Synthesis, DFT Study and Biological Activities of Ternary Metal Complexes Using Quinolones With Amino acid

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MS THESIS WORK

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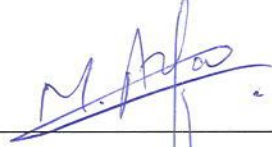
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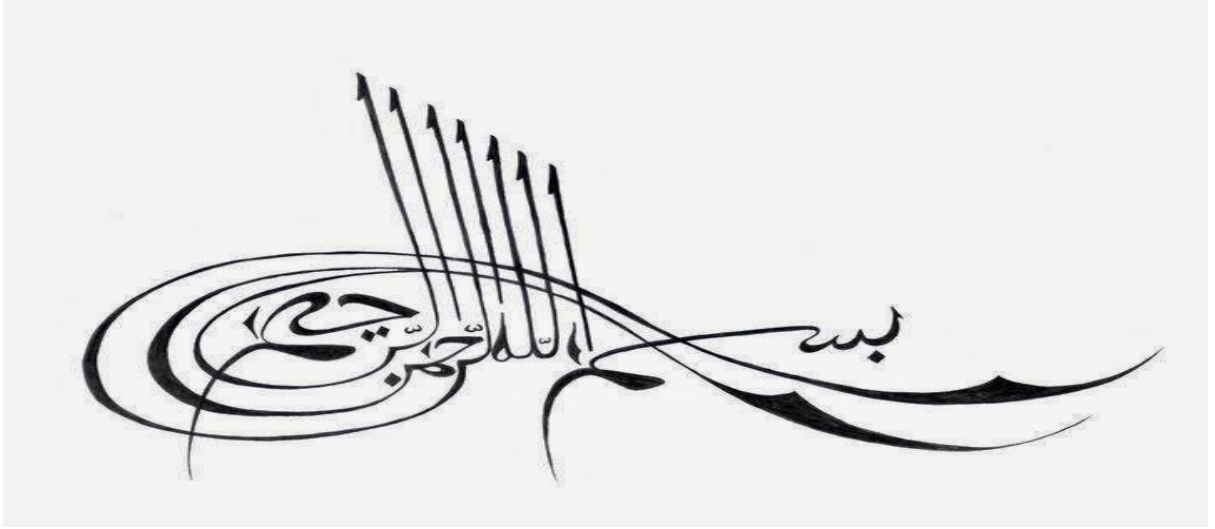
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In the Name of Allah, the Most Beneficent, the Most Merciful

Dedicated to

Who is cold breeze in hot

Who is like drizzle after swelter

Who is shelter in tough times

Who gives soothing comfort

Who takes all my pain

Who on earth is only

"My Mother"

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List of Abbreviations

B3LYP	Becke 3-parameter exchange functional with Lee-Yang-Parr correlation functional
°C	Degree Celsius
CO	Carbonyl
CO_2	Carbon dioxide
CH_3CONa	Sodium Methoxide
CIP/ cprf	Ciprofloxacin
DMSO	Dimethyl Sulfoxide
DFT	Density Functional Theory
en	Ethylene Diamine
FTIR	Fourier Transform Infrared
Gly	Glycine
H_4CyTA	Cyclohexanediaminotetraacetic Acid
KOH	Potassium Hydroxide
LEV/ Lvx / LFLH	Levofloxacin
MOX/ MFL	Moxifloxacin
MOFs	Metal-Organic Frameworks
M. P.	Melting Point
MRSA	Methicillin Resistant Staphylococcus aureus
nalH	Nalidixic Acid
py	Pyridine
SARs	Structure‒Activity-Relationships

SCCs	Supramolecular Coordination Complexes
UV-vis	Ultra violet visible
%	Percentage

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Abstract

Complex formation in pharmaceutical chemistry improves the pharmacological and physico-chemical properties of drugs. Twelve novel ternary metal complexes of biologically important ligands quinolones and glycine have been synthesized and characterized by FT-IR and UV-vis. Quinolones act as primary ligand and glycine act as secondary ligand. General formula of complexes is $[M(MOX)(Gly)(H_2O)_2]$, $[M(LEV)(Gly)(H_2O)_2]$, $[M(CIP)(Gly)(H_2O)_2]$ where M = Ni(II), Cu(II) and $[M(MOX)(Gly)(H_2O)_2]Cl$, $[M(LEV)(Gly)(H_2O)_2]Cl$, $[M(CIP)(Gly)(H_2O)_2]Cl$ where M = Fe(III), Co(III) with octahedral structure. Density functional theory (DFT) is used to optimize the geometry, bond length, bond angle and stable energy of complexes. *In-vitro* antimicrobial activity of ternary complexes was measured higher than reference drugs ciprofloxacin and kanamycin. Also, these complexes were screened for cytotoxicity and the results showed that all complexes are non-toxic. So, these results indicated that after further studies these complexes may be proved as lead compounds to be used as effective antibiotic drugs.

Chapter 1

1.1. Introduction

In modern chemical sciences, the field of coordination chemistry is one of the most interesting, attractive and experimentally demanding frontier. Since half century, it has grown from a limited area into the most active research field of Chemistry. Coordination products are applicable in vast range of areas like fungicides, paints, pigments, polymers, pharmaceuticals, catalysis, and photoconductors [1]. For both qualitative and quantitative analysis of metals, complexation reactions are used. The role of coordination compounds is also important in colorimetric, spectrophotometric and polarographic analysis [2]. The beauty of coordination chemistry lies in variety of bond properties around metal. That's why nature use metals on special places in enzymes for highly define activities. For most industrial synthesis of chemicals, use of metal catalysis is common [3]. After the discovery of the anticancer properties of cisplatin, a coordination compound, medicinal inorganic chemistry as a discipline is considered to have lifted. Coordination complexes applications to medicine are a rapidly developing, field and new therapeutic and diagnostic metal complexes are now having an impact on medicinal practice [4-5]. Metal complexes or coordination complexes are made up of a central metal atom or ion, also called the coordination center, and a surrounding array of bound molecules or ions, which are called ligands [6]. Metal is an electron pair acceptor which forms coordinate covalent bond. Metals show preferred coordination numbers, which not only vary from one metal to other metal, but can change for a particular metal. Ligand is the second interactant, which donates the pair of electron used for bonding. It is also called electron pair donor which may be monoatomic (F^-) or as big as polymer. When a ligand has more than one binding site and occupies more than one coordination positions so that a ring structure formed which is called chelate. The more rings that are formed, the more stable the complex is. Chelating agents having three, four and six donor atoms are familiar and are termed as tridentate, tetradentate and hexadentate ligands respectively [7].

$$M^+ + xL \rightarrow [ML_x]^{n+}$$
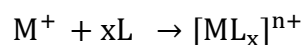
1.2. History

Alfred Werner is regarded as the father of Coordination Chemistry; this does not mean that coordination compounds were not known before his work. Instead, metal complexes have a long and well known history [8]. The solution of transition metal bonding was successfully given by Alfred Werner who received Noble Prize in 1913. His theory provided the foundation for field of metal coordination chemistry. He gave concept of a double valence for transition-metal ions, a primary valence to fulfill principles of neutrality, and a secondary valence giving a "coordination number" [9]. The work of A. Werner opened a new gateway in field of coordination chemistry providing concepts about their formation, structure and reactivity of complexes. New disciplines of coordination chemistry have emerged, for example metal-organic frameworks (MOFs) and supramolecular coordination complexes (SCCs) [10].

1.3. Ligands

1.3.1. Quinolones

Quinolones is a general term used for quinolone carboxylic acids or 4-quinolones. These are groups of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton [11]. Since 1960s clinical practice of nalidixic acid (nalH), made it possible to isolate number of structurally related highly potent broad-spectrum antibacterial agents [12-13]. On the basis of structure-activity-relationships (SARs), modifications were made on nalidixic acid (nalH). Fluorine atom at position 6 and a piperazine ring at position 7 greatly enhance the spectrum of activity. Such quinolones are called fluoroquinolones. These are useful for the treatment of infections, like urinary tract, soft tissue, respiratory and bone-joint infections and diseases like typhoid fever, sexually transmitted diseases, community acquired pneumonia, acute bronchitis and sinusitis [12-14]. Turel studied interaction of metal ions with diverse deprotonated quinolones as ligands [11]. Complex formation increases the bioavailability of metal ion or the ligand drug, or both due to increase in hydrotropy and liposolubility which amplify the ability of drug molecules in crossing the cell membrane, thus raised the biological utilization ratio and activity of the drug [15].
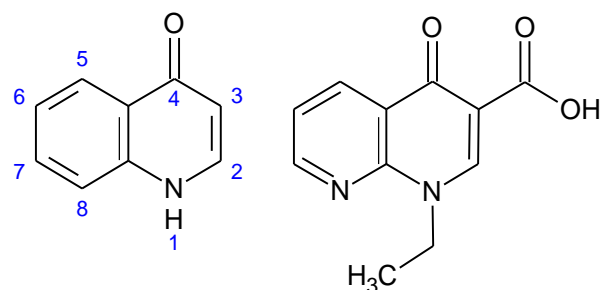
4-oxo-1 and 4
dihydroquinolone

Nalidixic acid

Fig. 1.1: Structure of Nalidixic Acid

Table 1.1: Three Important Fluoroquinolones

Ciprofloxacin	Moxifloxacin	Levofloxacin
1-cyclopropyl-6-fiuoro-4-oxo-7-(l-piperazinyl)-1,4-dihydroquinoline-3-carboxylic acid [16].	1-cyclopropylo-6-fluoro-1,4-dihydro-8-methoxy-7-[(4a*S*,7aS)-octa-hydro-*6H*-pyrrolo[3,4-b] pyridine-6-yl]-4-oxo-3-quinoline carboxylic acid [17].	(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4 methylpiperazin-1-yl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid [18].
Second generation antibacterial fluoroquinolone.	Fourth generation antibacterial fluoroquinolone [19].	On the basis of chemical structure, it is second generation fluoroquinolone [20] but on the basis that it is twice active against Gram-positive bacteria it is considered as a third-generation quinolone [21-22].
Ciprofloxacin and metal ions interaction have been studied mainly due to its biological and chemical usage [23].	In literature numbers of studies have been described between metal ions and moxifloxacin which show that moxifloxacin utilize 4-carbonyl and carboxyl oxygens as donor atoms [11, 24].	Until now various metal complexes of levofloxacin were synthesized and studied [25].

Ciprofloxacin   **Moxifloxacin**

Levofloxacin
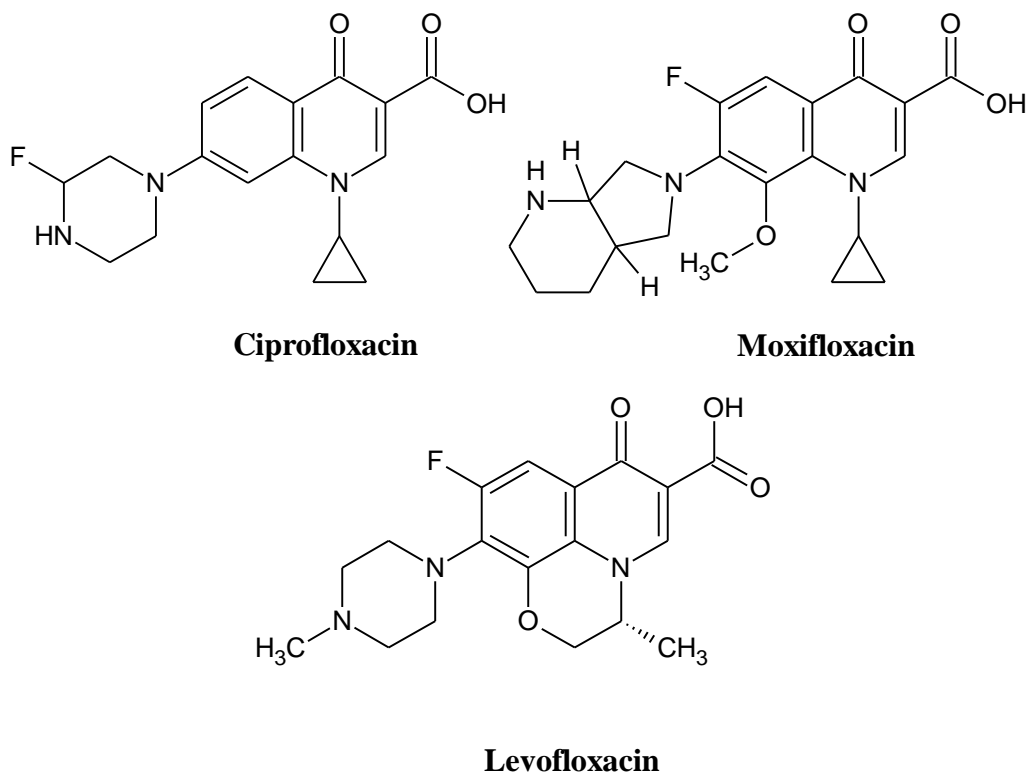
Fig. 1.2: Structure of Quinolones

1.3.2. Amino Acid

Twenty natural amino acids form the building blocks of proteins, which are chemical species vital for number of biological functions [26]. Glycine is the neutral, aliphatic, optically inactive non-essential amino acid [27-31]. It can be synthesized by glycine synthase from CO_2 and NH_3 or transamination of glyoxylate and in metabolism of serine and choline [32]. In various biological processes like oxygen conveyer, electron transfer and oxidation, complexes of transition metals with amino acids in proteins and peptides are used. In such processes, enzymes's active site forms complexes with divalent metal ions [33].

1.4. Types of Metal Complexes

1.4.1. On the Basis of Ligand Nature

1.4.1.1. Classical or Werner Complexes

Ligands bind to metal with their lone pairs of electrons on the main group atoms of ligand. Typical ligands are H_2O, NH_3, pyridine and CN^- examples of such complexes are: [Co $(NH_3)_6$] Cl_3, [Co $(en)_2X_2$] $^+$ (X = Cl, Br *etc.*) [9].

1.4.1.2. Organometallic Complexes

Organometallic compounds contain a metal-carbon bond between an organic molecule, ion, or radical and a metal. Typical ligands are alkenes, alkynes, alkyls, phosphines (PPh$_3$), hydride and CO [34].
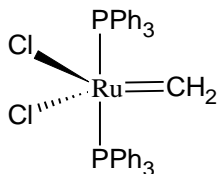
Fig. 1.3: Structure of Organometallic Complex

1.4.1.3. Bioinorganic Complexes

They are biological ligands for metal ions. Typical ligands are amino acids and porphyrins. Example: hemoglobin and vitamin B$_{12}$ [35].

