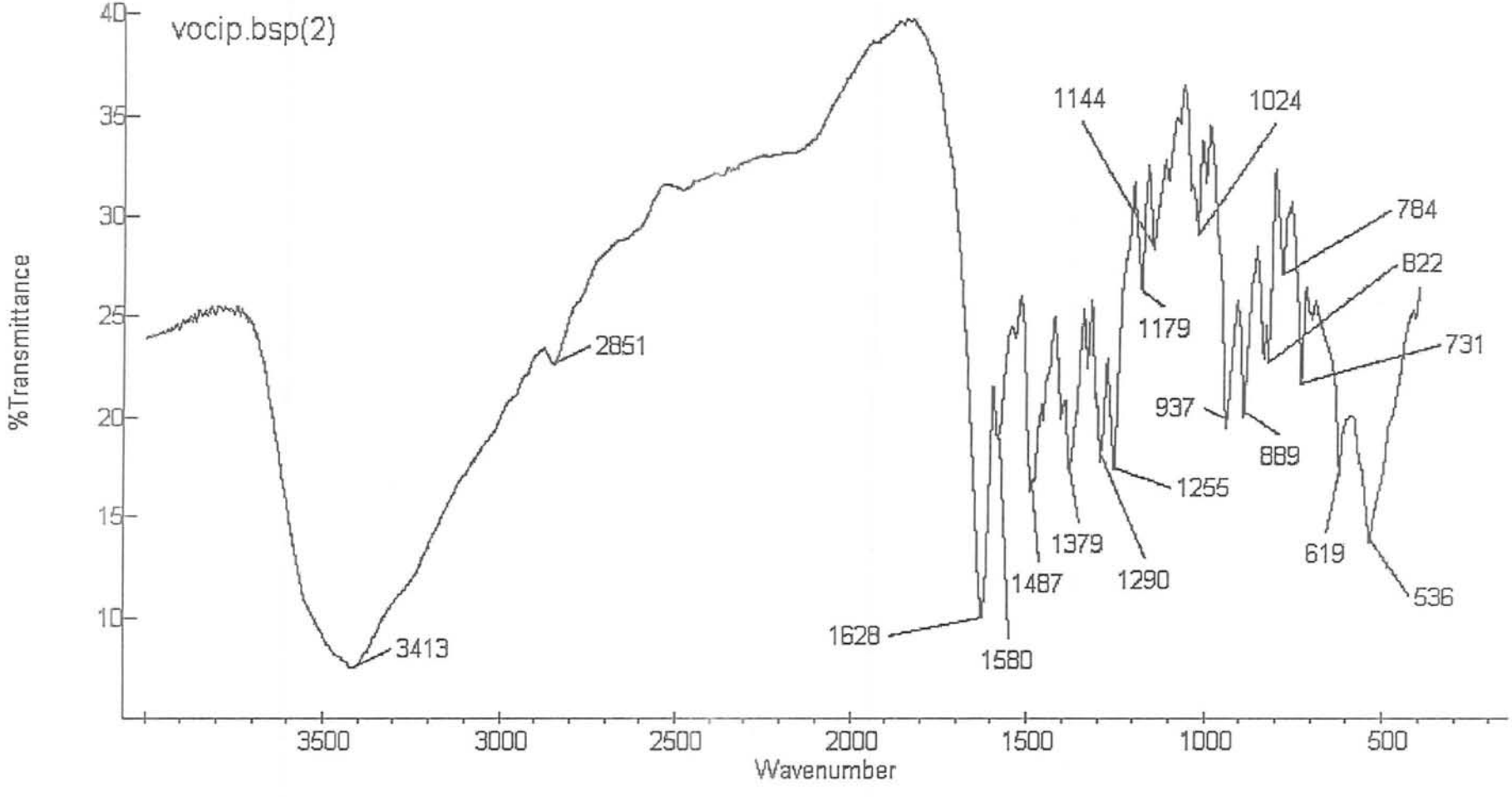


Fig 6.4
FT-IR Spectrum of Manganese Ciprofloxacin

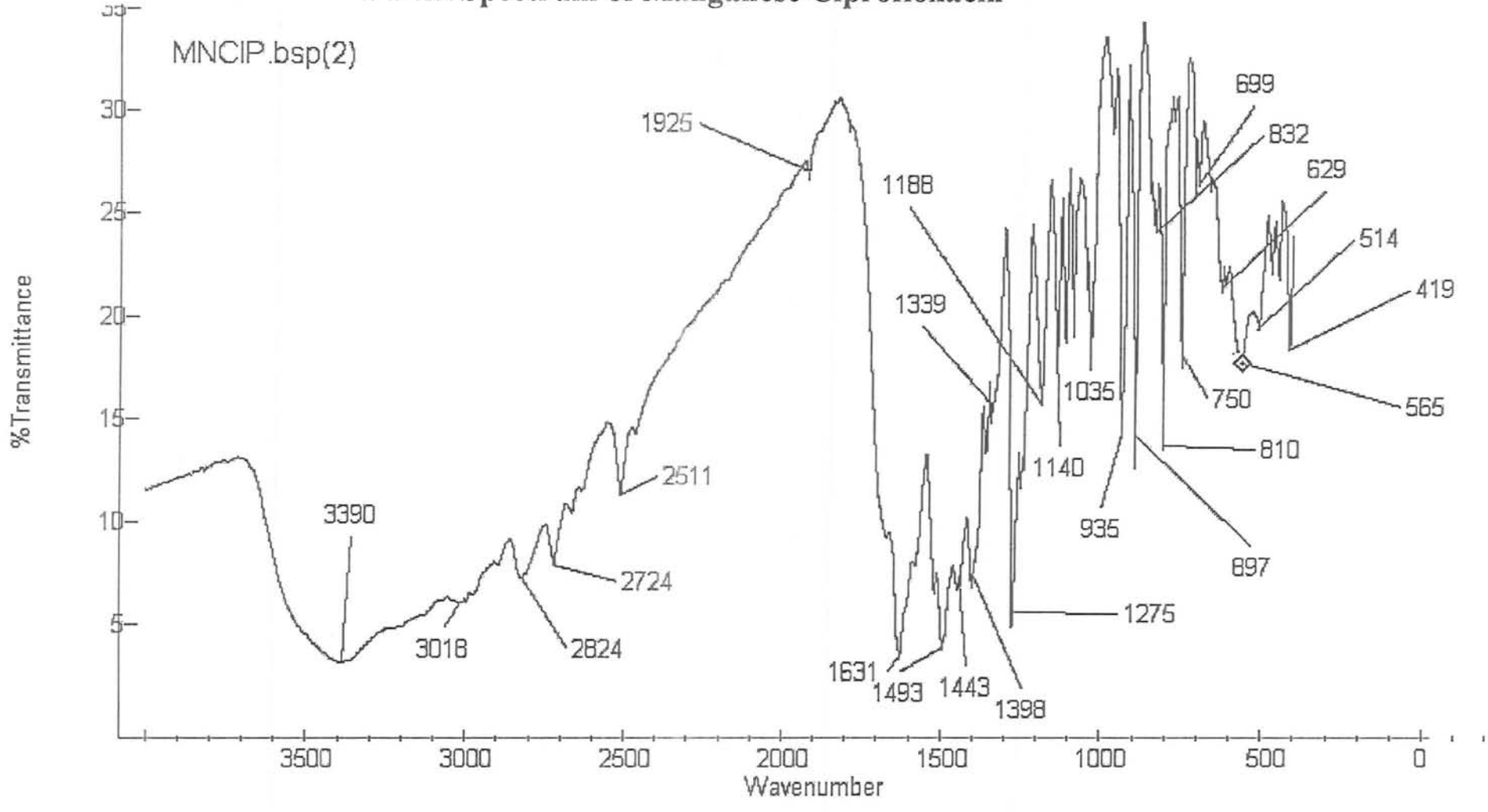


Fig 6.5
FT-IR Spectrum of Chromium Ciprofloxacin

