CHAPTER I

INTRODUCTION
1.1 - INTRODUCTION

Coordination chemistry is one of the most active and important branches of inorganic chemistry. The coordination compounds find innumerable applications in all walk of life and infact all chemical and biochemical processes can be related to coordination chemistry. Transition metal chemistry has long been part of coordination chemistry and subject of study for many workers in diverse disciplines related to both theoretical and experimental aspects. Considerable interest in the transition metal complexes originated from their many fold applications in chemical, analytical, medicinal, biological, industrial, agricultural and many other fields.

The work reported here is a part of a comprehensive study aimed at isolating and characterizing coordination compounds formed between non-steroidal anti-inflammatory drugs (NSAIDs) and transition metal ions. The drugs taken as ligands are namely: Piroxicam, Isoxicam, Aspirin and Diclofenac sodium. It will be worthwhile to discuss the more important aspects of the mixed ligand complexes viz. definition, classification, modes of formation and factors governing stability. A brief review of studies on drug-metalloelement complexes are also given in this chapter.

The complexes where two or more different ligands are disposed in regular or pseudo-regular geometry around the central metal ion in any of its possible oxidation state are termed as mixed ligand complexes. In the mixed ligand complexes both the coordinated (primary and secondary) ligands may be neutral, one of them may be neutral and other anionic or both of them may be anionic nature. These complexes are quite different
from binary complexes because there is a profound change in the coordinated ligand as a consequence of significant change in electron density at various atoms of coordinated species and also in molecular orbital energies of ligands. These lead to an increase in their reaction selectivity.

Formation of these complexes is known to be very important in biological processes (1-7). Since there are a number of potent ligands to compete with metal ions such as Na(I), K(I), Mg(II), Ca(II), Mn(II), Ni(II), Co(II), Cu(II) and Zn(II) (8-9), for example, the complex Cu(II) (histidine, threonine) has been isolated from normal human serum 10. Some well known examples of mixed ligand complexes found in biological systems are oxyhemoglobin, vit.-B12 (cyanocobalamine) and related co-enzymes. Metalloenzymes have been shown to react to substrates through mixed ligand complex formation (11,12). Mixed ligand complexes are known to show comparatively higher antimicrobial activity than their respective binary chelates (13-17).

All catalytical activities pass through mixed ligand complex formation (18-26). These complexes are known to act as intermediate in ligand displacement 27, ligand catalyzed complex formation and redox reaction where metal ion being simultaneously coordinated to two different ligands by promoting electron transfer from reductant to oxidant (28-31).

The formation of mixed ligand complex may result in marked changes in the physical properties of the original complex and hence the biological effect of the complex is also changed. Cu(II) complex of
NSAIDs have been shown to be more effective in comparison to parent agent \(^{(32,33)}\).

There are very few possible ways in which the mixed ligand complex formation can be considered. In the first case, the inability of single ligand to fulfill all the coordination positions is responsible for second ligand to interact in order to satisfy remaining coordination sites \(^{(34-37)}\). In the second case, all the coordination sites may be satisfied by the first ligand in such a way that simple binary complex so formed still possesses an overall positive charge of the original metal ions and it is free to combine with ligand of proper anionic charge to form mixed ligand complex \(^{(38-39)}\). In the third case where both ligands have the capacity to satisfy the metal charge and its coordination number, the following equilibria may exist:

\[
\begin{align*}
M^{2+} + A^- & \rightleftharpoons MA^+ & (1) \\
M^{2+} + B^- & \rightleftharpoons MB^+ & (2) \\
MA^+ + B^- & \rightleftharpoons MAB & (3) \\
MB^+ + A^- & \not\rightleftharpoons MBA & (4) \\
MA^+ + A^- & \not\rightleftharpoons MA_2 & (5) \\
MB^+ + B^- & \not\rightleftharpoons MB_2 & (6)
\end{align*}
\]

Beside above complexes there may exist \([M (AH) (B)]^+, [M (BH) (A)]^+, [M (AH) (BH)]^{2+}, [M (A) (OH)], [M (B) (OH)] and [M (OH)_2]\). But at normal pH (7-8) these are formed in negligible amount and only mixed ligand complexes of the type (3) and (4) are statistically favored \(^{(40-44)}\).

In addition to the statistical factor, electronic structure \(^{(45-46)}\), ionic radius of central metal ion, mutual polarizability of metal ion and
ligands (47-49) and symmetry relation of their electronic orbitals 50 are the other factors which governs the formation of mixed ligand complexes. The thermodynamic factors, entropy 51 and enthalpy (52-53) favours the formation of mixed ligand or complexes rather than simple binary chalets.

Beside the above physical factors, there are some extra-molecular and intra-molecular effects that govern the stability and formation of mixed ligand complexes.