1.4.1.4. Cluster Complexes

Complex compounds in which central metal atom is a three-dimensional cell of directly bonded metal atoms. Perhaps the cluster group of metal atoms is combined with ligands [36].

1.4.2. On the Basis of Central Metal Atom

1.4.2.1. Mononuclear Complexes

Complexes with single metal atom or ion surrounded by ligands. As *trans*-[(py)$_4$TiCl$_2$] [37].

1.4.2.2. Polynuclear Complexes

The complexes with two metal or more atoms surrounded by ligands. C. H. Zhang *et al*. reported six polynuclear complex synthesized by main group and transition metals, polyoxotungstates (SiW$_{12}$O$_{40}$)$^-$ and *trans-N,N,N,N*-1,2-cyclohexanediaminotetraacetic acid (H$_4$CyDTA),

(1) (NH$_4$)$_3$[Ni$_4$Na- (H$_2$O)10(CyDTA)$_2$][SiW$_{12}$O$_{40}$]·10H$_2$O

(2) (NH$_4$)$_2$[Cu$_3$Na$_2$ (HCyDTA)$_2$(H$_2$O)$_{13}$][SiW$_{12}$O$_{40}$]·5H$_2$O

(3) (NH$_4$)$_2$[Zn$_5$(CyDTA)$_2$(H$_2$O)$_{16}$][SiW$_{12}$O$_{40}$]·8H$_2$O

(4) (NH$_4$)$_4$[Cd$_4$(CyDTA)$_2$(H$_2$O)$_8$][SiW$_{12}$O$_{40}$]·6H$_2$O

(5) $(NH_4)_4[Sr_3(HCyDTA)_2(H_2O)_{14}][SiW_{12}O_{40}] \cdot 2H_2O$

(6) $[Ca_4(H_2CyDTA)_2(H_2O)_{22}][SiW_{12}O_{40}] \cdot 8H_2O$ [38].
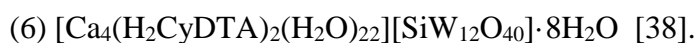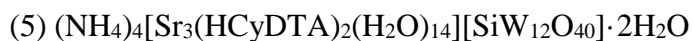
1.4.3. Number of Species

1.4.3.1. Binary Complexes

Metal complexes consist of two molecules metal and ligand [39].

1.4.3.2. Ternary Complexes

These complexes mainly involved the interaction of metal ion with a primary ligand and a secondary ligand. Now there has been increased interest in the mixed chelation as it occurs frequently in biological fluids. Mixed complexes contain millions of possible ligands which compete for metal ions [40-41]. Ternary coordination complexes play significant part in biological processes for example enzymes are known to be activated by metal ions [42]. Ternary complexes are involved in the storage and transfer of active substances through membranes. An increased in the stability of ternary complexes have been observed by Siegel *et al.* (1975) if the ligand contained a hetero atom nitrogen base (basic character) and an oxygen donor ligand [43]. Mixed ligand ternary complexes in which one ligand is aromatic is also more stable [44].

Fluoroquinolone metal complex synthesis has been carried out as an attempt to interpret their physico-chemical properties and some antibacterial activity studies present that these complexes grant the transformation of the effectiveness and specificity of fluoroquinolones [45-49].

The metal complexes together with medicinal drugs and amino acids play a main part in the biological and chemical activity. As the main focus of the synthesis of any biologically active compound is to inhibit the disease-causing agent without any side effect on the patient. And if it is used as chemotherapeutic agent it would destroy only cancerous cell not normal cells. Soliman *et al.* reported the antimicrobial activity of complexes with drug and amino acid then compared their activities with reference drug [50]. The cytotoxic activity of complexes with drug and amino acid were reported by Mohamed *et al.* [51]. Metal complex stability controls the synergy of metal and ligands in complex media [52]. The metal ligand selectivity and vigor of metal ligand bonds depends on stability constants [53]. Mixed ligands copper complexes studies (including

quinolone) just as the antibacterial drugs oxolinic acid, enrofloxacin, flumequine and gatifloxacin in the existence of nitrogen donor heterocyclic ligands have exposed that the quinolone ligands act as deprotonated bidentate ligands and are combined to the metal ion through the pyridone and one carboxylate oxygen atoms [54–57]. Due to extensive use, there has been an increasing risk of bacterial resistance to quinolones [58], which needed to enhance existing antimicrobial drugs and develop new ones; metal complexes could be a substitute to conventional drugs, as new derivatives of fluoroquinolones [59-60].

Quinolones combine with the enzyme-DNA complex forming a drug-enzyme-DNA complex that inhibits progression and the replication processes [22,61]. Gameiro *et al*. synthesized binary and ternary complexes of nor-floxacin and ofloxacin (quinolones) with copper (II) ion in the presence and absence of phenanthroline. Very high values of stability constant of the binary and ternary copper (II) complexes showed that the ternary complexes were more stable than the binary ones [62]. The results show that the ternary copper complexes of fluoroquinolone can be seen as manner to form new antibacterial drugs [63].

1.5. Synthesis

1.5.1. Metal Complexes with Quinolones Ligands (Binary Complexes)

Ciprofloxacin metal complexes were prepared in aqueous medium, by mixing with stirring, a solution containing sodium ciprofloxacinate suspended acid. Instantly blue colored precipitate appeared which was filtered; turquoise-blue crystals were obtained by slow evaporation of the remaining solution at room temperature [64]. Psomas showed ciprofloxacin complexes with Mn(II), Ni(II), Fe(III) and MoO_2(II) ions having formula $[M(cprf)_2(H_2O)_2]$. Complexes were prepared by methanolic solution of ciprofloxacin, deprotonated with KOH was added to a methanolic solution of metal chloride. nH_2O. The mixture was refluxed for 2 hours. The solution was filtered and left for slow evaporation. After few days, a microcrystalline product was obtained [65].
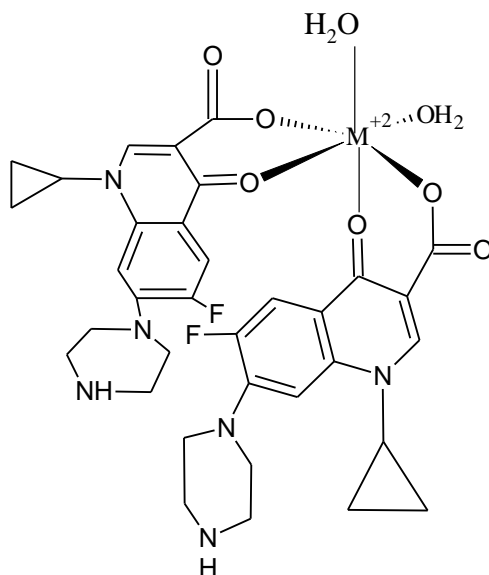
Fig. 1.4: Structure of Binary Complex

Huber *et al.* reported the synthesis of following metal complexes [Cu(C$_{18}$H$_{19}$FN$_3$O$_4$)$_2$].2H$_2$O, [Cu(C$_{17}$H$_{17}$FN$_3$O$_3$)$_2$].2H$_2$O, [Zn(C$_{18}$H$_{19}$FN$_3$O$_4$)$_2$].2H$_2$O and [Zn(C$_{17}$H$_{17}$FN$_3$O$_3$)$_2$]. 2H$_2$O through an aqueous solution of copper II or zinc nitrate, by adding stirring solution of levofloxacin in methanol and ciprofloxacin in water [66].

I A, **M** = Cu(II)
I B, **M** = Zn(II)

II A, **M** = Cu(II)
II B, **M** = Zn(II)

I A Copper levofloxacin
I B Zinc levofloxacin

II A Copper ciprofloxacin
II B Zinc ciprofloxacin
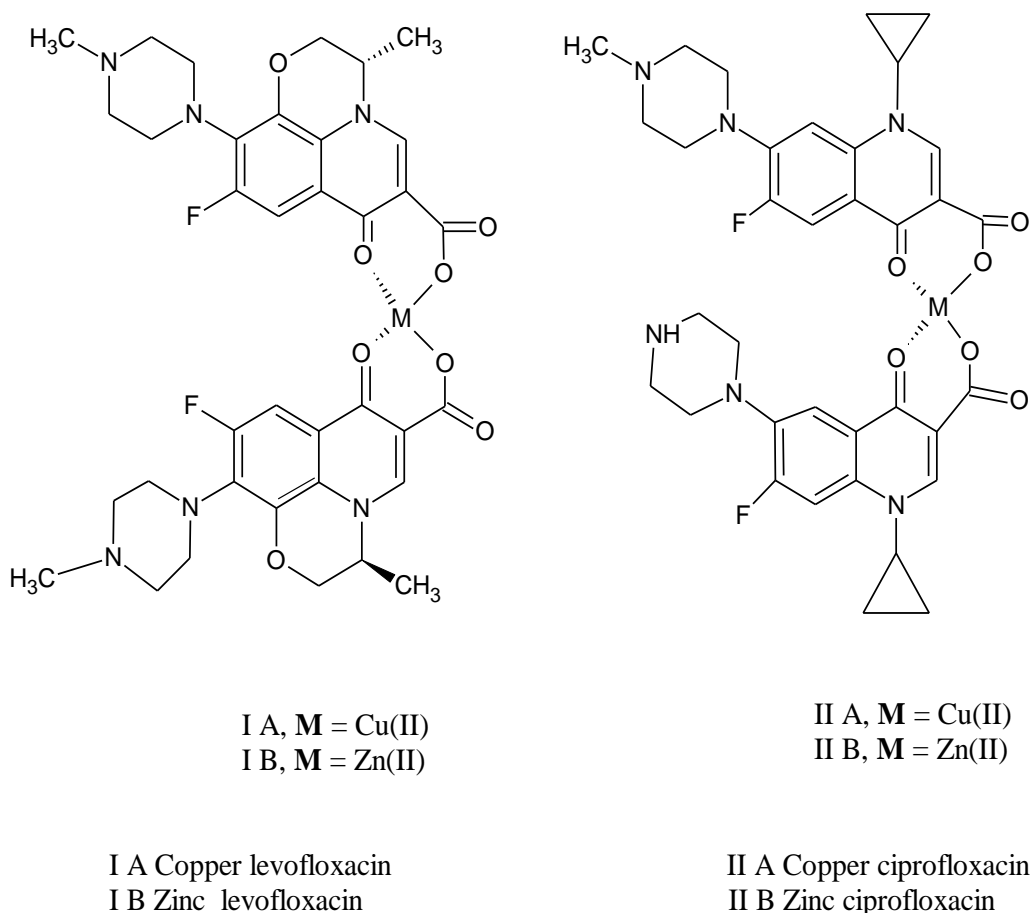
Fig. 1.5: Proposed Structure for Synthesized Complexes

1.5.2. Complexes with Quinolones and *N*- Donor Ligands (Ternary Complexes)

For the Synthesis of $[Cu(lvx)(phen)(H_2O)]NO_3.2H_2O$, to a solution of levofloxacin and NaOH, 1,10-phenanthroline were added. To this mixture under constant stirring aqueous solution of Cu $(NO_3)_2$ was added. The green solution was concentrated on a rotovapor, and then left to stand at room temperature. After a few hours dark green crystals were formed [67]. Almost same method of synthesis for copper(II) complex of ofloxacin and phenanthroline was reported by Chen *et al*. [68]. Ruı´z *et al.* outlined the synthesis of copper(II) with norfloxacin and 1,10-phenantroline [69].

Ternary complex of copper with ciprofloxacin and phenanthroline was prepared by suspension of sodium ciprofloxacinate with 1,10-phenantroline. A solution of Cu $(NO_3)_2$. $3H_2O$ was added. With 1 M NaOH the pH was maintained to 7.8 and the solution was stirred and filtered off. Crystals were formed after 10-15 days.

Another copper complex is prepared when sodium ciprofloxacinate and 1 M HNO_3 were added to an aqueous solution of 1,10-phenantroline. An aqueous solution of $Cu(NO_3)_2.3H_2O$ was added, then a solution of 1 M NaOH was added. The pH of solution is maintained at 5.5. Through filtration the resulting suspension was removed. The crystals that had formed were filtered off and air dried after 8 days [70].

A methanolic solution of fluoroquinolone (GFL/MFL) in presence of sod. methoxide was added to a methanolic solution of $CuCl_2.2H_2O$ and stirred for 30 min, then methanolic solution of the neutral bidentate ligand (phenanthroline/bipyridyl) is added. By dilute solution of sod. methoxide the pH was adjusted to 6.2. The solution was refluxed for 2 hour on a steam bath, followed by concentrating it to half of its volume. The microcrystalline coloured product of obtained was washed with ether and dried. The general composition of the complexes was $[Cu(A)(L)Cl].5H_2O$, A= moxifloxacin/gatifloxacin and L=nitrogen donor ancillary ligands phenanthroline/bipyridyl [71].