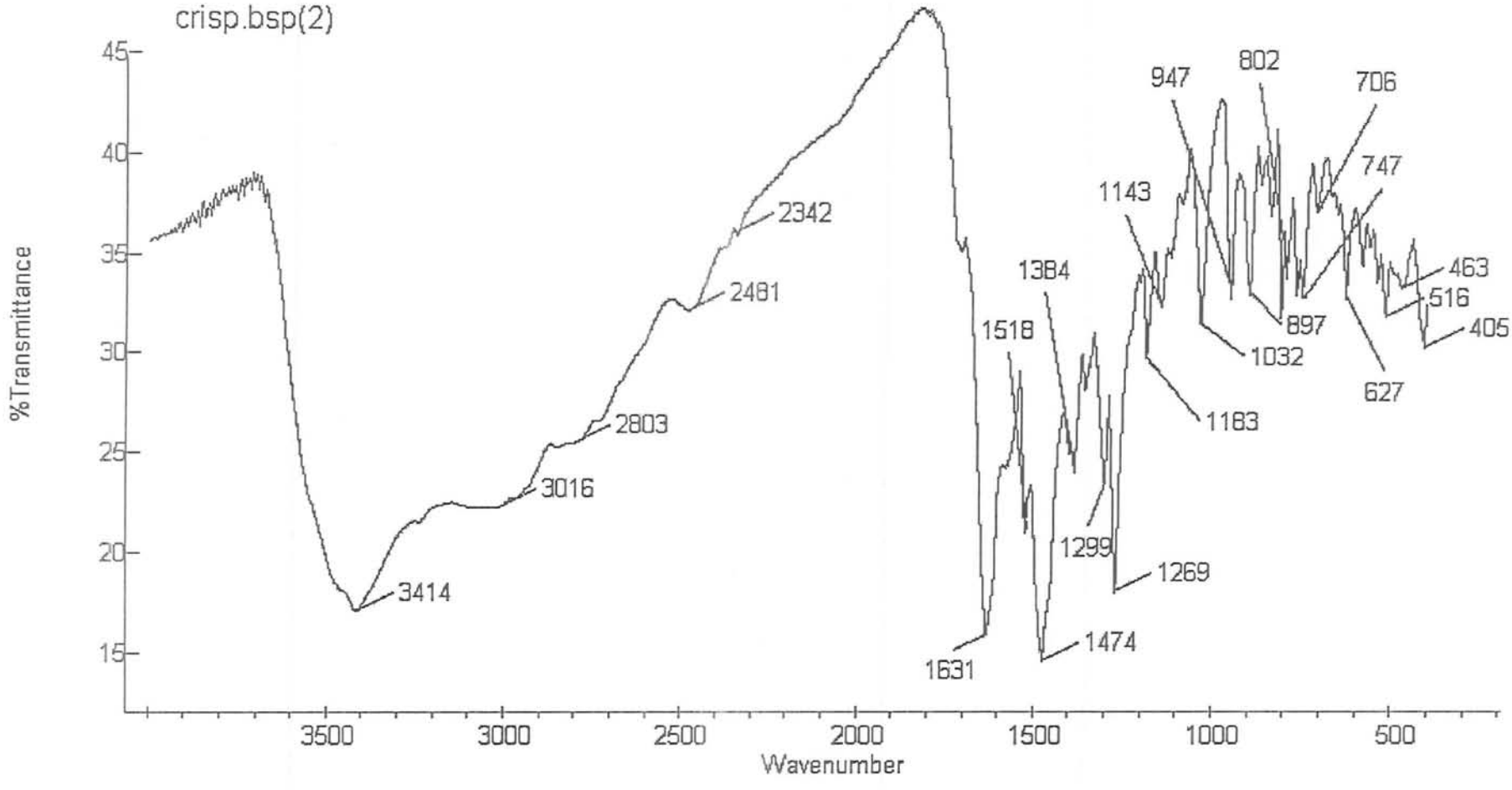


Fig 6.6
FT-IR Spectrum of Nickel Ciprofloxacin Bipyridyl

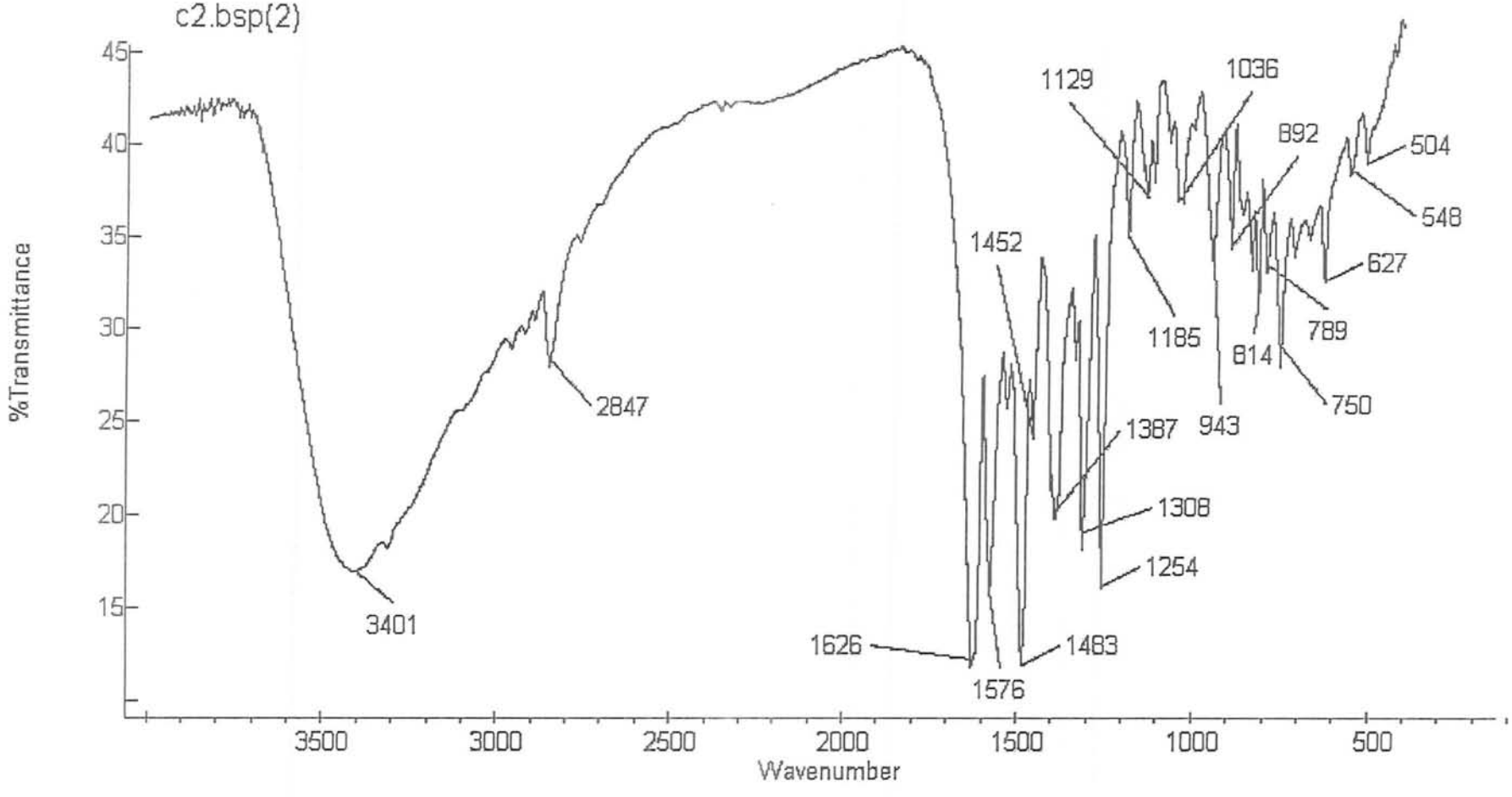


Fig 6.7