The extra-molecular effect involves the environment of the complex like solvent, ionic strength of the medium (54-55), pH, temperature 51 and concentration (56-58) of reacting species etc. Fridman et al. have observed that stability constant of Cu(acac)(oxin) [acac= acetylacetonate and oxin= β-hydroxyquinolinate] varies in different solvents. This difference is due to the difference in polarizability of coordinate bonds or appearance of dipole in the mixed ligand or ternary complexes at expense of binary complexes. The solvent effect is independent of compound and charge of coordination sphere and it is more pronounced with increasing difference in electronegativities of the ligand pairs (59-61).

Sigel and coworkers (62,63) have reported that nitrogen containing ligands form mixed ligand complexes at lower pH than those with oxygen donating ligands.

Intra-molecular effect involves the ligand field strength, mode of bonding of ligand size of chelate ring formed as a consequence of complexation and steric hindrance. Conversion of binary complexes into mixed ligand or ternary complexes involve two major intra-molecular
change viz. change in the geometry of the molecule and change in bond energy. According to Kidda (64-66) mixed ligand or ternary complex formation encounter difficulty when geometrical structure and bond type differ from each other. The plot of binding energy of the ligand versus ligand strength led to the conclusion that any pair of the ligands which form only high spin or low spin complexes with transition metal ions, can form more compatible ligands. In the case of non-compatibility of ligands, ternary or mixed ligand complexes are found to be less stable in the solution than the binary complexes.

Further σ and π bonding capacity of the constituent ligands is also one of the major factors for stabilization of the mixed ligand complexes. The σ bonding increases the electron density on the metal atom and its effect is assumed to work in the same direction as the electrostatic effect in the ionic complexes. The π bonding allows back donation of charge density on the metal ion. Thus when both of the ligand A and B form σ bonding, the mixed ligand complexes MAB is preferentially formed because of the destabilization caused by inter-ligand repulsion is smaller in it than the parent complexes (56, 69, 74). On the contrary, when both ligand A and B are π acceptors, formation of mixed ligand complex is less favoured since the electron accepting tendency of one ligand tends to reduce that of other (74). Inspite of the fact that repulsion between metal dπ electrons and ligands lowers under such circumstances. Lastly, if one ligand is σ bonding and another π bonding, the former adds charge to the metal atom, decreases its electronegativity while the later removes the charge from the metal atom, increases its electronegativity leading to mutual enhancement of their bonding capacities (62, 63, 75-79). This is termed as cooperative effect, consequently the ternary complex formed is highly
stabilized. Thus when A is a $\pi$ bonding ligand with N-N coordinating atom and B is a $\sigma$ bonding ligand with O' - O' or N - O' or N - N coordinating atoms the stabilization of the mixed ligand complex MAB is in the order.

$$O' - O' > N - O' > N - N$$

The extra stabilization of the mixed ligand complex with O' - O' donor as compared to AB$_2$ is due to decreased electronic repulsion between metal d$\pi$ electrons and lone pairs present on oxygen atoms caused by $\pi$ bonding effect of ligand A. Since there is no extra lone pair of electrons present on $\sigma$ bonding N atom hence this decreased repulsion is not felt in N - N coordinating ligands ($^{62,63,75-80}$).

Size of chelate ring also play an important role in stability of mixed ligand complexes. Binary complex of 3d metal ion with five member chelate ring are known to be more stable than with six member rings (in case of aliphatic ligands). The mixed ligand complexes containing one five and one six member chelate rings are more stable than those containing either six or five member rings $^{80}$.

All the factors governing mixed ligand complex formation and their stability are interconnected with each other and may influence one another $^{81}$. A complete satisfactory explanation for stabilization of the mixed ligand complexes is still lacking. Statistically, mixed ligand complex formation is favoured but in most cases experimentally determined stability constants deviate from calculated values. Marculs et al. have explained these deviations assuming a polarized metal ion model for ternary complex. They suggested that metal ions are polarized
to a greater extent in the field of different ligands rather than that of identical ligands \(^{(82,83)}\).

The coordination chemistry of first transition metal with physiologically active ligand containing nitrogen and oxygen donors have received a great deal of attention because of the possible relationship with many biological problems and valuable catalytic applications. Moreover iron ions have played many biological activities \(^{84}\) such as oxygen transport in plasma, oxygen storage in muscle, iron transport in plasma, iron storage in cells, metabolism of hydrogen peroxide and terminal oxidation. Iron-sulphur proteins ruberdoxins are participate in biological redox reactions\(^{85}\). Cobalt is core of vit. B\(_{12}\) and used in treatment of pernicious anemia. Certain anaerobic bacteria used methyle cobaltamine in a cyclic way to produce methane, cobalt containing vit. B\(_{12}\) works as a co-factor for a number of enzymes \(^{86}\). A deficiency disease in sheeps of Florida, Australia, Britain and New Zeland was traced to a lack of Co in soil \(^{87}\).