A = Moxifloxacin / Gatifloxacin
L = Bipyridyl/Phenantroline
R = $C_7N_2H_{14}$
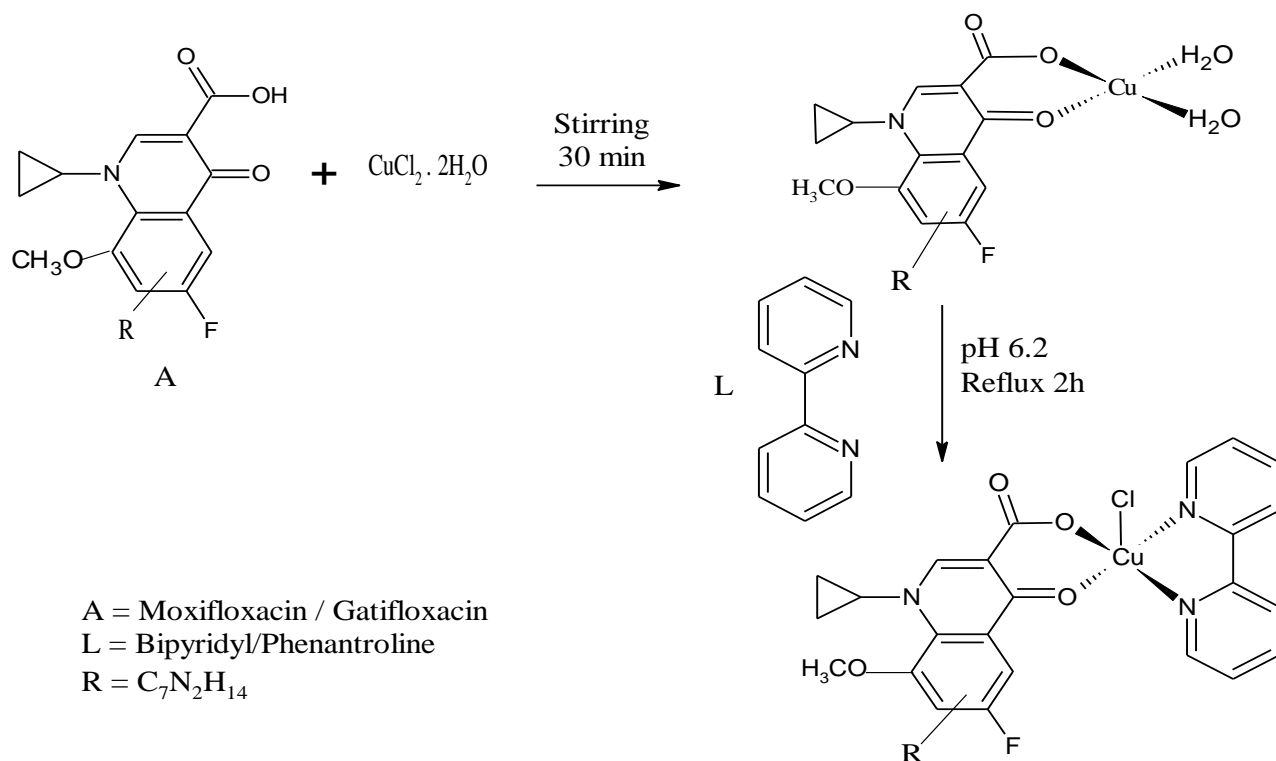
Scheme 1.1: Schematic Representation of Synthesis of Complexes

A methanolic solution of $CuCl_2.2H_2O$ was added to a methanolic solution of *NN* donor (bipyridyl/phenanthroline) ligand, then solution of LFLH (levofloxacin) in methanol in presence of CH_3ONa was added. Using dilute solution of CH_3ONa pH was adjusted to 6.2. The solution was refluxed for 1 hour on a steam bath; volume of the solution is reduced to half. Fine amorphous coloured product obtained was washed with ether/hexane and dried in vacuum desiccator [72].

1.5.3. Complexes of Quinolones and Glycine

Mohamed *et al.* prepared mixed complexes by combining equal amounts of hot saturated ethanolic solution of the first ligand (lomefloxacin) and second ligand (glycine) with the same amount of metal chloride or nitrate salts. The mixture was then refluxed. The solid was filtered and washed with hot ethanol till the filtrates become clear. The solid were dried in desiccator over anhydrous calcium chloride [51]. Soliman *et al.* reported almost similar method for synthesis of enrofloxacin and glycine complexes [50]. In another paper Soliman *et al.* published almost same method for the synthesis of sparfloxacin and glycine [73].
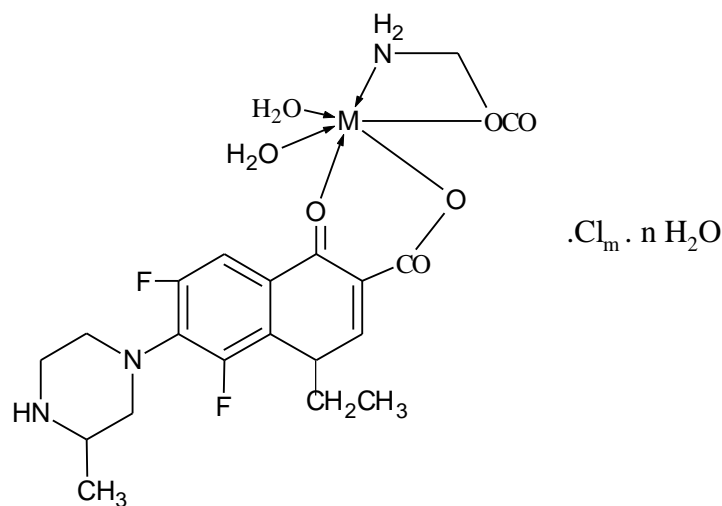
M = Cr(III), Mn(II),Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Th(IV)
m = 1-3, n = 0-2

Fig. 1.6: Structure of Ternary Metal Complexes

1.6. Density Functional Theory (DFT)

DFT is an extensive tool to understand and foretell the behavior of chemical, physical, and biological phenomena in the field of coordination chemistry. Tsipis outlined the effect of modern computational technology in the progress of Chemistry [74]. Platas-Iglesias *et al*. [75] reported different applications of DFT to determine the structure, dynamics, vibrational spectra, NMR, hyperfine interactions, excited states, and magnetic properties of lanthanide(III) complexes. DFT help to perform following operations.

• Geometry optimization

• Single-point energy calculation

• Predicting reaction mechanisms

• Calculation of bonding properties

• Calculation of atomic charges, dipole moments, multipole moments, electrostatic potentials, polarizabilities, etc

 • Calculation of NMR chemical shifts

• Calculation of ionization energies and electron affinities

• Simulating EPR spectra

• Simulating X-ray absorption spectra

• Noncovalent interactions in extended molecular systems [76].

1.7. Biological Activities of Ternary Complexes

Ternary Complexes are biologically and chemically active moiety, due to which it shows large number of pharmaceutical and industrial applications.

1.7.1. Anti-Cancer Activity

Discovery of drugs as anti-cancer agents is developing area of pharmaceutical research [77]. Now proteins are also considered to be one of the chief molecular targets in the activity of anticancer agents. Zhang *et al*. have reported the ternary complex of 1,10-

phenanthroline and L-threonine, which exhibited cytotoxicity [78]. Patitungkho and co-workers reported anti-tumor activity of ternary moxifloxacin–copper complexes against breast cancer cell lines [79]. Ternary copper complexes of moxifloxacin and gatifloxacin against lung cancer cells were described by Singh *et al*. [71].

1.7.2. Anti-Oxidant Activity

Patel *et al*. synthesized different ternary metal complexes which exhibited anti-microbial, anti-oxidant and anti-tubercular activities [80]. Patel *et al*. reported ternary complexes of fourth generation flouroquinolone which act as anti-oxidant [81].

Cu(II) Complexes   **Ni(II) Complexes**

R = H (L ^{1}), p-Cl (L ^{2}), p-NO$_2$ (L ^{3}), p-OH (L ^{4})
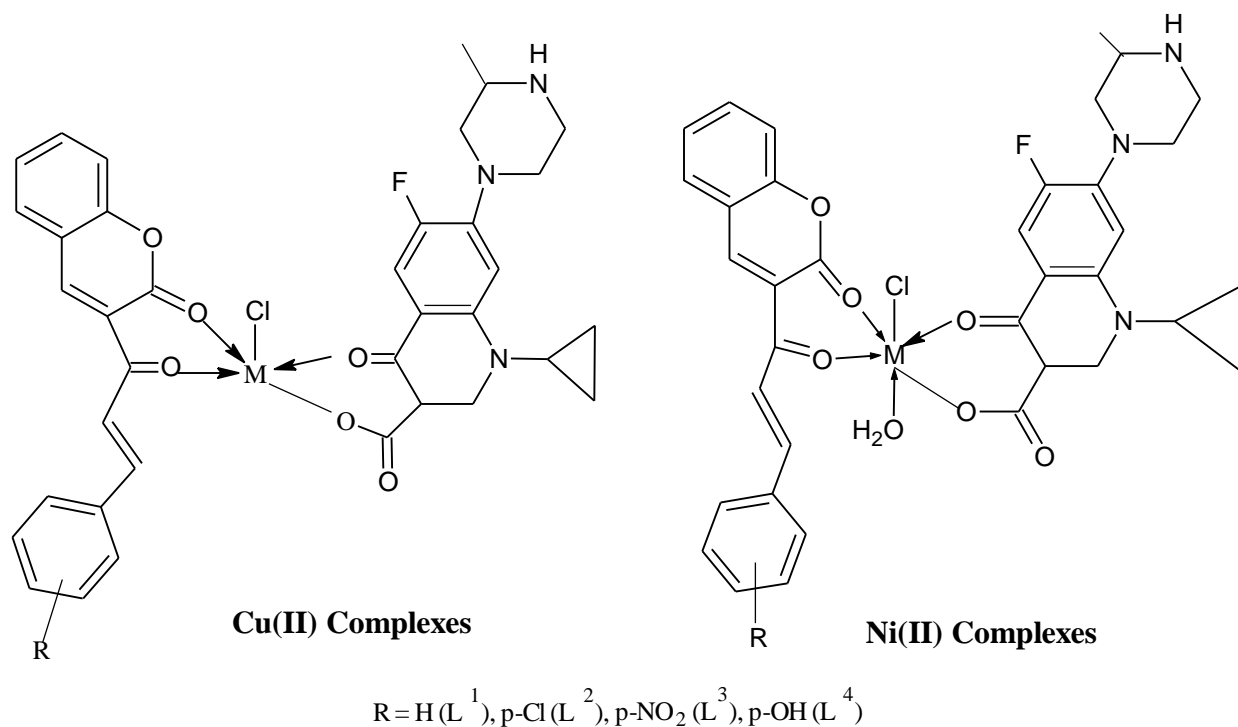
Fig. 1.7: Anti-oxidant Metal Complexes

1.7.3. Anti-Microbial Activity

Mohamed *et al*. reported mixed ligand complexes of lomefloxacin drug and glycine with transition metals as antifungal and antimicrobial agent [51]. El-Gamel and Zayed described the synthesis of binary and ternary complexes of sparfloxacin with *dl*-alanine and reported their anti-microbial activity [82].
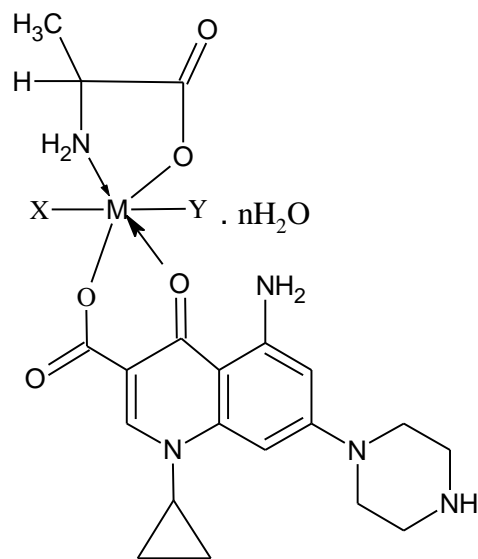
M = Cr (III),Fe (III), X = Cl,Y = H_2O, n =1
M = Cu (II), Co (II), Ni (II), Mn (II), X = Y = H_2O, n = 0
M = La (III), X = NO_3 Y = H_2O, n =1

Fig. 1.8: Anti-microbial Metal Complexes

1.8. Objective of the Work

a) To conduct the synthesis of ternary complexes of Ni (II), Cu (II), Co (III), Fe (III) by using ligands, flouroquinolones (ciprofloxacin, levofloxacin and moxifloxacin) with amino acid (glycine) as complexing agents.

b) To characterize and determine the geometry of synthesized complexes, using melting point, FTIR, UV-vis spectroscopic techniques and DFT study.

c) To carry out biological screening of synthesized complexes.

Chapter 2

2.1. Experimental

2.2. Chemicals

Analytical grade chemicals were used. The drugs used were: Levofloxacin, Moxifloxacin, and Ciprofloxacin. Nickel chloride hexa-hydrate ($NiCl_2.6H_2O$), copper nitrate tri-hydrate ($Cu(NO_3)_2.3H_2O$), cobalt chloride hexa-hydrate ($CoCl_3.6H_2O$), ferric chloride hexa-chloride ($FeCl_3 .6H_2O$), glycine, sodium hydroxide (NaOH) and solvents like distilled water, ethanol, methanol, diethyl ether (Aldrich).

2.3. Instruments

The compounds were weighed through electronics analytical balance ATY224. The indication of formation of synthesized complexes was checked by determining their respective melting points on melting point apparatus (SMP10, SN: R000111020). Bruker ATR FTIR spectrophotometer was used to record the infrared (FTIR) spectra in the range of 4000-400 cm^{-1}. UV 2800 spectrophotometer was used to record the UV-vis spectra of complexes in the range 800-200 nm.

2.4. Synthesis of Ternary Metal Complexes

Various ternary complexes were prepared by using four different metal salts, three quinolones drugs and amino acid.

Following steps are involved to synthesize desired compound.

i)   Reaction between metal salt and drug.
ii)   Reaction of drug salt complex with amino acid.