FT-IR Spectrum of Copper Ciprofloxacin Bipyridyl

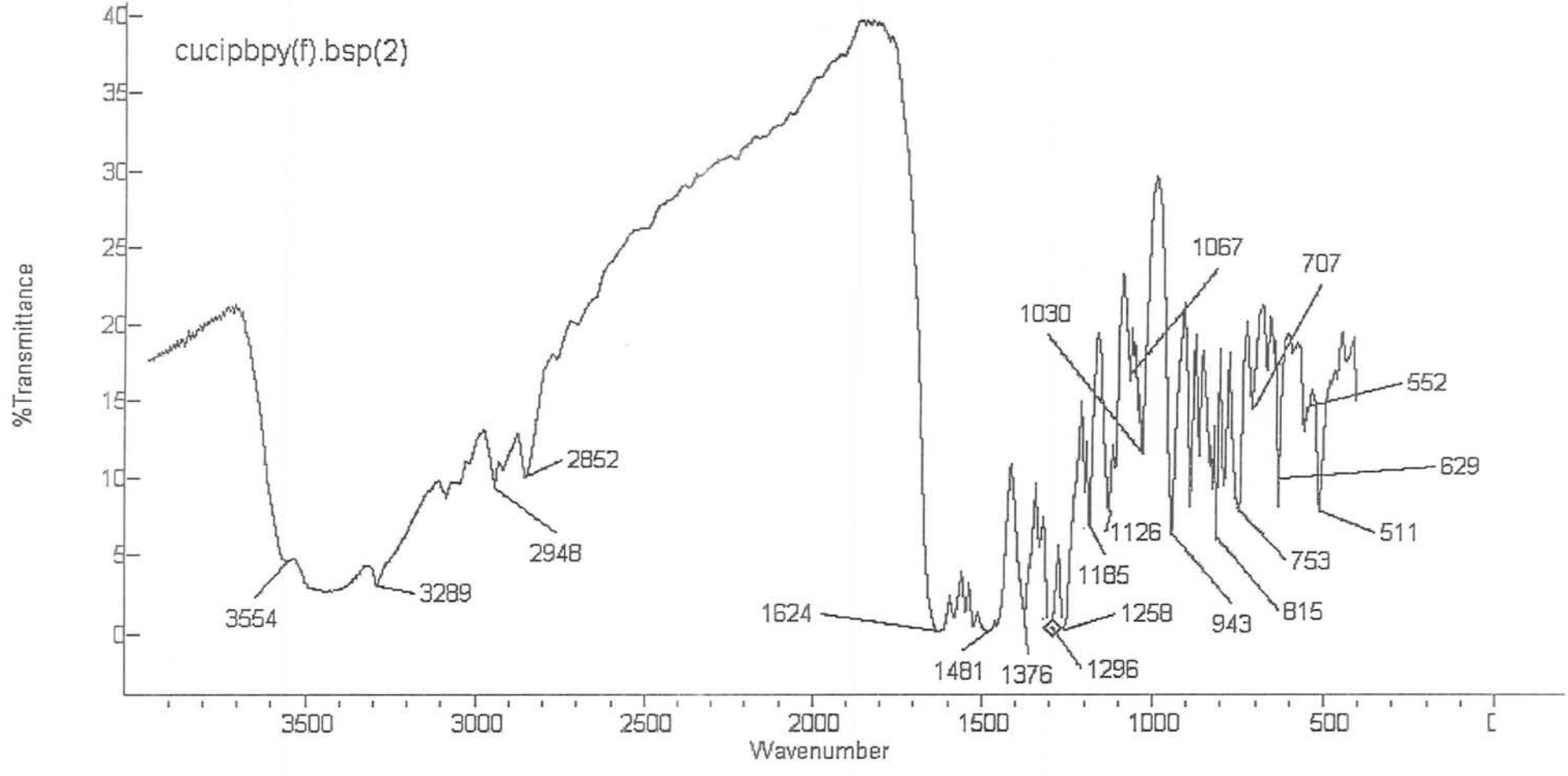


Fig 6.8

FT-IR Spectrum of Norfloxacin

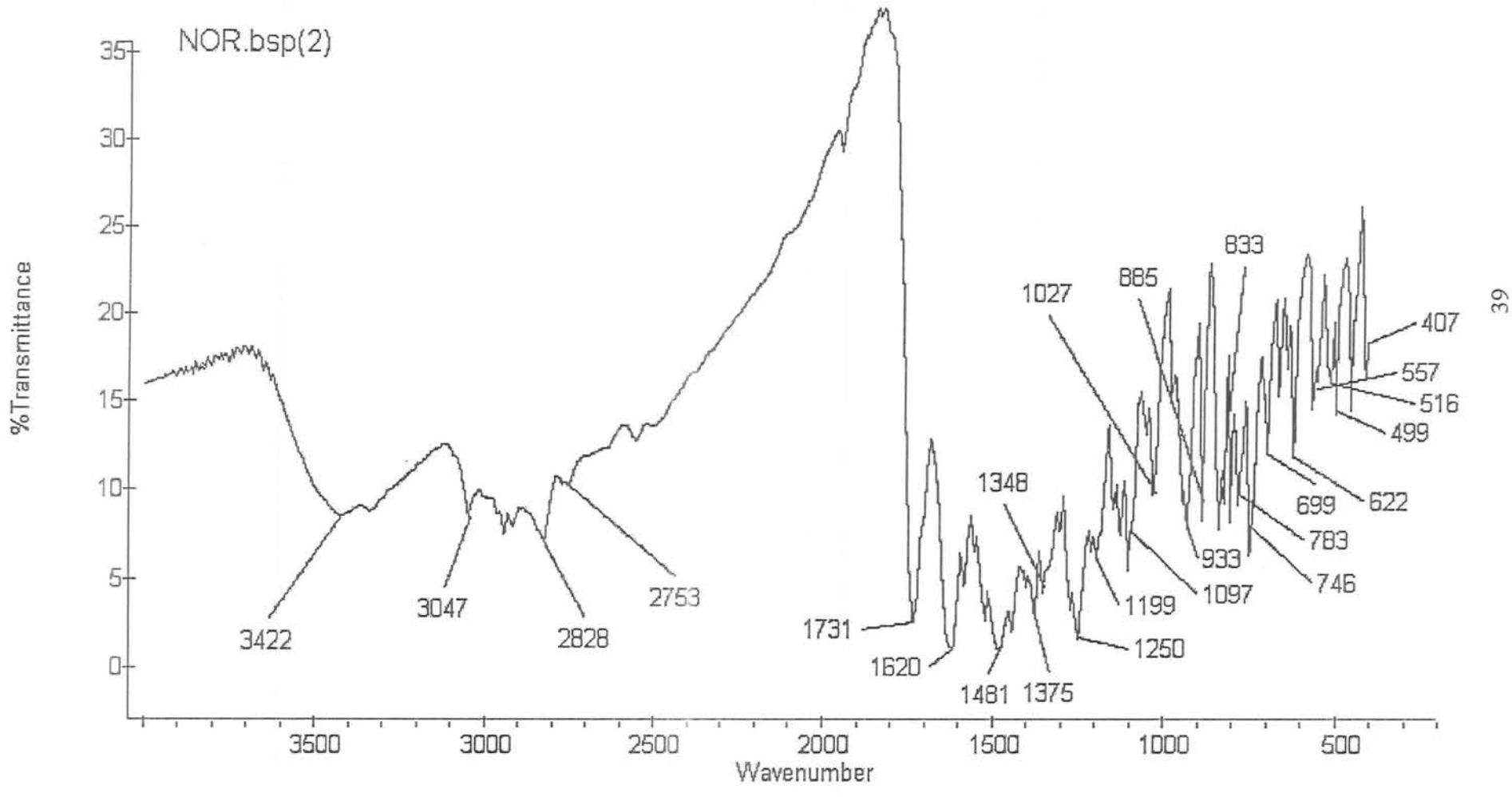


Fig: 6.9