Nickel has many biological as well as catalytic role in various types of reaction. Although it's widespread occurrence in bacterial hydrogenase and plant urease may be of greatest long term importance \(^{(88,89)}\). Chicks and rats raised on deficient diet show impaired liver function and morphology, it also stabilized to coiled ribosomes \(^{90}\).

Copper was first shown to be an essential biological element in the 1920s when anemia was found to result from Cu-deficient diets in animals \(^{92}\) and addition of Cu slats corrected this affliction \(^{(91,92)}\). It is now recognized as an essential trace element for many biological functions \(^{(93,94)}\). It serves as a catalytic component in many enzymes, e.g.
it is an important constituent of metalloproteins (exhibiting oxidative reductase activity, e.g. oxidases or hydroxylases) and in such enzymes as lysyl oxidase (required for connective tissue) and cytochrome oxidase (electron transport protein).

Copper also influence specific gene expression in mammalian cells nerve mydality and endorphin action with Cu deficiency impairing immunity. The role of trace metallic elements, such as Cu in inflammation, is of great interest because their function as cofactors in metabolic processes involving articular/connective tissue and the immune system and their effect on PG synthesis. The daily intake of Cu in humans is ~1.5-3.0 mg per day.

A potential scientific basis for the anti-inflammatory Cu bracelet remedy emerged when it was shown that metallic Cu can dissolve in sweat and be absorbed through skin. Copper is believed to possess anti-inflammatory activity. Patents with RA and osteoarthritis exhibit changes in the Cu distribution in the blood. It is proposed that there is increased demand for Cu during inflammatory conditions, which is compensated for by enhanced intestinal absorption and decreased intestinal excretion of Cu. It has long been suggested that the mode of action of salicyclates and other such anti-inflammatory drug may involve the chelation of bioactive metal ions such as Cu(II), so facilitating the transfer of the metal to and from or site of inflammation or pain.

Interest in the possible beneficial effects of Cu-complexes was renewed by Sorenson's report in 1976. Sorenson reported that Cu-complexes of the anti-inflammatory drugs were more active in animal
models than either their parent inorganic Cu(II) salt on the parent NSAID. The pharmacological activity was proposed to be due to the complex itself rather than just that of its constituents since the ambient of Cu in such complexes does not correlate with anti-inflammatory activity (118, 119).

Since coordinated forms of Cu are always more stable forms compared to ionized forms, it exist in biological systems as a variety of complexes (120-124). According to pharmacological theory, the Cu-chelates were the product of chemical transformation in vivo, these chelates would be more active than either cupric acetate or the parent chelating agents (as shown in Fig.1.1).

**ANTI-INFLAMMATORY ACTIVITY**

![Diagram](https://via.placeholder.com/150)

COPPER CHELATE

CUPRIC ACETATE

ANTI-INFLAMMATORY AGENT (CHELATING COMPOUNDS)

Fig. 1.1 Rational for the hypothesizes active forms of anti-arthritic compounds

If these Cu-chelates were more active than Cu, in the form of cupric acetate, and the parent chelating compounds, they might be correctly considered as the active forms of the anti-inflammatory agents.
The chemistry of complexation has also got great importance in the field of pharmaceutical science. If the drug (as ligand) form a stable, water-soluble metal chelate, it is said to be a sequestering agent. Sequestration (Latin: to remove) is the suppression of the property or reaction of a metal without removal of the metal from the system or phase by any process of precipitation or extraction and is usually accomplished by chelation. Sequestering agents are used in the treatment of urinary calculi, calciferous corneal deposits, and hypercalcemia. EDTA may be used as an in vitro anticoagulant for blood. In lead poisoning, salts of EDTA form a stable lead chelate, which is inert, non-toxic and rapidly eliminated. EDTA also increases the absorption of iron in the gastrointestinal tract. An iron in the chelate of 8-hydroxy quinoline has antibacterial action. The binding of polyvalent metal cations by the tetracyclines has been shown to markedly reduce their efficiency. Dithizone (a dye) forms coloured complex with many metals and is useful in the estimation of trace quantities of lead and zinc. The complex of Fe(II) ion with o-phenanthroline is used as indicator in titration utilizing ceric sulphate.

Biological materials are often dependent on formation of metal chelates. The stabilization of insulin with zinc; the enzymatic bond formation and rupture process of carbohydrates and nucleoproteins, the iron in heme, Mg in chlorophyll and Co in vitamin B_{12} are example of a system in which metal chelate complexes are essential for biological activity. Many enzymes contain metals, which are essential for activity of the enzyme system. Lack of atom or removing it can inactivate the enzyme and therefore trace amounts of Cu, Zn, Mn, Co etc. are required for biological processes \(^{(125, 126)}\). Copper is found to be associated with
enzyme tyrosinase, zinc with carbonic anhydrase, molybdenum in xanthine dehydrogenase.