2.4.1. General Procedure for the Synthesis of Ternary Metal Complexes

1mL of 1M NaOH was added to 1mmol of drug (0.37 g LEV, 0.41 g MOX, 0.36 g CIP), further 1mmol methanolic solution of metal salt (0.237g $NiCl_2.6H_2O$, 0.241g $Cu(NO_3)_2.3H_2O$, 0.237g $CoCl_3.6H_2O$, 0.270 g $FeCl_3 .6H_2O$) was added and stirred for 30 minutes. After that 1mmol (0.075 g) methanolic solution of glycine at pH 9 was added

to metal drug complex. The overall pH of the reaction mixture was maintained at 6.2. It was then refluxed for 3 hours. The resulting solution was concentrated to half of its volume by evaporation under reduced pressure. The coloured product obtained was washed with ether and air dried.
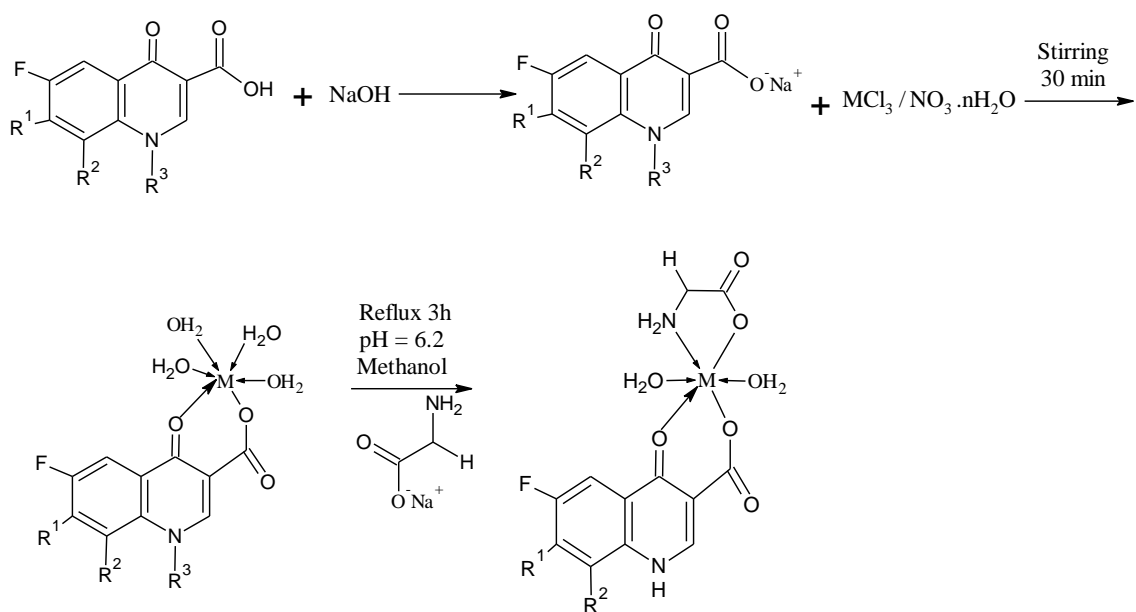
MOX: $R_1 = C_7N_2H_{14}$, $R_2 = OCH_3$, $R_3 = C_3H_6$

LEV: $R_1 = C_5N_2H_{12}$, and R_2-C_5OH_{12}-R_3

CIP: $R_1 = C_4N_2H_{10}$, $R_2 = H$, $R_3 = C_3H_6$

Scheme 2.1: General Reaction of Synthesis of Metal Complexes

2.4.1.1. [Ni(MOX)(Gly)(H₂O)₂] (1)

Nickel(II) glycine moxifloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, Green colour microcrystalline. Yield 95 %. m. p. 273 °C. $C_{23}H_{31}N_3FNiO_8$ (555 g/mol). FT-IR (ν_{max}, cm^{-1}): 1706 (C=O), 558 (Ni-O), 456 (Ni-N).

2.4.1.2. [Ni(LEV)(Gly)(H₂O)₂]   (2)

Nickel(II) glycine levofloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, Dark green colour powder. Yield 92%. m. p. 260 °C. $C_{20}H_{27}FN_4NiO_8$ (529 g/mol). FT-IR (v_{max},cm^{-1}) : Disappear (C=O), 578 (Ni-O), 435 (Ni-N).

2.4.1.3. [Ni(CIP)(Gly)(H₂O)₂]   (3)

Nickel(II) glycine ciprofloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, Light green powder. Yield 66 %. m. p. 240 °C. $C_{19}H_{25}FN_4NiO_7$ (499 g/mol). FT-IR (v_{max}, cm^{-1}): Disappear (C=O), 540 (Ni-O), 455 (Ni-N).

2.4.1.4. [Co(MOX)(Gly)(H₂O)₂].Cl   (4)

Cobalt(III) glycine moxifloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes Reaction time: 3 hours 30 min, light orange powder. Yield 87%. m. p. 231 °C. $C_{23}H_{31}N_3FCoO_8Cl$ (591 g/mol). FT-IR (v_{max}, cm^{-1}): 1700 (C=O), 544 (Co-O), 440 (Co-N). UV-vis (H₂O, λ_{max}, nm): 292, 336, 685.

2.4.1.5. [Co(LEV)(Gly)(H₂O)].Cl   (5)

Cobalt(III) glycine levofloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, dark purple shiny solid. Yield 84 %. m. p. 245 °C. $C_{20}H_{27}FN_4CoO_8Cl$ (565 g/mol). FT-IR (v_{max}, cm^{-1}): disappear (C=O), 517 (Co-O), 430 (Co-N). UV-vis (H₂O, λ_{max}, nm): 276, 317, 678.

2.4.1.6. [Co(CIP)(Gly)(H₂O)].Cl   (6)

Cobalt(III) glycine ciprofloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, sky blue powder. Yield 82 %. m. p. 267 °C. $C_{19}H_{25}FN_4CoO_7Cl$ (535 g/mol). FT-IR (v_{max}, cm^{-1}): 1700 (C=O), 440 (Co-O), 432 (Co-N). UV-vis (H₂O, λ_{max}, nm): 287, 335, 684.

2.4.1.7. [Fe(MOX)(Gly)(H₂O)].Cl   (7)

Iron(III) glycine moxifloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, dark brown microcrystalline. Yield 89 %. m. p. 226 °C. $C_{23}H_{31}N_3FFeO_8Cl$ (588 g/mol). FT-IR (v_{max}, cm^{-1}): 1700 (C=O), 540 (Fe-O), 432 (Fe-N). UV-vis (H₂O, λ_{max}, nm): 292, 363, 501.

2.4.1.8. [Fe(LEV)(Gly)(H₂O)].Cl   (8)

 Iron(III) glycine levofloxacin reaction complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, black brown shiny microcrystalline. Yield 81 %. m. p. 218 °C. $C_{20}H_{27}FN_4FeO_8Cl$ (562 g/mol). FT-IR (v_{max}, cm^{-1}): disappear (C=O), 542 (Fe-O), 441 (Fe-N). UV-vis (H₂O, λ_{max}, nm): 227, 294, 505.

2.4.1.9. [Fe(CIP)(Gly)(H₂O)].Cl   (9)

Iron(III) glycine ciprofloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, red brown powder. Yield 74 %. m. p. 280 °C. $C_{19}H_{25}FN_4FeO_7Cl$ (532 g/mol). FT-IR (v_{max}, cm^{-1}): 1700 (C=O), 536 (Fe-O), 430 (Fe-N). UV-vis (H₂O, λ_{max}, nm): 275, 354, 445.

2.4.1.10. [Cu(MOX)(Gly)(H₂O)₂]   (10)

Copper(II) glycine moxifloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, green powder. Yield 88 %. m. p. 230 °C. $C_{23}H_{31}N_3FCuO_8$ (560 g/mol). FT-IR (v_{max}, cm^{-1}): 1700 (C=O), 549 (Cu-O), 420 (Cu-N). UV-vis (H₂O, λ_{max}, nm): 294, 335, 603.

2.4.1.11. [Cu(LEV)(Gly)(H₂O)₂]   (11)

Copper (II) glycine levofloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, sea green powder. Yield 93%. m. p. 214 °C. $C_{20}H_{27}FN_4CuO_8$ (534 g/mol). FT-IR (v_{max}, cm^{-1}): disappear (C=O), 550 (Cu-O), 421 (Cu-N). UV-Vis (H₂O, λ_{max}, nm): 292, 324, 573.

2.4.1.12. [Cu(CIP)(Gly)(H₂O)₂]   (12)

Copper (II) glycine ciprofloxacin complex was prepared as mentioned in the general procedure for the synthesis of complexes. Reaction time: 3 hours 30 min, blue microcrystalline. Yield 61 %. m. p. 260 °C. $C_{19}H_{25}FN_4CuO_7$ (504 g/mol). FT-IR (v_{max}, cm^1): disappear (C=O), 560 (Cu-O), 470 (Cu-N). UV-Vis (H₂O, λ_{max}, nm): 272, 239, 638.

2.5. Computational Studies

Geometry optimization was carried out for all the complexes of Ni(II), Co(III), Cu(II) and Fe(III). All the optimized structure established stable ground state conformers. For all complexes density functional theory (DFT) method comprising the becke 3-parameter exchange functional with Lee-Yang-Parr correlation functional (B3LYP) combined with 6-31G (d) basis set was used [83-84]. It produces geometry similar to crystallographic data from experiments. Guass view 5.0 software was used to draw complexes. Gaussian 09 software was used for geometry optimization [85].

2.6. Biological Activities

2.6.1. Antimicrobial Activity

The antimicrobial activity of the complexes was assayed against the bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella Pnuemonae*, *Pseudomonas aeroginosa*, *Acenitobacter* and MRSA using well diffusion method and Disk diffusion test. The complexes **1, 2, 4, 5, 7, 8, 10** and **11** were dissolved in methanol while complexes **6** and **9** were dissolved in autoclaved water while complexes **3** and **12** were dissolved in methanol for disk diffusion method and in DMSO for well diffusion study.

2.6.1.1. Well Diffusion Test

Each complex was dissolved in small volume of solvent to make sample solution. Sample size for all the compounds was fixed. The wells were made by agar medium in a petri dish, which was already inoculated with the microorganisms. The solution of each complex was added in the wells and petri dishes were subsequently incubated.

Kanamycin and Ciprofloxacin were used as standard drugs for antimicrobial study. Zone of inhibition produced by each complex was measured in mm.

2.6.1.2. Disk Diffusion Test

In this test agar plates were inoculated with microorganism. Then, filter paper discs, containing complexes were placed on the agar surface. The Petri dishes are incubated under favorable conditions. Complexes diffuse into the agar and inhibit germination and growth of the test microorganism and then the diameters of inhibition growth zones were measured. Kanamycin and Ciprofloxacin were also used as standard drugs for this test.

2.6.2. Cytotoxic Assay

The cytotoxicity assay was carried out with larvae *Artemia Salina*. All the samples were diluted in 50% Cell culture DMSO in ratio of 5mg/mL and 10 µL of this sample prepared was used in cytotoxicity assay. The larvae were then distributed in solution of complexes to be tested. The mortality was determined after 24 hours of exposition of the larvae to the solution. The experimental design was random.

Chapter 3

3.1. Results and Discussion

In this chapter, physical data and the results related to spectral studies such as FT-IR, UV-vis, DFT and biological studies of the metal complexes are discussed. A systematic study of reactions of metal salts with quinolone drugs and amino acid with mole ratio 1:1:1 in methanol may be represented by the following equation.

$$MCl_3 /NO_3.nH_2O + L_1 \xrightarrow[\substack{ii) L_2, \ pH = 6.2 \\ Reflux \ 3 \ h}]{i)30 \ min \ Stirring} [M \ L_1L_2]^{n+}$$
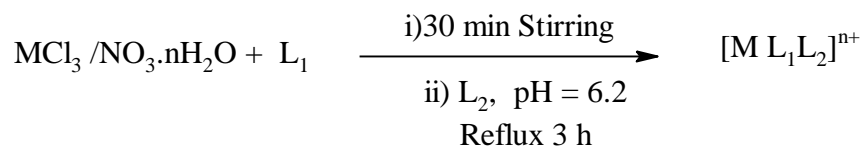
Where,
M = Ni (II), Co (III), Fe (III) and Cu (II)

L_1 = MOX, LEV, CIP
L_2 = Gly

Scheme3.1: General Reaction of Synthesis of Complexes

The reaction was reported procedure by Singh *et al.* 2012 [71]. They prepared only ternary complexes of copper. We used four different metal salts for synthesis i.e. $Cu(NO_3)_2 .3H_2O$, $NiCl_3.6H_2O$, $FeCl_3.6H_2O$, $CoCl_3.6H_2O$. Furthermore, the ligands used in our synthesis of ternary complexes are entirely different, quinolone drugs (MOX, LEV, CIP) and amino acid (glycine). The reaction conditions are optimized by 3 hours reflux and pH is maintained at 6.2 using few drops of 1M NaOH. The obtained microcrystalline complexes are found to be stable in air. Along with this the anti-bacterial activity of synthesized complexes is checked. The techniques by which efforts have been made to through light on the synthesis and geometry of synthesized ternary complexes are UV-vis, FT-IR along with DFT studies.

3.2. Physical Characteristics

All ternary complexes are colored, thermodynamically stable at room temperature and non-hygroscopic. Melting point and the color of the complexes may be linked to the coordination to the metal ions.

Table 3.1: General Physical Properties of Ternary Complexes (1-12)

Sr No:	Compound	Colour	Physical appearance	MW (g/mol)	M. P. (ºC)	Yield (%)	Solubility
1	[Ni(MOX)(Gly)(H$_2$O)$_2$]	Green	Microcrystalline	555	273	95	H$_2$O, CH$_3$OH, DMSO
2	[Ni(LEV)(Gly)(H$_2$O)$_2$]	Dark green	Powder	529	260	92	H$_2$O, CH$_3$OH, DMSO
3	[Ni(CIP)(Gly)(H$_2$O)$_2$]	Light green	Powder	499	240	66	DMSO
4	[Co(MOX)(Gly)(H$_2$O)$_2$]Cl	Light orange	Powder	591	231	87	H$_2$O, CH$_3$OH, DMSO
5	[Co(LEV)(Gly)(H$_2$O)2].Cl	Dark purple	Shiny solid	565	245	84	H$_2$O, CH$_3$OH, DMSO
6	[Co(CIP)(Gly)(H$_2$O)].Cl	Sky blue	Powder	535	267	82	DMSO
7	[Fe(MOX)(Gly)(H$_2$O)].Cl	Dark brown	Shiny microcrystalline	588	226	89	H$_2$O, CH$_3$OH, DMSO
8	[Fe(LEV)(Gly)(H$_2$O)].Cl	Black brown	Shiny microcrystalline	562	218	81	H$_2$O, CH$_3$OH, DMSO
9	[Fe(CIP)(Gly)(H$_2$O)].Cl	Red brown	Powder	532	280	74	H$_2$O, DMSO

10	[Cu(MOX)(Gly)(H$_2$O)$_2$]	Green	Powder	560	230	88	H$_2$O, CH$_3$OH, DMSO
11	[Cu(LEV)(Gly)(H$_2$O)$_2$]	Sea green	Powder	534	214	93	H$_2$O, CH$_3$OH, DMSO
12	[Cu(CIP)(Gly)(H$_2$O)$_2$]	Blue	Microcrystalline	504	260	61	DMSO

3.3. FT-IR Spectra

Information regarding the nature of the binding mode and functional groups attached to the metal ion is obtained from FT-IR spectra. So, the IR spectra of the free ligands were compared with the spectra of the all complexes as compiled in Table 3.2. The FTIR data of free ligands and their ternary complexes were carried out in the range 4000-400 cm^{-1}. Strong band in the region of 1725-1709 cm^{-1} corresponds to the stretching vibrations of carboxyl carbonyls (C=O in COOH). This band is found in the spectra of complexes at 1700-1739 cm^{-1} or disappeared in some complexes (Fig: 3.1-3.2) showing coordination of carbonyl oxygen with metal [86]. IR spectra of complexes have strong broad band at the region 3400 cm^{-1} was assigned to the -OH which indicated the presence of coordinated water molecule [51]. The COO asymmetrical stretching vibrations were observed at 1591cm^{-1} for free ligands CIP. There is a shift in position of COO asymmetrical stretch 1530-1590 cm^{-1} indicating coordination of carboxylate oxygen atom with metal [87].