FT-IR Spectrum of Nickel –Norfloxacin- Bipyridyl

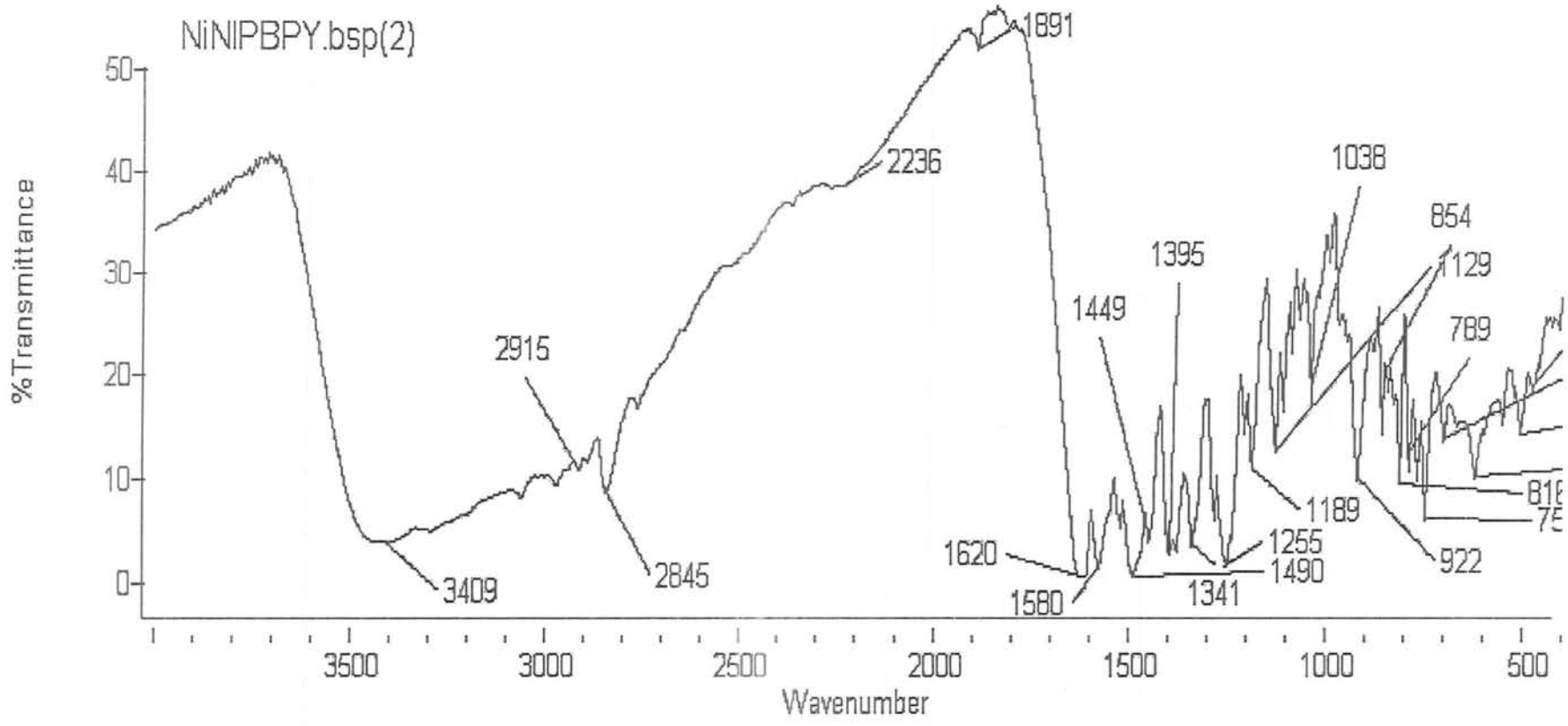


Fig 6.10

FT-IR Spectrum of Cobalt Norfloxacin bipiridyl

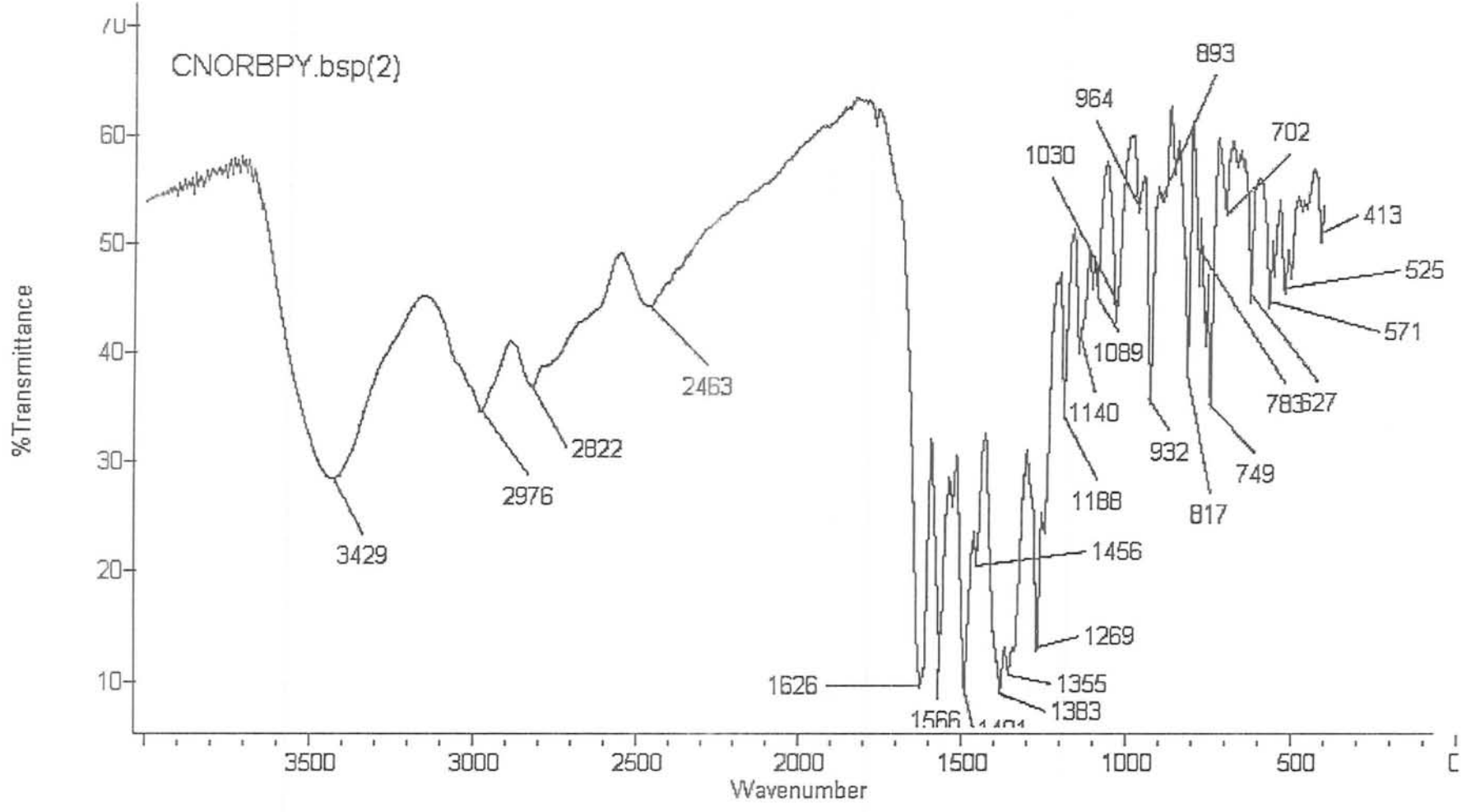


Fig 6.11

FT-IR Spectrum of Zirconium -Norfloxacin

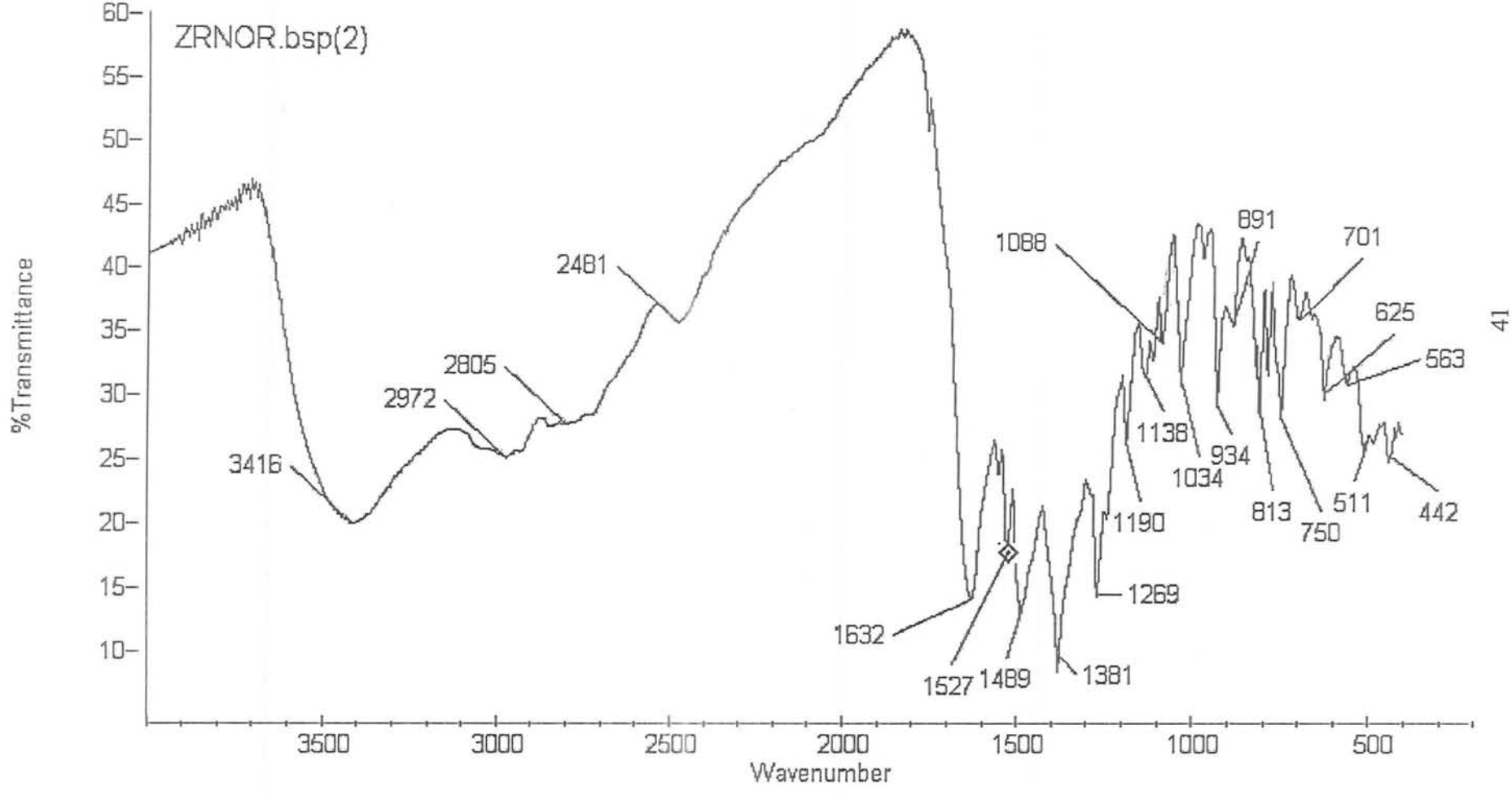


Fig 6.12
FT-IR Spectrum of Chromium Norfloxacin

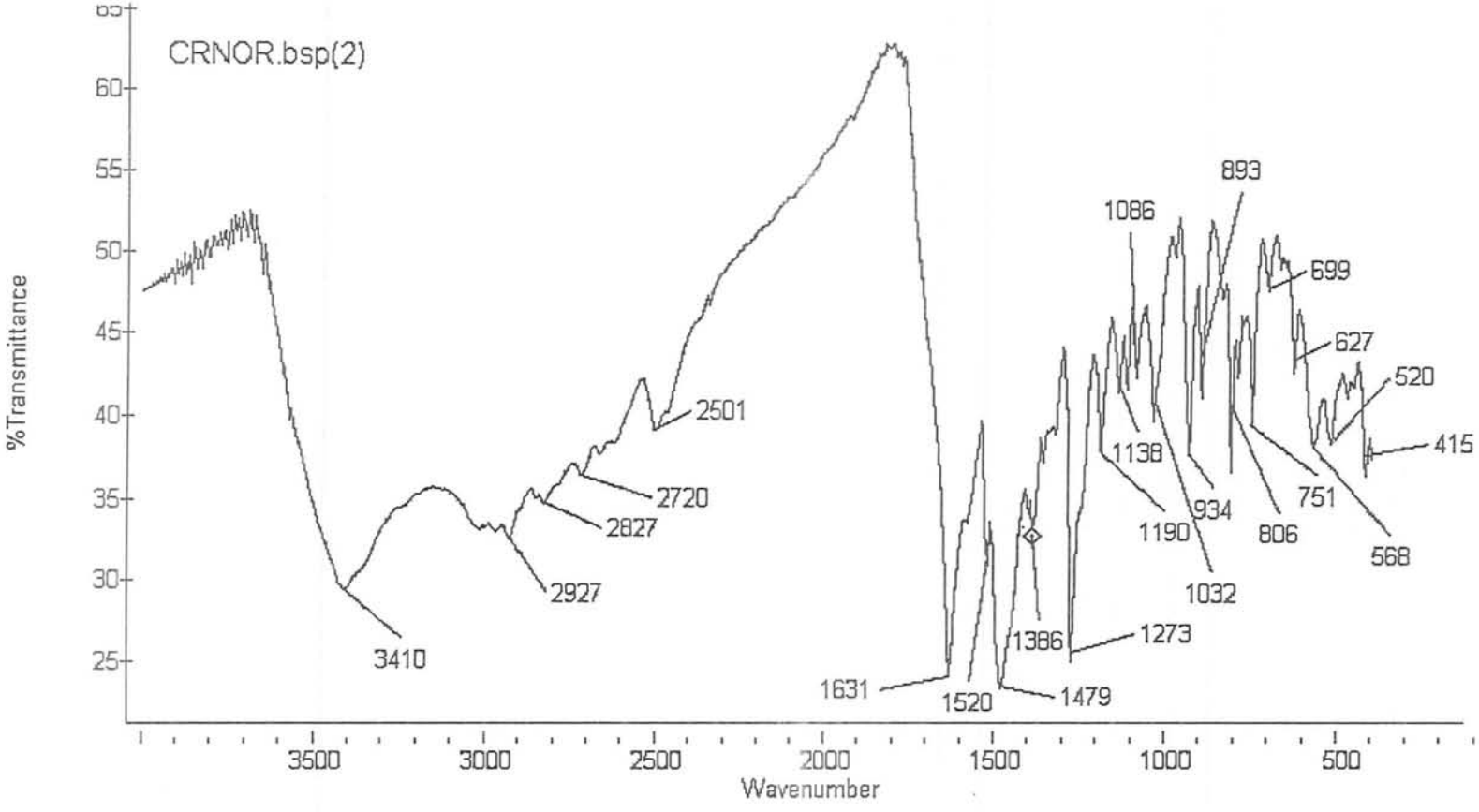