In free ligands MOX and LEV the COO stretching vibration is not observed but on coordination with metal two strong bands are observed as asymmetric and symmetric vibrations indicating the coordination to the metal ion. The difference between these two bands indicates a monodentate coordination mode of the carboxylate group [88-89]. Free amino acid act as zwitterions, so its IR frequency lies in the range of 3130-3030 cm^{-1} due to NH$_3$ stretching. In complex formation NH$_3$ gets deprotonated and binds to metal by NH$_2$ group in the range from 3500-3300 cm^{-1} [91]. FT-IR spectra of all ternary complexes showed a band in range 3300-3450 cm^{-1} indicated that nitrogen of amino group was involved in coordination. In contrast to free amino acid, the COO asymmetric

Table 3.2: FT-IR Spectral Data of the Ligands and its Complexes

Sr No:	Compound	Asym. NH_2 + OH Stretch cm^{-1}	C=O stretch cm^{-1}	COO asym cm^{-1}	M-O cm^{-1}	M-O in coordinated water cm^{-1}	M-N cm^{-1}
1.	MOX	3531s	1709s	-	-	-	-
2.	LEV	3269s	1725s	-	-	-	-
3.	CIP	3525s	1708s	1591m	-	-	-
4.	Glycine	3169s	1703s	1556m	-	-	-
5.	[Ni(MOX)(Gly)(H$_2$O)$_2$]	3343 br	1706m	1514m	558sm	520sm	456sm
6.	[Ni(LEV)(Gly)(H$_2$O)$_2$]	3355sm	Disappear	1525m	578sm	450sm	435sm
7.	[Ni(CIP)(Gly)(H$_2$O)$_2$]	3399br	Disappear	1570m	540sm	480sm	455sm
8.	[Co(MOX)(Gly)(H$_2$O)$_2$].Cl	3350br	1700s	1517m	544sm	450sm	440sm
9.	[Co(LEV)(Gly)(H$_2$O)$_2$].Cl	3344br	Disappear	1567m	517sm	460sm	430sm
10.	[Co(CIP)(Gly)(H$_2$O)$_2$].Cl	3340m	1700m	1569m	540sm	440sm	432sm
11.	[Fe(MOX)(Gly)(H$_2$O)$_2$].Cl	3350br	1700s	1509m	552sm	518sm	430sm
12.	[Fe(LEV)(Gly)(H$_2$O)$_2$].Cl	3340br	Disappear	1573m	552sm	509sm	441sm
13.	[Fe(CIP)(Gly)(H$_2$O)$_2$].Cl	3350br	1700s	1575m	536sm	511sm	430sm
14.	[Cu(MOX)(Gly)(H$_2$O)$_2$]	3349br	1700m	1519m	549sm	485sm	420sm
15.	[Cu(LEV)(Gly)(H$_2$O)$_2$]	3434br	Disappear	1572m	532sm	508sm	421sm
16.	[Cu(CIP)(Gly)(H$_2$O)$_2$]	3435br	Disappear	1560m	580sm	540sm	470sm

s = strong, sm = small, br = broad, m = medium

vibrations in complexes show shift in values indicating carboxylate group was chelated to metal. In far-IR range 580-400 cm^{-1}, low intensity bands were observed due to M-O and M-N stretching vibrations.

From FT-IR study, it is indicated that MOX, LEV and CIP act as bidentate ligand via carbonyl oxygen and carboxylic oxygen. Glycine also behaves as bidentate ligand and chelated to metal via amino group N and carboxylic oxygen.
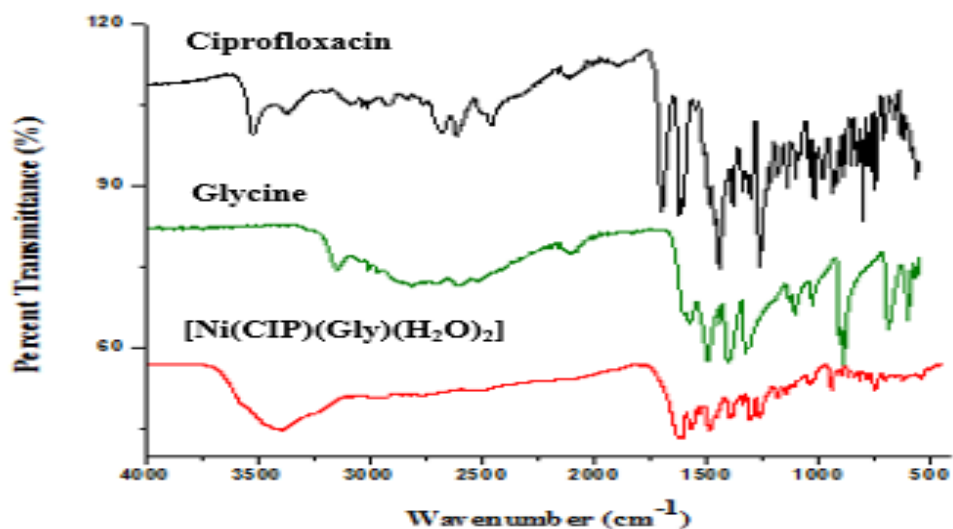
Fig 3.1:   FT-IR Spectrum of [Ni(CIP)(Gly)(H$_2$O)$_2$] (3)
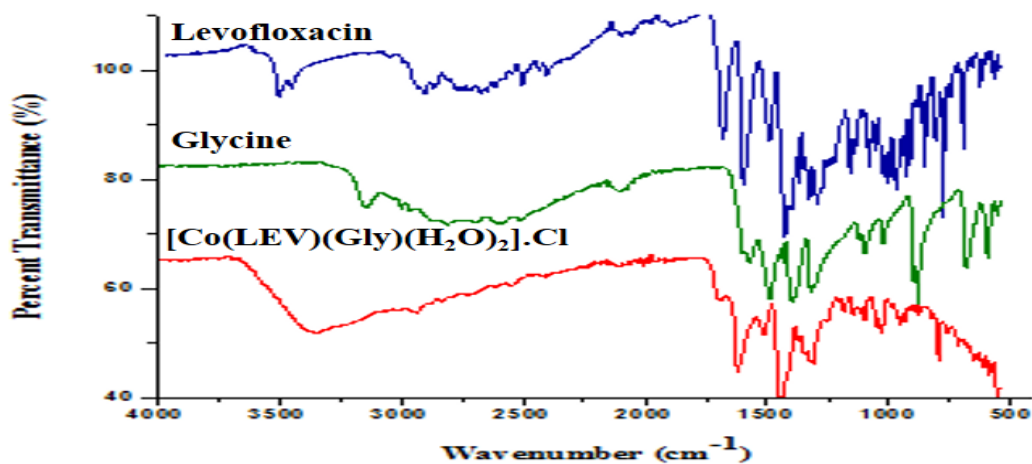
Fig 3.2:   FT-IR Spectrum of [Co(LEV)(Gly)(H$_2$O)$_2$].Cl (9)

3.4. UV-vis Data of Complexes (4-12)

Electronic spectra measurements are convenient for allocating the stereochemistry of the metal complexes on the basis of position of d-d transitions arising due splitting of these orbitals. It is also useful in the analysis of results obtained by other methods of structural

evaluation [91]. The complexes UV-vis spectral data were recorded in water in the wavelength that ranges from 800-200 nm at room temperature.

In the electronic spectrum of the complexes there are two strong absorption bands, which could be allocated to n→π* and π→π* transitions. These intraligand transitions are due to π→π* transition of aromatic system in the ligand and n→π* transition is due to the carboxylate oxygen of ligands [92]. Such transitions are emerged to be fluctuating towards lower and higher frequencies, verifying the coordination of ligands with the metal ions [93].

Table 3.3 UV-vis Spectral Data of Ligands and Complexes

Sr No:	Compound	Solvent	Wavelength (nm)	Band Assignment	Geometry
1.	MOX	H_2O	289	π→π*	-
			340	n→π*	
2.	LEV	H_2O	288	π→π*	-
			332	n→π*	
3.	CIP	H_2O	272	π→π*	-
			326	n→π*	
4.	Glycine		215	π→π*	-
5.	[Co(MOX)(Gly)(H_2O)].Cl	H_2O	292	π→π*	Octahedral
			336	n→π*	
			685	d-d	
7.	[Co(LEV)(Gly)(H_2O)].Cl	H_2O	276	π→π*	Octahedral
			317	n→π*	
			672	d-d	
8.	[Co(CIP)(Gly)(H_2O)].Cl	H_2O	287	π→π*	Octahedral

			335	n→π*	
			684	d-d	
9.	[Fe(MOX)(Gly)(H_2O)].Cl	H_2O	292	π →π*	Octahedral
			363	n→π*	
			501	d-d	
10.	[Fe(LEV)(Gly)(H_2O)].Cl	H_2O	227	π →π*	Octahedral
			294	n→π*	
			505	d-d	
11.	[Fe(CIP)(Gly)(H_2O)].Cl	H_2O	275	π →π*	Octahedral
			354	n→π*	
			445	d-d	
12.	[Cu(MOX)(Gly)(H_2O)$_2$]	H_2O	296	π →π*	Octahedral
			335	n→π*	
			603	d-d	
13.	[Cu(LEV)(Gly)(H_2O)$_2$]	H_2O	294	π →π*	Octahedral
			324	n→π*	
			573	d-d	
14.	[Cu(CIP)(Gly)(H_2O)$_2$]	H_2O	272	π →π*	Octahedral
			239	n→π*	
			638	d-d	
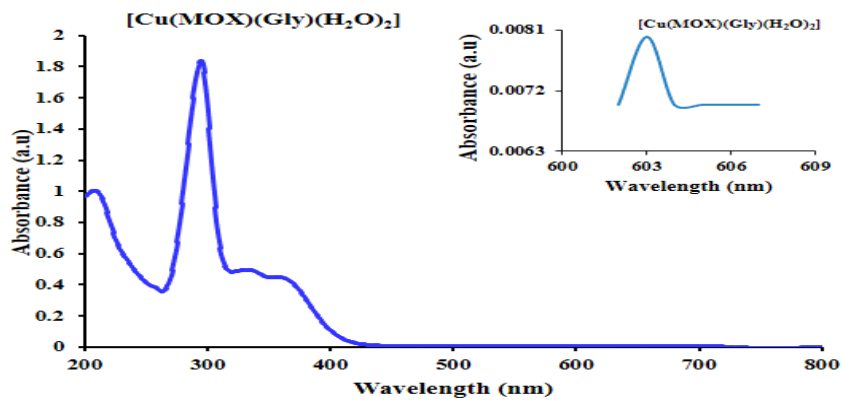
Fig. 3.3: UV-vis Spectrum of [Cu(MOX)(Gly)(H_2O)$_2$]
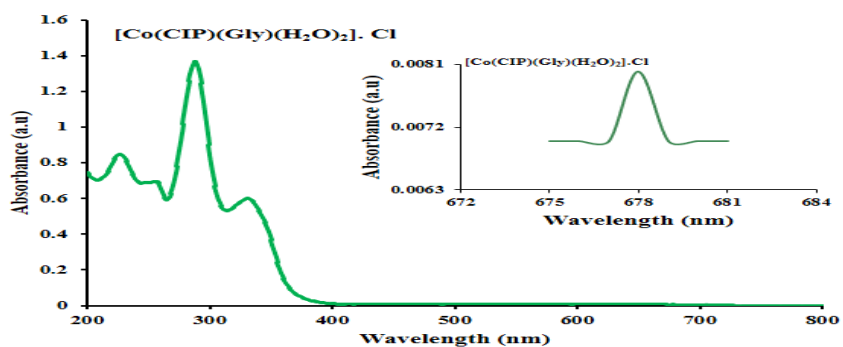
Fig. 3.4: UV-vis Spectrum of [Co(CIP)(Gly)(H$_2$O)].Cl
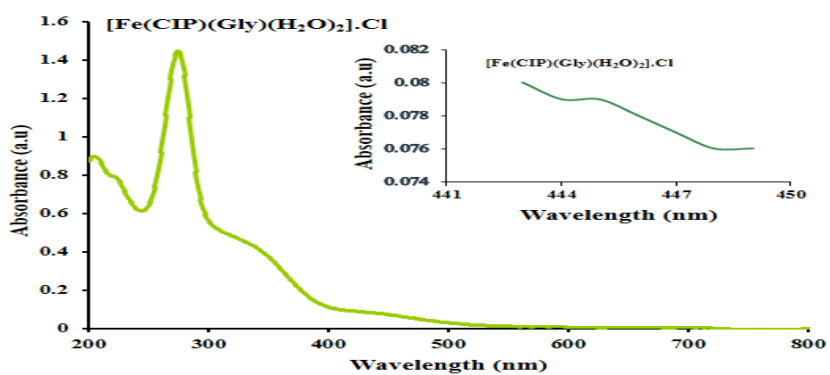
Fig. 3.5: UV-vis Spectrum of [Fe(CIP)(Gly)(H$_2$O)].Cl
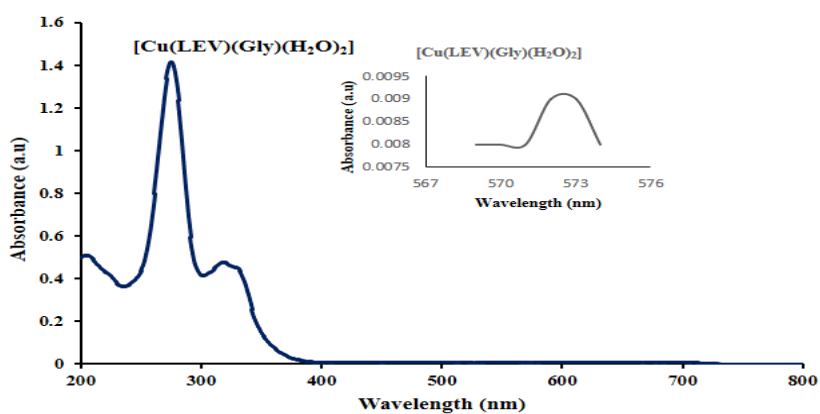
Fig. 3.6: UV-vis Spectrum of [Cu(LEV)(Gly)(H$_2$O)$_2$]

The complexes of copper show a probable d-d transition band a small peak has appeared at 570-638 nm which explains their octahedral geometry [73,94]. The complexes of cobalt show a probable d-d transition at 678-685 nm [95] and complexes of iron have d-d transition at start of visible region range from 445-505 nm [51,73]. This shows all ternary complexes have octahedral geometry.