Fig 6.13

FT-IR Spectrum of Iron Norfloxacin

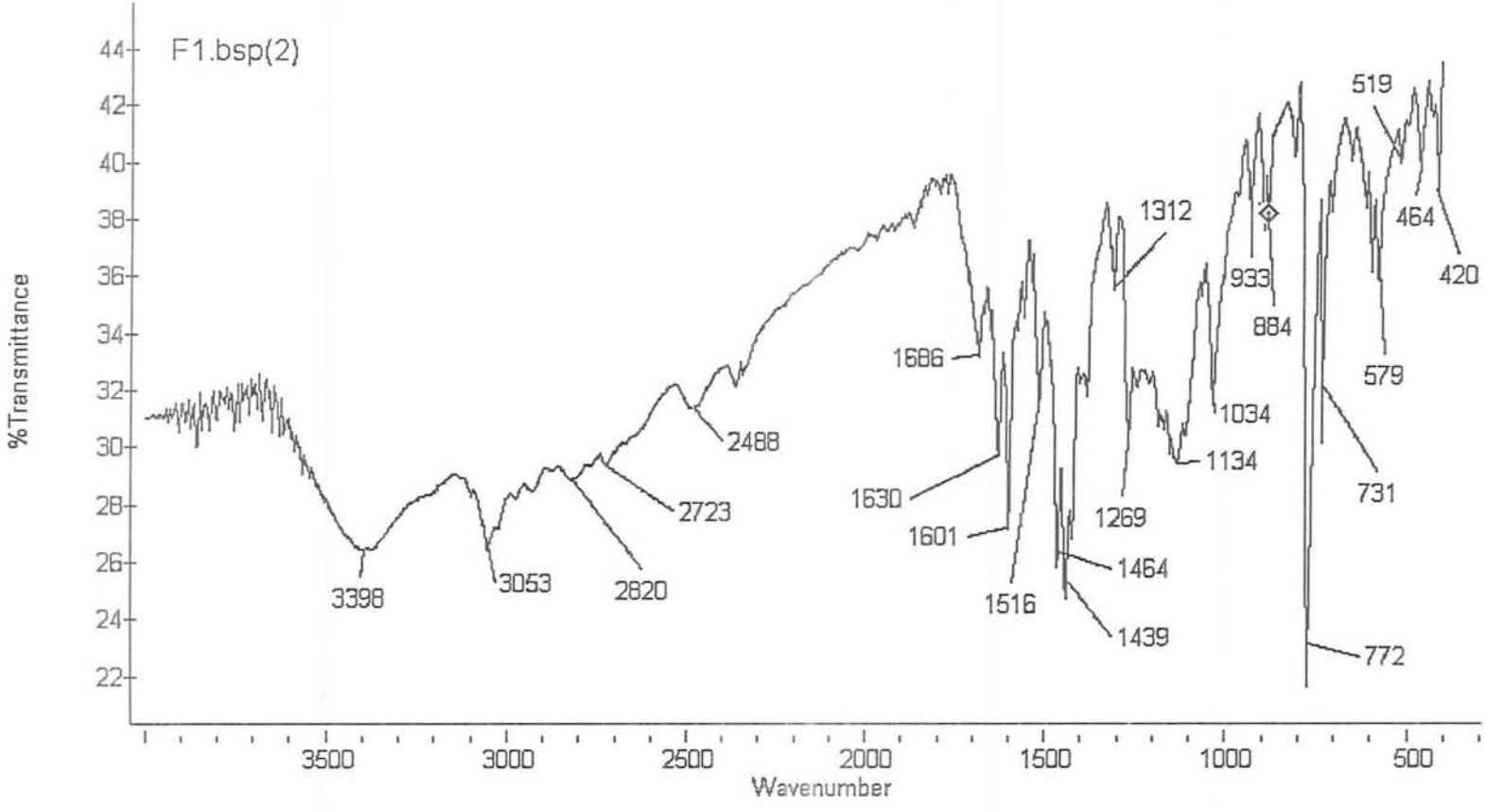


Fig6.14
FT-IR Spectrum Of Copper-Norfloxacin- Bipyridyl