3.5. DFT Studies

In order to have better understanding of bonding we have carried out DFT calculations on all the complexes. Selected bond distances and angles for all the complexes are compiled in Table 3.4-3.7. Results of DFT study, shows complexes adopts octahedral geometry, where quinolone drugs (MOX, LEV and CIP) and amino acid (glycine) act as bidentate ligand. Around metal ions the ligands gave square planar arrangement. While the axial sites were being occupied by water molecules.
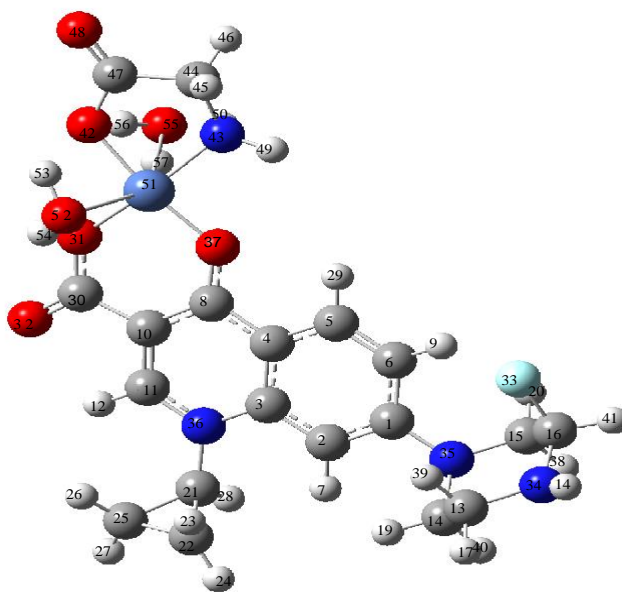
Fig. 3.7: Optimized Structure of [Ni(CIP)(Gly)(H₂O)₂] (3)
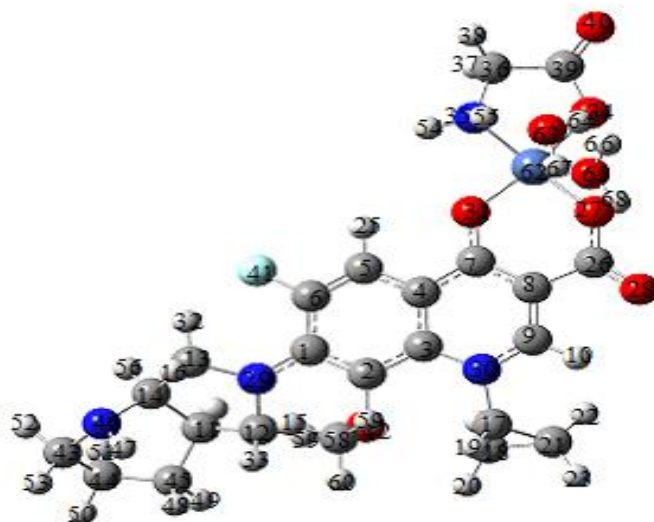
Fig. 3.8: Optimized Structure of [Ni(MOX)(Gly)(H₂O)₂] (1)
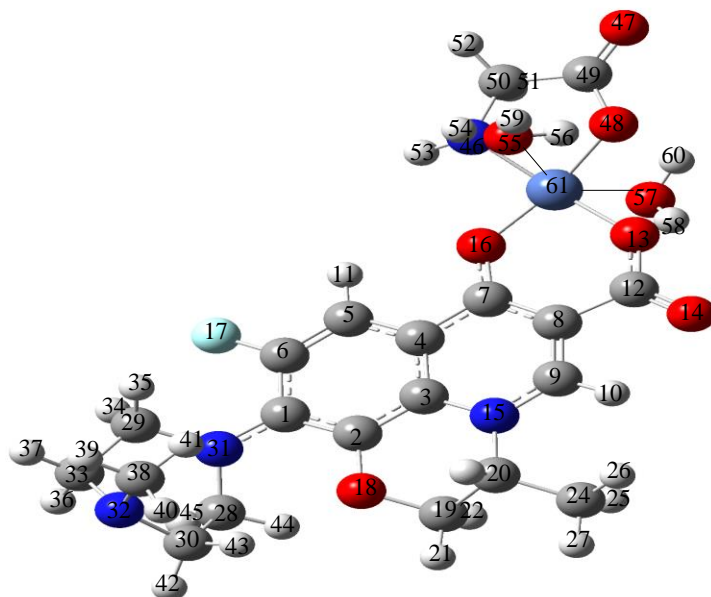
Fig. 3.9: Optimized Structure of [Ni(LEV)(Gly)(H₂O)₂] (2)

Carbon   Oxygen   Nickel   Hydrogen   Nitrogen

Table 3.4: Calculated Bond Distances (Å), Angles (°) and Hartee-Fock Energy (kcal mol^{-1}) of the Ni(II) Complexes (1-3) Computed by B3LYP/6-31G (d)

Parameter	Complex	Parameter	Complex	Parameter	Complex
Bond distance (Å)	Ni-CIP-Gly	Bond distance (Å)	Ni-MOX-Gly	Bond distance (Å)	Ni-LEV-Gly
$Ni_{51}-N_{43}$	1.847	$Ni_{62}-N_{35}$	1.846	$Ni_{61}-N_{46}$	1.853
$Ni_{51}-O_{31}$	1.833	$Ni_{62}-O_{34}$	1.849	$Ni_{61}-O_{48}$	1.839
$Ni_{51}-O_{37}$	1.785	$Ni_{62}-O_{31}$	1.786	$Ni_{61}-O_{16}$	1.786
$Ni_{51}-O_{42}$	1.835	$Ni_{62}-O_{27}$	1.822	$Ni_{61}-O_{13}$	1.829
$Ni_{51}-O_{52}$ (H_2O)	2.447	$Ni_{62}-O_{63}$(H_2O)	2.767	$Ni_{61}-O_{55}$ (H_2O)	2.780
$Ni_{51}-O_{55}$(H_2O)	2.813	$Ni_{62}-O_{65}$(H_2O)	2.453	$Ni_{61}-O_{57}$ (H_2O)	2.423
Bond angles (°)		Bond angles (°)		Bond angles (°)	
$O_{37}-Ni_{51}-N_{43}$	87.98	$O_{34}-Ni_{62}-N_{35}$	84.14	$O_{16}-Ni_{61}-N_{46}$	87.53
$O_{31}-Ni_{51}-O_{42}$	91.05	$O_{31}-Ni_{62}-O_{27}$	96.82	$O_{13}-Ni_{61}-O_{48}$	89.69
$N_{43}-Ni_{51}-O_{42}$	83.82	$O_{31}-Ni_{62}-N_{35}$	88.54	$O_{48}-Ni_{61}-N_{46}$	83.40
$O_{31}-Ni_{51}-O_{37}$	96.52	$O_{27}-Ni_{62}-O_{34}$	92.51	$O_{13}-Ni_{61}-O_{16}$	96.62
$O_{52}-Ni_{51}-O_{42}$	79.76	$O_{63}-Ni_{62}-O_{34}$	69.63	$O_{55}-Ni_{61}-O_{48}$	67.17
$O_{52}-Ni_{51}-N_{43}$	126.24	$O_{63}-Ni_{62}-N_{35}$	68.74	$O_{55}-Ni_{61}-N_{46}$	65.95
$O_{55}-Ni_{51}-O_{42}$	67.73	$O_{65}-Ni_{62}-O_{31}$	103.69	$O_{57}-Ni_{61}-O_{48}$	83.20
$O_{55}-Ni_{51}-O_{31}$	88.38	$O_{65}-Ni_{62}-O_{27}$	82.19	$O_{57}-Ni_{61}-O_{16}$	108.99
Hartee-Fock Energy (kcal mol^{-1})	-1,940,703.8		-2,075,162.85		-2,002,341.68

Table 3.5: Calculated Bond Distances (Å), Angles (°) and Hartee-Fock Energy (kcal mol^{-1}) of the Co(III) Complexes (4-6) Computed by B3LYP/6-31G (d)

Parameter	Complex	Parameter	Complex	Parameter	Complex
Bond distance (Å)	Co-CIP-Gly	Bond distance (Å)	Co-MOX-Gly	Bond distance (Å)	Co-LEV-Gly
$Co_{50}-N_{42}$	1.918	$Co_{68}-N_{35}$	1.926	$Co_{61}-N_{46}$	1.928
$Co_{50}-O_{31}$	1.885	$Co_{68}-O_{34}$	2.010	$Co_{61}-O_{48}$	1.998
$Co_{50}-O_{36}$	1.817	$Co_{68}-O_{31}$	1.838	$Co_{61}-O_{16}$	1.907
$Co_{50}-O_{41}$	1.905	$Co_{68}-O_{27}$	1.896	$Co_{61}-O_{13}$	1.839
$Co_{50}-O_{52}$ (H_2O)	2.206	$Co_{68}-O_{62}$ (H_2O)	2.193	$Co_{61}-O_{55}$ (H_2O)	2.216
$Co_{51}-O_{53}$ (H_2O)	2.302	$Co_{68}-O_{64}$ (H_2O)	2.195	$Co_{61}-O_{57}$ (H_2O)	2.182
Bond angles (°)		Bond angles (°)		Bond angles (°)	
$O_{41}-Co_{50}-N_{42}$	84.99	$O_{34}-Co_{68}-N_{35}$	82.79	$O_{16}-Co_{61}-N_{46}$	86.29
$O_{31}-Co_{50}-O_{36}$	96.52	$O_{31}-Co_{68}-O_{27}$	94.90	$O_{13}-Co_{61}-O_{48}$	99.37
$N_{42}-Co_{50}-O_{36}$	88.83	$O_{31}-Co_{68}-N_{35}$	86.18	$O_{48}-Co_{61}-N_{46}$	82.90
$O_{31}-Co_{50}-O_{41}$	92.92	$O_{27}-Co_{68}-O_{34}$	99.68	$O_{13}-Co_{61}-O_{16}$	94.90
$O_{52}-Co_{50}-O_{41}$	74.44	$O_{63}-Co_{68}-O_{34}$	76.21	$O_{55}-Co_{61}-O_{48}$	76.25
$O_{52}-Co_{50}-N_{42}$	113.30	$O_{62}-Co_{68}-N_{35}$	86.41	$O_{55}-Co_{61}-N_{46}$	85.51
$O_{53}-Co_{50}-O_{41}$	84.86	$O_{64}-Co_{68}-O_{31}$	98.82	$O_{57}-Co_{61}-O_{48}$	73.80
$O_{53}-Co_{51}-O_{31}$	75.27	$O_{64}-Co_{68}-O_{27}$	86.97	$O_{57}-Co_{61}-O_{16}$	99.46
Hartee-Fock Energy (kcal mol^{-1})	-1,852,463.04		-1,996,778.27		-1,923,957.74

Table 3.6: Calculated Bond Distances (Å), Angles (°) and Hartee-Fock Energy (kcal mol^{-1}) of the Fe(III) Complexes (7-9) Computed by B3LYP/6-31G (d)

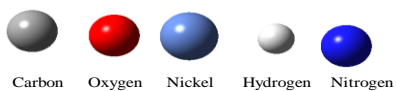| Parameter	Complex	Parameter	Complex	Parameter	Complex
Bond distance (Å)	Fe-CIP-Gly	Bond distance (Å)	Fe-MOX-Gly	Bond distance (Å)	Fe-LEV-Gly
Fe_{57}-N_{43}	1.951	Fe_{68}-N_{35}	1.956	Fe_{61}-N_{46}	1.956
Fe_{57}-O_{31}	1.895	Fe_{68}-O_{34}	1.964	Fe_{61}-O_{48}	1.966
Fe_{57}-O_{37}	1.833	Fe_{68}-O_{31}	1.837	Fe_{61}-O_{16}	1.837
Fe_{57}-O_{42}	1.844	Fe_{68}-O_{27}	1.930	Fe_{61}-O_{13}	1.929
Fe_{57}-O_{51} (H_2O)	1.988	Fe_{68}-O_{62} (H_2O)	1.999	Fe_{61}-O_{55} (H_2O)	1.998
Fe_{57}-O_{52} (H_2O)	3.171	Fe_{68}-O_{64} (H_2O)	2.023	Fe_{61}-O_{57} (H_2O)	2.025
Bond angles (°)		Bond angles (°)		Bond angles (°)	
O_{37}-Fe_{57}-N_{43}	103.02	O_{34}-Fe_{68}-N_{35}	82.95	O_{16}-Fe_{61}-N_{46}	86.92
O_{31}-Fe_{57}-O_{42}	131.67	O_{31}-Fe_{68}-O_{27}	93.79	O_{13}-Fe_{61}-O_{48}	95.98
N_{43}-Fe_{57}-O_{42}	84.53	O_{31}-Fe_{68}-N_{35}	86.65	O_{48}-Fe_{61}-N_{46}	82.95
O_{31}-Fe_{57}-O_{37}	93.71	O_{27}-Fe_{68}-O_{34}	96.57	O_{13}-Fe_{61}-O_{16}	94.10
O_{51}-Fe_{57}-O_{42}	76.82	O_{62}-Fe_{68}-O_{34}	75.24	O_{55}-Fe_{61}-O_{48}	75.27
O_{51}-Fe_{57}-N_{43}	157.13	O_{62}-Fe_{68}-N_{35}	90.23	O_{55}-Fe_{61}-N_{46}	89.81
O_{52}-Fe_{57}-O_{42}	89.43	O_{64}-Fe_{68}-O_{31}	101.29	O_{57}-Fe_{61}-O_{48}	82.67
O_{52}-Fe_{57}-O_{31}	54.38	O_{64}-Fe_{68}-O_{27}	76.66	O_{57}-Fe_{61}-O_{16}	100.98
Hartee-Fock Energy (kcal mol^{-1})	-1,778,107.57		-1,922,423.81		-1,849,603.02

Table 3.7: Calculated Bond Distances (Å), Angles (°) and Hartee-Fock Energy (kcal mol^{-1}) of the Cu(II) Complexes (10-12) Computed by B3LYP/6-31G (d)

Parameter	Complex	Parameter	Complex	Parameter	Complex
Bond distance (Å)	Cu-CIP-Gly	Bond distance (Å)	Cu-MOX-Gly	Bond distance (Å)	Cu-LEV-Gly
Cu_{51}-N_{43}	1.938	Cu_{68}-N_{35}	1.926	Cu_{17}-N_{47}	1.953
Cu_{51}-O_{31}	1.935	Cu_{68}-O_{34}	2.010	Cu_{17}-O_{49}	1.934
Cu_{51}-O_{37}	1.925	Cu_{68}-O_{31}	1.838	Cu_{17}-O_{16}	1.907
Cu_{51}-O_{42}	1.835	Cu_{68}-O_{27}	1.896	Cu_{17}-O_{13}	1.919
Cu_{51}-O_{52} (H_2O)	1.979	Cu_{68}-O_{62} (H_2O)	2.193	Cu_{17}-O_{56} (H_2O)	2.967
Cu_{51}-O_{53}(H_2O)	2.915	Cu_{68}-O_{64} (H_2O)	2.195	Cu_{17}-O_{58} (H_2O)	2.065
Bond angles (°)		Bond angles (°)		Bond angles (°)	
O_{37}-Cu_{51}-N_{43}	94.69	O_{34}-Cu_{68}-N_{35}	82.79	O_{16}-Cu_{17}-N_{47}	87.45
O_{31}-Cu_{51}-O_{42}	107.98	O_{31}-Cu_{68}-O_{27}	94.90	O_{13}-Cu_{17}-O_{49}	99.39
N_{43}-Cu_{51}-O_{42}	82.61	O_{31}-Cu_{68}-N_{35}	88.54	O_{49}-Cu_{17}-N_{47}	81.01
O_{31}-Cu_{51}-O_{37}	92.77	O_{27}-Cu_{68}-O_{34}	92.51	O_{13}-Cu_{17}-O_{16}	91.72
O_{52}-Cu_{51}-O_{42}	74.04	O_{63}-Cu_{68}-O_{34}	69.63	O_{56}-Cu_{17}-O_{48}	59.62
O_{52}-Cu_{51}-N_{43}	131.74	O_{63}-Cu_{68}-N_{35}	68.74	O_{56}-Cu_{17}-N_{47}	61.79
O_{53}-Cu_{51}-O_{42}	74.93	O_{65}-Cu_{62}-O_{31}	103.69	O_{58}-Cu_{17}-O_{48}	100.76
O_{53}-Cu_{51}-O_{31}	61.31	O_{65}-Cu_{62}-O_{27}	82.19	O_{58}-Co_{61}-O_{16}	119.85
Hartee-Fock Energy (kcal mol^{-1})	-2,013,390.13		-2,084,876.15		-2,002,341.68

From the DFT study, it is concluded that complexes have octahedral geometry, where quinolones (MOX, LEV, CIP) acts as a bidentate ligand via the carboxylate and carbonyl oxygen [M-$O_{(COO)}$ = 2.010-1.833 Å and M-$O_{(C=O)}$ = 1.907-1.785 Å]. The amino acid acts as bidentate ligand with amino N and deprotonated carboxylate O of glycine [M-$O_{(COO)}$= 1.822-1.964 Å and M-$N_{(NH2)}$ = 1.846-1.956 Å]. Two water molecules are in trans position. Hartee-Fock energy value suggests that more stable complexes are

formed with MOX ligand and with respect to metals copper ternary complexes are more stable. Mulliken charges values show the cationic property of metal atoms and anionic property of the ligands surrounding the metal [96] as complied in Table 3.8-3.11.

Table 3.8: Dipole Moments and Mulliken Charges of Ni(II) Complexes (1-3)

Parameters	Complexes		
	Ni-CIP-Gly	**Ni-MOX-Gly**	**Ni-LEV-Gly**
Dipole moment (D)	19.26	12.25	10.43
Mulliken charges	Ni: 0.548	Ni: 0.883	Ni: 0.523
	N_{43}: -0.792	N_{35}: -0.075	N_{46}: -0.806
	O_{42}: -0.622	O_{34}: -0.591	O_{48}: -0.603
	O_{31}: -0.636	O_{31}: -0.696	O_{13}: -0.643
	O_{37}: -0.640	O_{27}: -0.618	O_{16}: -0.654
	O_{52}: -0.789	O_{63}: -0.662	O_{55}: -0.652
	O_{55}: -0.822	O_{65}: -0.664	O_{57}: -0.610

Table 3.9: Dipole Moments and Mulliken Charges of Co(III) Complexes (4-6)

Parameters	Complexes		
	Co-CIP-Gly	**Co-MOX-Gly**	**Co-LEV-Gly**
Dipole moment (D)	11.4	16.36	13.36
Mulliken charges	Co: 0.965	Co: 0.561	Co: 0.559
	N_{42}: -0.793	N_{35}: -0.776	N_{46}: -0.778
	O_{41}: -0.584	O_{34}: -0.614	O_{48}: -0.616
	O_{31}: -0.654	O_{31}: -0.650	O_{13}: -0.653
	O_{36}: -0.718	O_{27}: -0.655	O_{16}: -0.649
	O_{52}: -0.622	O_{62}: -0.639	O_{55}: -0.638

Table 3.10: Dipole Moments and Mulliken Charges of Fe(III) Complexes (7-9)

Parameters	Complexes		
	Fe-CIP-Gly	**Fe-MOX-Gly**	**Fe-LEV-Gly**
Dipole moment (D)	11.94	15.47	11.01
Mulliken charges	Fe: 0.658	Fe: 0.652	Fe: 0.652
	N_{43}: -0.806	N_{35}: -0.757	N_{46}: -0.758
	O_{42}: -0.653	O_{34}: -0.622	O_{48}: -0.621
	O_{31}: -0.683	O_{31}: -0.645	O_{13}: -0.647
	O_{37}: -0.633	O_{27}: -0.649	O_{16}: -0.644
	O_{51}: -0.627	O_{62}: -0.639	O_{55}: -0.640
	O_{52}: -0.656	O_{64}: -0.623	O_{57}: -0.622

Table 3.11: Dipole Moments and Mulliken Charges of Cu(II) Complexes (9-12)

Parameters	Complexes		
	Cu-CIP-Gly	**Cu-MOX-Gly**	**Cu-LEV-Gly**
Dipole moment (D)	17.89	16.36	15.31
Mulliken charges	Cu: 0.548	Cu: 0.561	Cu: 0.551
	N_{43}: -0.100	N_{35}: -0.776	N_{47}: -0.797
	O_{42}: -0.625	O_{34}: -0.614	O_{49}: -0.589
	O_{31}: -0.623	O_{31}: -0.650	O_{13}: -0.657
	O_{37}: -0.617	O_{27}: -0.655	O_{16}: -0.629
	O_{52}: -0.143	O_{62}: -0.639	O_{56}: -0.652
	O_{53}: -0.096	O_{64}: -0.615	O_{58}: -0.643

3.6. Structural Interpretation

Structures of the ternary complexes of MOX, LEV, CIP and glycine with Ni(II), Co(III), Fe(III) and Cu(II) were proved by FT-IR, UV-vis and DFT data. Octahedral geometry is proposed for investigated complexes from all the above observations. It is generally inferred that recommended structural formula of the complexes are compiled as:

a) Proposed structure for complexes of Ni(II), Co(III), Fe(III) and Cu(II) with MOX and glycine is:

M = Ni(II), Cu(II)   **M** = Co(III), Fe(III)
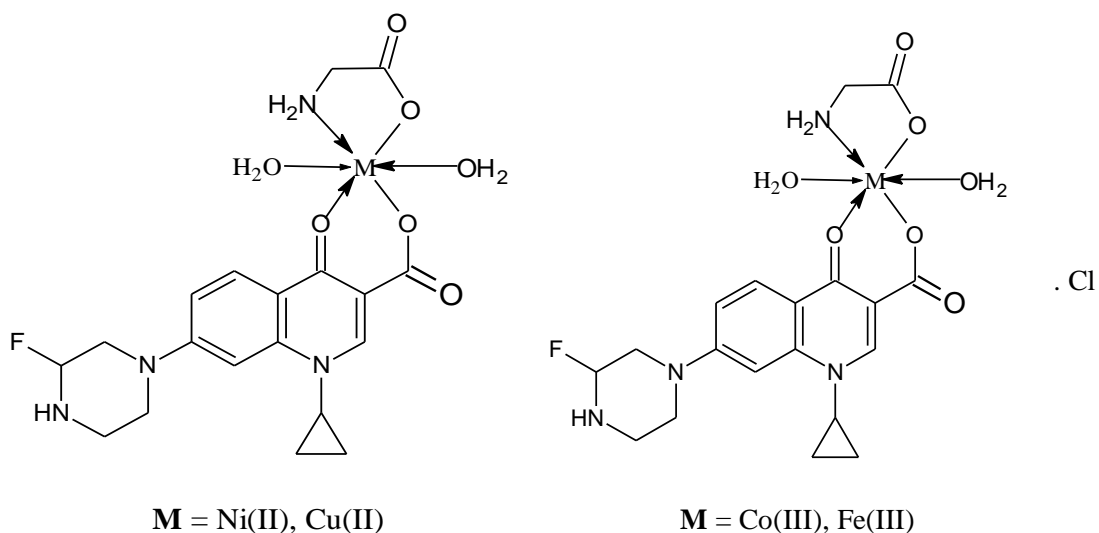
b) Proposed structure for complexes of Ni(II), Co(III), Fe(III) and Cu(II) with LEV and glycine is:

M = Cu(II), Ni(II)   **M** = Co(III), Fe(III)
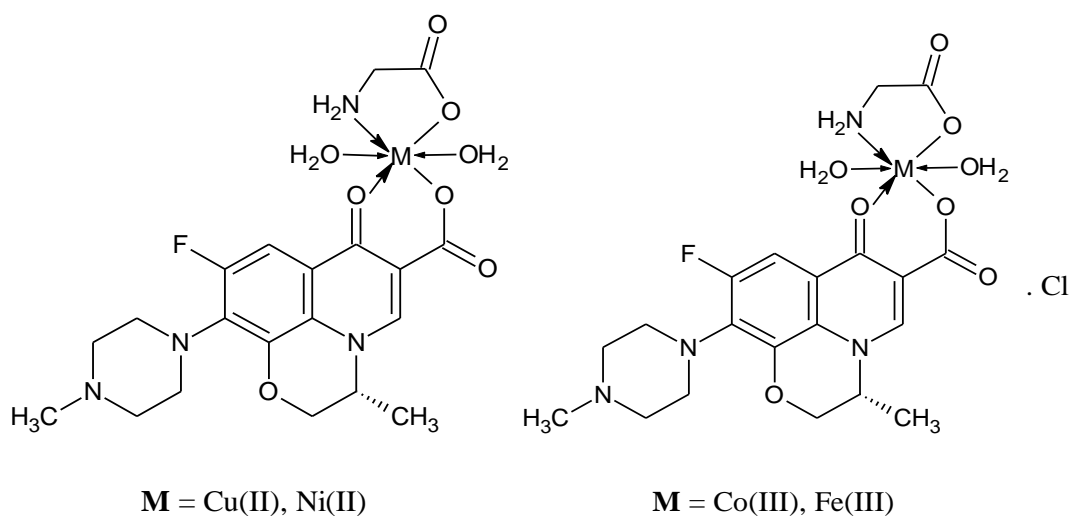
c) Proposed structure for complexes of Ni(II), Co(III), Fe(III) and Cu(II) with CIP and glycine is:

M = Ni(II), Cu(II) M = Co(III), Fe(III)
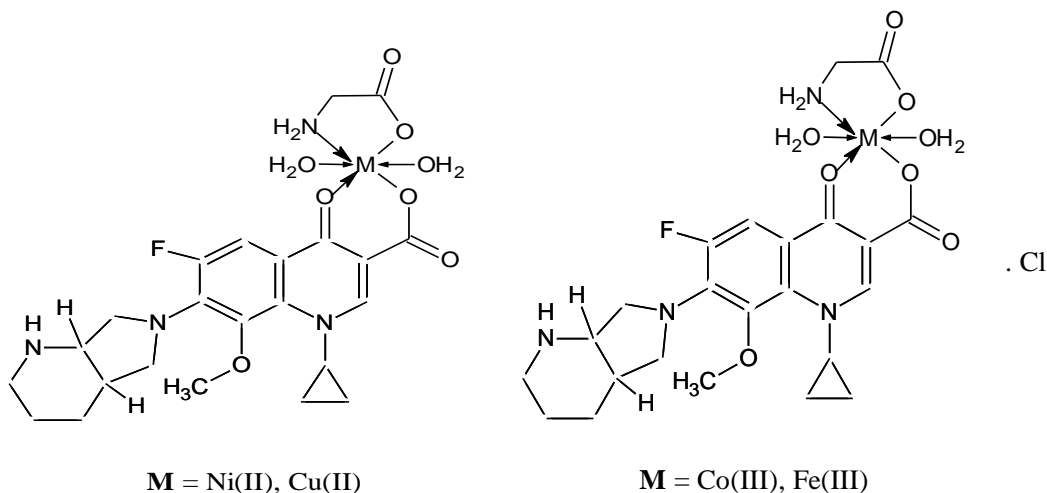
3.7. Biological Studies

3.7.1. Anti-microbial Activity

Generally, following aspects are considered when metal complex's anti-microbial activity [97] may be evaluated:

i) Chelate effect

ii) Ligand's nature

(iii) Charge on complex; there is a decrease in the antimicrobial activity in the following sequence cationic > neutral > anionic complex.