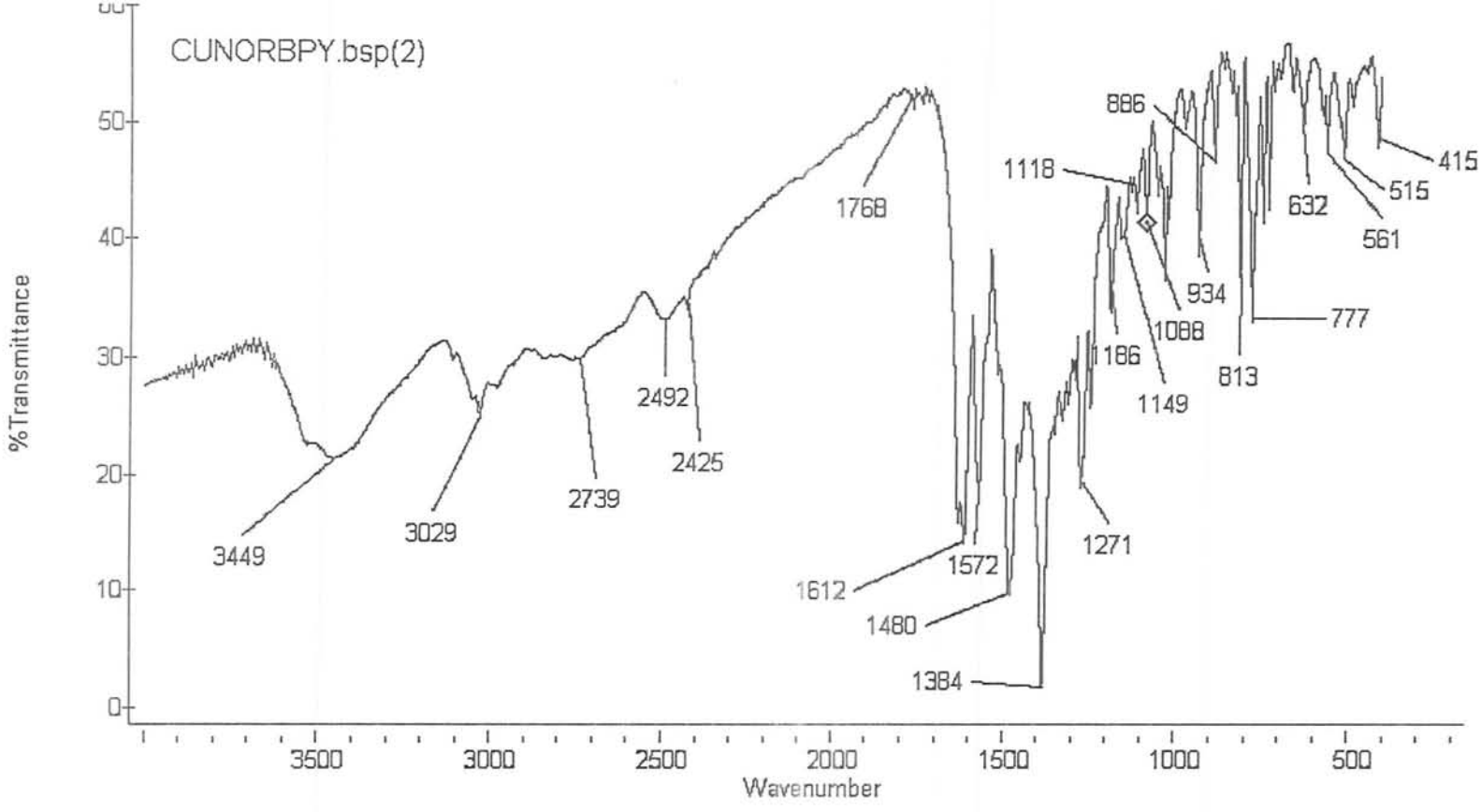


Fig 6.15
FT-IR Spectrum of Iron-Norfloxacin -Bipyridyl

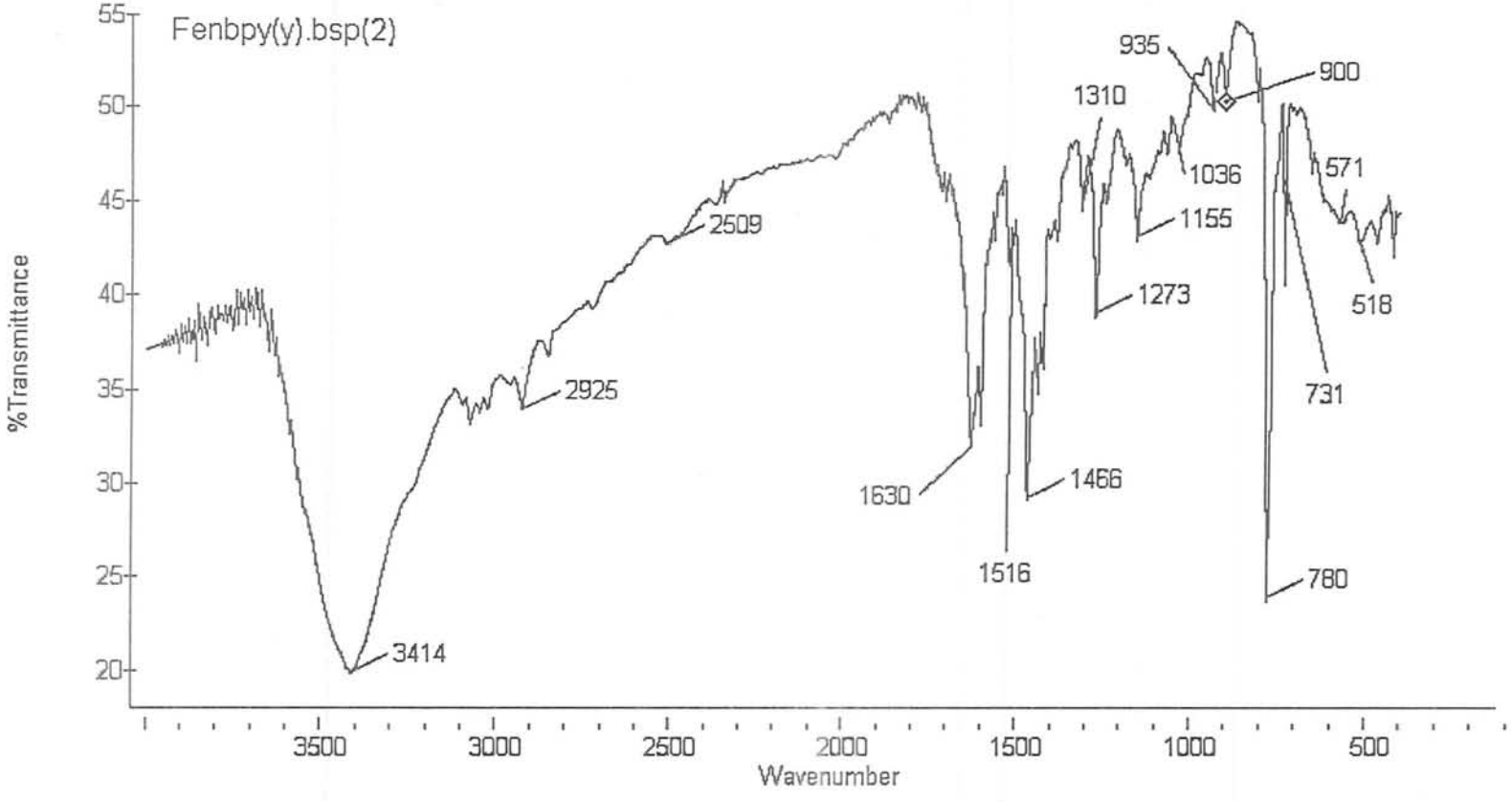


Fig 6.16

FT-IR Spectrum of Zinc Norfloxacin

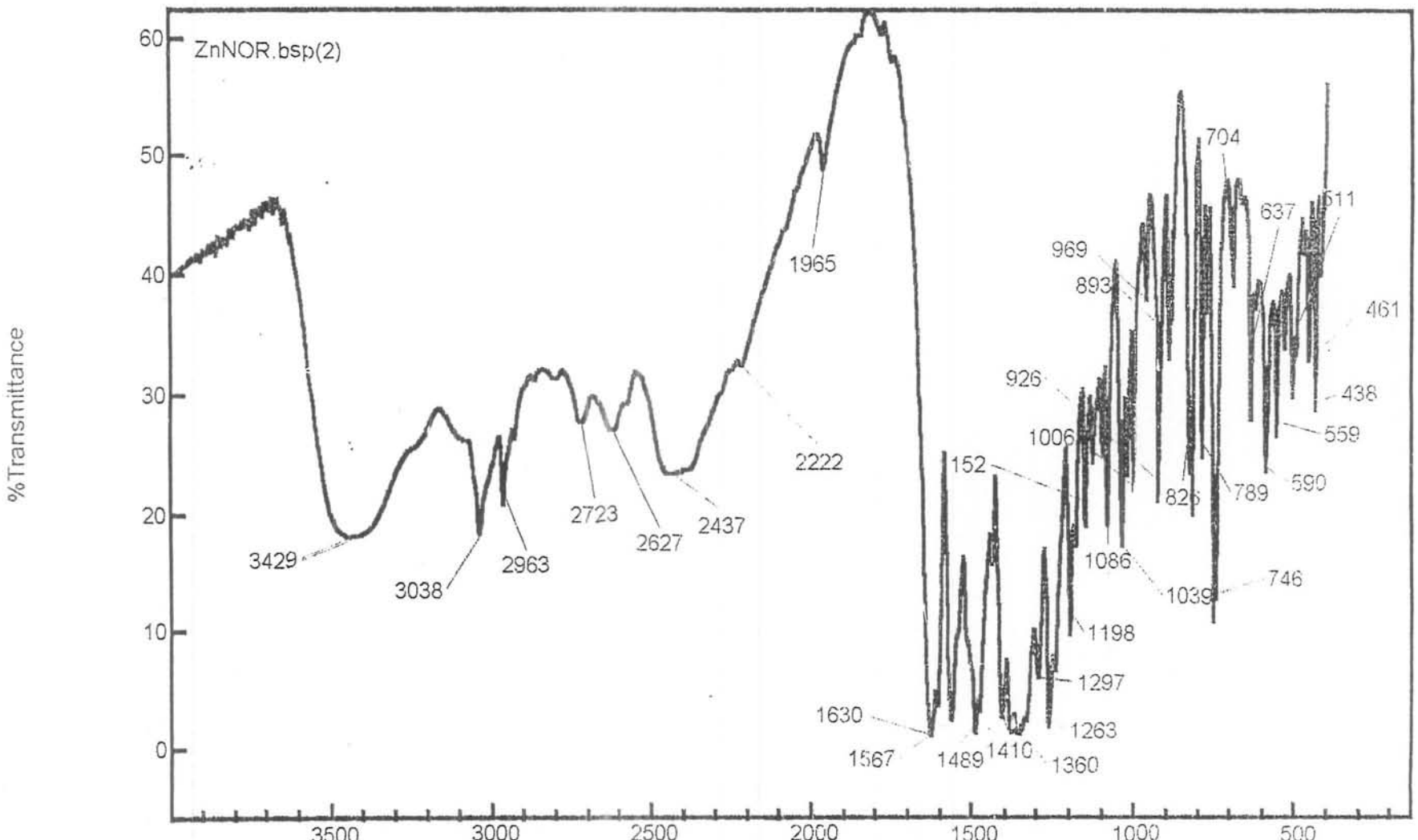
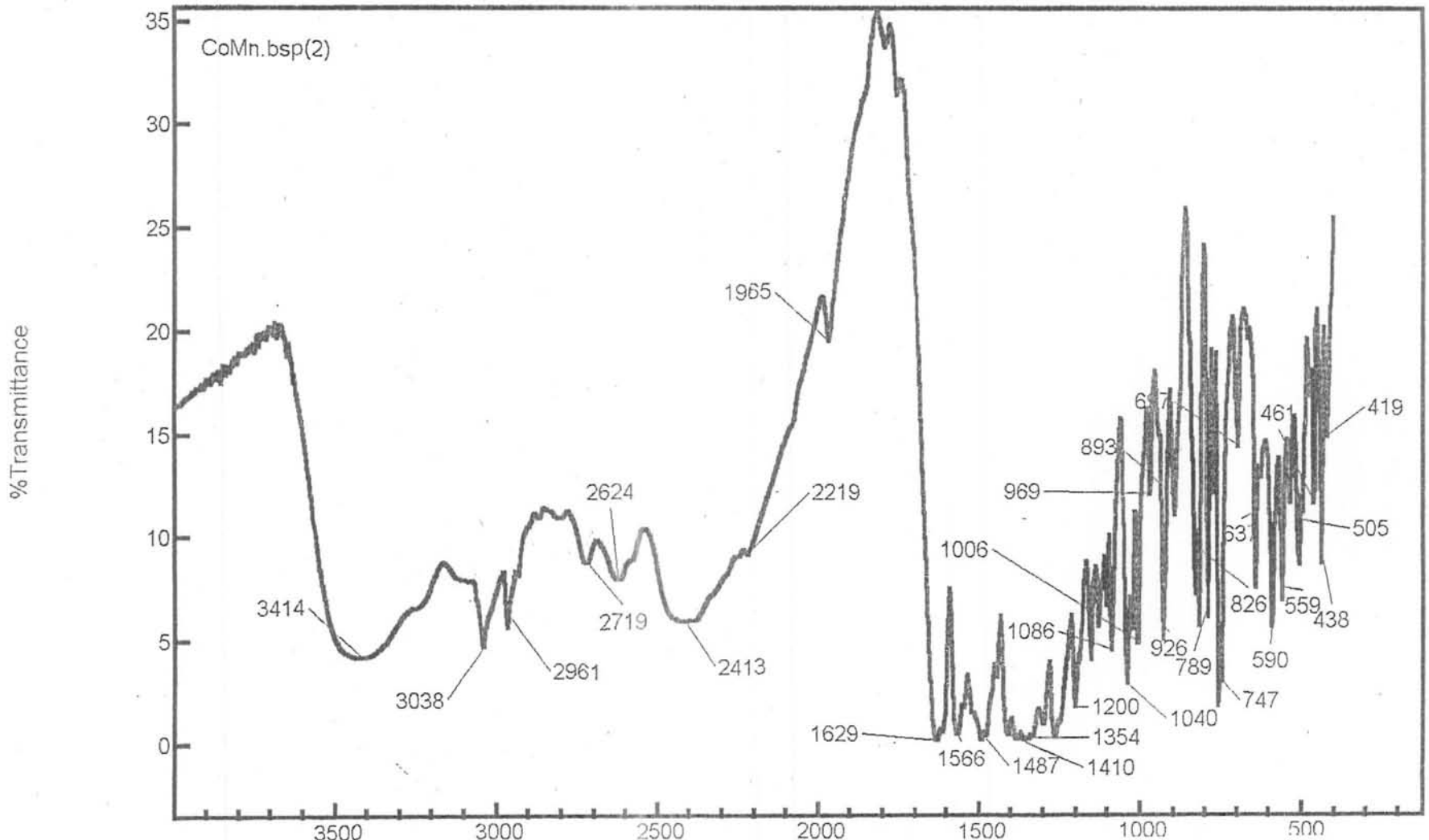


Fig 6.17

FT-IR Spectrum of Cobalt Norfloxacin



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