(iv) Counter ion's nature in the ionic complexes.

(v) Metal center nuclearity in complex; more activity of dinuclear centers is observed as compared to mononuclear ones.

Results of antimicrobial activities by well diffusion and disk diffusion method are shown in Figures 3.7-3.12 and presented in Table 3.12-3.13. Chelation and pie electron

delocalization favours permeation in bacterial membrane as a result organism died [98]. According to chelation theory increased activity is due to overlap of ligand orbital with the metal ion orbital. pi-electron delocalization increases the complexes' lipophilicity which may disintegrate cell's permeability barrier [51]. So, the ternary complexes are more active than standard drugs Kanamycin and Ciprofloxacin. The complexes are more active against gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Acenitobacter*, MRSA) by well diffusion method and they show good result against *Escherichia coli, Klebsiella pnuemoniae, Pseudomonas aeroginosa* and MRSA by disk diffusion method. Some complexes, did not exhibit much increase in activity, which can be described by the degree of their solubility which is directly related to the permeability across the micro-organism's lipoid layer [99]. The order of decrease in antimicrobial activity in terms of metals is

Co(III) complexes > Fe(III) complexes > Cu(II) complexes > Ni(II) complexes

This order can be explained on the basis of counter ion effect and chelate effect.

3.7.1.1. Well Diffusion Test
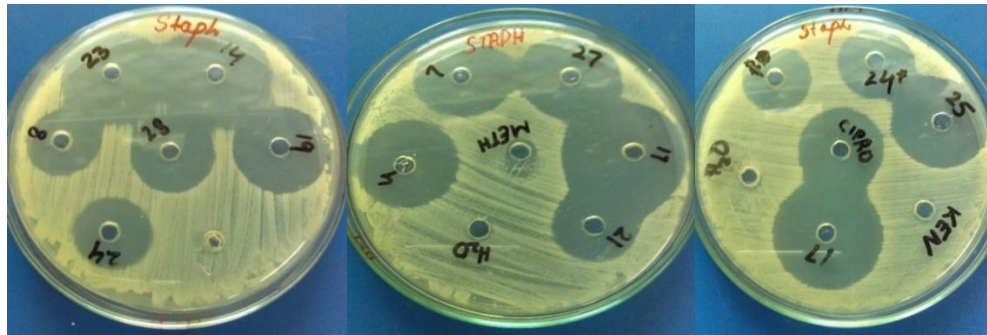
Fig 3.10: Anti-microbial Activity of Ternary Complexes by Well Diffusion Test against *Staphylococcus aureus*
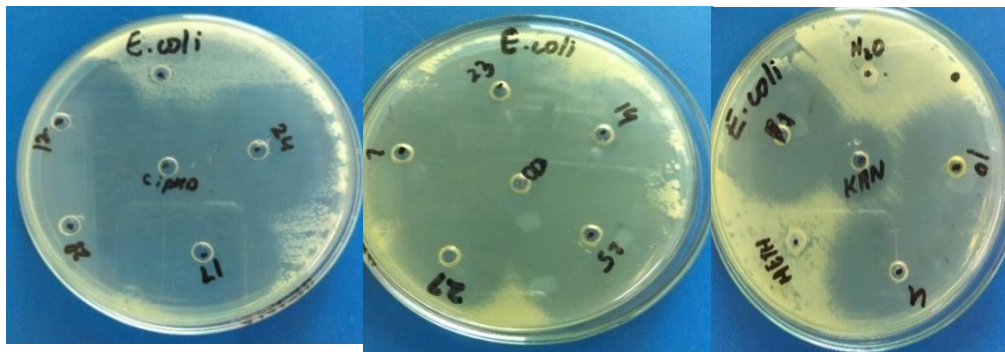
Fig 3.11: Anti-microbial Activity of Ternary Complexes by Well Diffusion Test against *Escherichia coli*
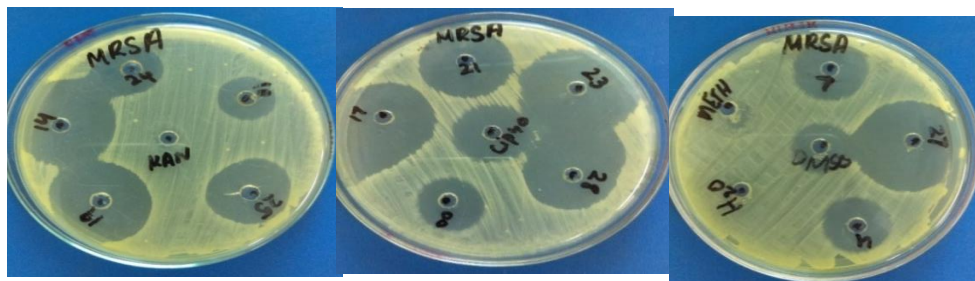
Fig 3.12: Anti-microbial Activity of Ternary Complexes by Well Diffusion Test against Methicillin Resistant *Staphylococcus aureus* (MRSA)

Table 3.12: Anti-microbial Activity of Ternary Complexes by Well Diffusion Test

Sr No:	Sample	*S. aureus*	*B. subtilis*	*E. coli*	*K. pnuemoniae*	*P. aerogi nosa*	*Acinetobac ter*	MRSA
		Zone diameter 'mm'	Zone diameter 'mm'	Zone diameter 'mm'	Zone diameter 'mm'	Zone diamet er 'mm'	Zone diameter 'mm'	Zone diameter 'mm'
1	Co-MOX-Gly	30	31	32	28	36	26	35
2	Cu-MOX-Gly	32	40	34	27	-	23	34
3	Ni-LEV-Gly	26	22	36	39	-	26	32
4	Fe-LEV-Gly	23	36	44	27	44	19	26
5	Co-CIP-Gly	22	31	38	30	48	11	18
6	Ni-CIP-Gly	28	36	40	29	-	19	20
7	Cu-CIP-Gly	26	31	39	36	-	20	23
8	Fe-MOX-Gly	28	34	30	25	-	18	33
9	Ni-MOX-Gly	33	40	38	22	38	24	30
10	Cu-LEV-Gly	22	35	46	31	38	19	27
11	Fe-CIP-Gly	21	30	32	31	-	12	17
12	Co-LEV-Gly	28	34	40	33	-	17	24
13	Methanol	9	0	0	19	0	7	0
14	Kanamycin	0	22	20	22	14	0	0
15	Ciprofloxacin	26	24	36	26	-	14	22
16	Autoclaved H_2O	0	0	0	0	0	0	0
17	DMSO	-	26	-	23	0	0	18

S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis,

E. coli = Escherichia coli, K. pneumoniae = Klebsiella pneumoniae,

P. aeruginosa = Pseudomonas aeruginosa.

3.7.1.2. Disk Diffusion Test

Fig 3.13: Anti-microbial Activity of Ternary Complexes by Disk Diffusion Test against *Pseudomonas aeroginosa*.
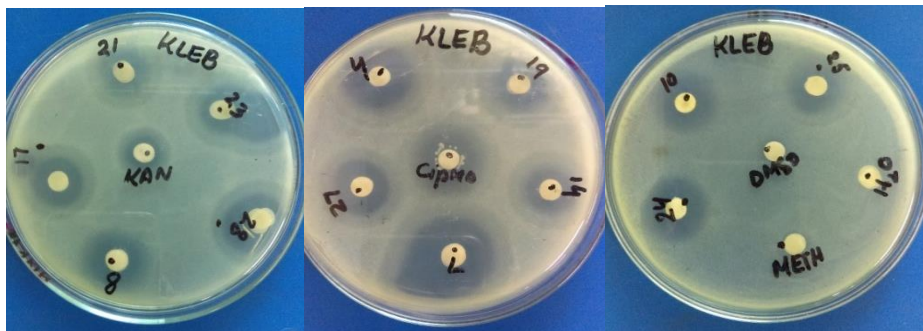
Fig 3.14: Anti-microbial Activity of Ternary Complexes by Disk Diffusion Test against *Klebsiella pneumoniae*.
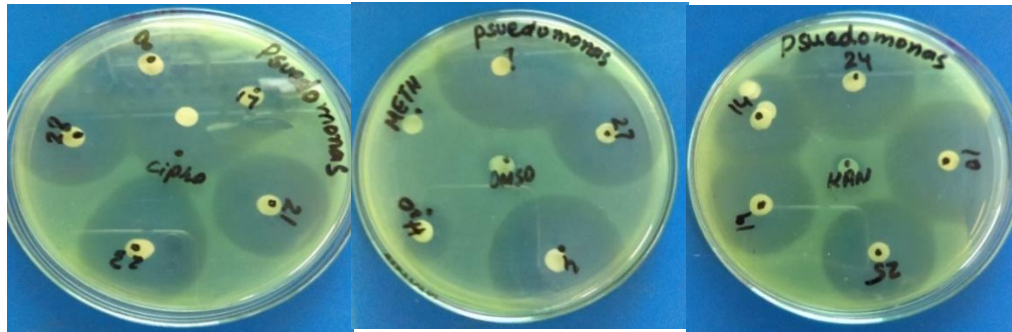

Fig 3.15: Anti-microbial Activity of Ternary Complexes by Disk Diffusion Test against *Bacillus subtilis*.

Table 3.13: Anti-microbial Activity of Ternary Complexes by Disk Diffusion Test.

Sr No:	Sample	*S. aureus*	*B. subtilis*	*E. coli*	*K. pnuemoniae*	*P. aeroginosa*	*Acinetobacter*	MRSA
		Zone diameter 'mm'	**Zone diameter 'mm'**	**Zone diameter 'mm'**	**Zone diameter 'mm'**	**Zone diameter 'mm'**	**Zone diameter 'mm'**	**Zone diameter 'mm'**
1	Co-MOX-Gly	29	20	34	14	32	15	25
2	Cu-MOX-Gly	28	30	32	12	27	13	20
3	Ni-LEV-Gly	21	20	38	14	31	11	13
4	Fe-LEV-Gly	23	24	30	19	30	13	13
5	Co-CIP-Gly	19	23	38	19	35	7	10
6	Ni-CIP-Gly	14	20	36	20	40	12	12
7	Cu-CIP-Gly	15	21	34	22	40	7	16
8	Fe-MOX-Gly	26	30	32	16	33	15	19
9	Ni-MOX-Gly	29	23	30	15	20	10	20
10	Cu-LEV-Gly	19	30	34	12	30	13	16
11	Fe-CIP-Gly	20	22	36	14	37	7	11
12	Co-LEV-Gly	18	22	40	15	33	11	16
13	Methanol	0	7	0	6	0	0	0
14	Kanamycin	0	19	21	10	8	0	0
15	Ciprofloxacin	19	22	10	14	34	8	10
16	Autoclaved H_2O	0	0	0	0	6	0	0
17	DMSO	-	-	-	6	0	6	0

S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis,

E. coli = Escherichia coli, K. pneumoniae = Klebsiella pneumoniae,

P. aeruginosa = Pseudomonas aeruginosa.

3.7.2. Cytotoxicity Assay

Studies proved that *Artemia salina* during its preliminary phases of development is more prone to toxins [100]. In most of the literature brine shrimp bioassay was coupled with lethality test called brime shrimp lethality test and proposed their use as anti-tumor, anti-cancer and anti-proliferative agent. In present work, only *In-vitro* cytotoxicity assay (brine shrimp bioassay) of the complexes was used to check the effectiveness of complexes as anti-microbial agent [101]. The cytotoxicity assays of all ternary

complexes were assessed and complied in Table 3.14. All the complexes show less/no toxicity against *Artemia salina,* or the complexes are totally non-functional (inert) against this assay [99] so there is a possibility of use of these synthesized compounds as lead compounds for antibiotic drugs.

Table 3.14: Results of Cytotoxicity Assay of Ternary Complexes Samples

Sample	Initial No. of dead larvae 'N'				No. of dead larvae after 24 hour 'A'				Total No. of larvae 'G'				Average No. of dead larvae in blind samples after 24 h 'B'	Mortality rate 'M' = $\frac{(A-B-N)\times100}{(G-N)}$
	R_1	R_2	R_3	Av	R_1	R_2	R_3	Av	R_1	R_2	R_3	Av		
Cu-MOX-Gly	0	0	0	0	18	25	18	20	20	32	25	26	23	0%
Fe-MOX-Gly	0	0	0	0	19	18	23	20	19	20	23	21	23	0%
Co-LEV-Gly	0	0	0	0	17	19	11	16	17	20	30	22	23	0%
Co-CIP-Gly	0	0	0	0	21	16	11	16	21	18	19	19	23	0%
Ni-MOX-Gly	0	0	0	0	8	5	8	7	17	22	19	19	23	0%
Ni-CIP-Gly	0	0	0	0	15	12	11	13	16	19	17	17	23	0%
Cu-MOX-Gly	0	0	0	0	19	12	17	16	21	16	27	21	23	0%
Co-MOX-Gly	1	0	0	0	10	20	25	18	19	36	26	27	23	0%
Fe-LEV-Gly	0	0	0	0	18	15	13	15	27	34	30	30	23	0%
Fe-CIP-Gly	0	0	0	0	18	13	12	14	18	31	24	24	23	0%
Ni-LEV-Gly	0	0	0	0	22	8	18	16	32	23	30	28	23	0%
Cu-LEV-Gly	0	0	0	0	12	16	13	14	18	19	14	17	23	0%

3.8. Conclusion

Synthesis and characterization of twelve new ternary metal complexes with second and fourth generation quinolone drugs along with amino acid glycine have been recognized by physical, spectroscopic and theoretical method. Complexes bound to metals through pyridine oxygen and carboxylate oxygen of quinolones and with amino N and deprotonated carboxylate O of glycine. Good molecular arrangement favors octahedral structure of all the complexes. Theoretical studies suggest that more stable complexes are formed with MOX ligand and with respect to metals; copper ternary complexes are more stable. All the complexes exhibit good antimicrobial activities as compared to standard drugs and their usage as possible antibacterial agents, in areas of disinfection, food packing and tap water. All the complexes have low toxicity. Future directions of this work are to carry out more biological activities of these ternary complexes as antitumor, anti-oxidant and anti-parasitic agents. *In-vivo* trials should be carried out and if the results will be encouraging it may be a new hope for researcher in developing new anti-bacterial drugs.

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