The drugs chosen as ligand in present curative research works are non-steroidal anti-inflammatory drugs (NSAIDs) namely: 4-hydroxy-2-methyl-N(2-pyridyl) 2-H-1, 2-benzothaizine-3-carboxamide-1,1-dioxide (PIROXICAM), 4-hydroxy-2-methyl-N (5-methyl-3-isoxazolyl)-2H-1,2-benzothiazine-1, 1-dioxide (ISOXICAM), o-acetoxybenzoic acid or acetylsalicylic acid (ASPIRIN) and [2-(2, 6-dichlorophenylamino)phenylacetate] mono sodium salt (DICLOFENAC).

Piroxicam is first NSAIDs chosen as a ligand in the present research work. Piroxicam (Feldene) is a derivative of 4-hydroxy benzothiazine-3-carboxamide-1, 1-dioxide is characterized by long acting and often very potent anti-inflammatory activity.

Piroxicam is a white or slightly yellow crystalline powder; fairly soluble in acetone, ethyl acetate, dimethyl formide, dimethyl sulphoxide and sparingly soluble in chloroform, water etc. and also shows polymorphism.

![Chemical Structure of Piroxicam](image)

**Fig. 1.2** - 4-hydroxy-2-methyl-N(2-pyridyl) 2-H-1, 2-benzothaizine-3-carboxamide-1,1-dioxide (PIROXICAM)
Piroxicam is a long acting NSAIDs with anti-inflammatory potency similar to indomethacin and analgesic action greater than that of Aspirin. It has useful antipyretic property. It is a reversible inhibitor of cyclooxygenase enzyme. It also decreases production of Ig rheumatoid factor and lower it’s plasma level in patients of rheumatoid arthritics. It also inhibits chemotaxis of leukocytes and decreases the ratio of T-helper to T-suppressor lymphocytes. Thus, it can inhibit inflammation in diverse ways.

Piroxicam is prescribed for treatment of rheumatoid arthritis, osteoarthritis, acute gout, ankylosing spondylitis, musculoskeletal disorder etc. used as analgesic for acute tenditis, bursitis primary dysmenorrhea and tooth extraction.

Isoxicam is a second important non-steroidal anti-inflammatory drugs taken as ligand in the present investigation. This new class of 4-hydroxy-2-methyl-N (5-methyl-3-isoxazolyl)-2H-1,2-benzothiazine-1, 1-dioxide (ISOXICAM) is characterized by long acting and often very potent anti-inflammatory activity.

Fig. 1.3 - 4-hydroxy-2-methyl-N (5-methyl-3-isoxazolyl)-2H-1,2-benzothiazine-1, 1-dioxide (ISOXICAM)
In short the possible mechanism by which Isoxicam appears to exert its therapeutic effects can be summarised as follows:

- Selective prostaglandin synthetase inhibition
- Inhibition of Superoxide anions from stimulated polymorphs
- Inhibition of platelet activating factor synthesis
- Prevention of Bradykinin/ cytokinine stimulation of nerves
- Scavenging of hypochlorous acid
- Blocking of histamine release \(^{127}\) and
- Prevention of cartilage damage by inhibition of metalloprotease synthesis.

Aspirin is the third important NSAIDs which is taken as a ligand in the present investigation. Aspirin is a derivative of salicylic acid. It is a colourless, feathery powder with a sweetish taste, soluble in boiling water and fairly soluble in alcohol and it melts within the range 158-161 °C.

![Fif. 1.4 - acetylsalicylic acid or o-acetoxybenzoic acid (ASPIRIN)]
Aspirin possesses a number of properties that make it the most often recommended drug. It is an effective analgesic, anti-inflammatory and antipyretic agent.

Diclofenac sodium, [2-(2, 6-dichlorophenylamino) phenylacetate] mono sodium salt, is a potent non-steroidal anti-inflammatory drug, therapeutically used in inflammatory and painful conditions of rheumatic and non-rheumatic origin. Diclofenac is white or slightly yellowish crystalline powder, slightly hygroscopic, sparingly soluble in water, fairly soluble in methanol, soluble in alcohol, slightly soluble in acetone and melts at about 280 °C with decomposition.

Diclofenac sodium was found to inhibit cyclooxygenase (COX) enzyme activity in vitro without any significant effect on phospholipase A2 or on lipoxygenase enzyme. In vitro inhibition of cyclooxygenase does not translate to in vivo inhibition, but it is suggested that like other NSAIDs diclofenac interact in vivo with arachidonic acid cascade at the level of COX since it is also found to decrease prostaglandin in synovial fluid in humans and in wine and renal medulla in rats and pigs.
Diclofenac sodium is highly protein bonded in plasma. It has been found to suppress adjuvant-induced arthritis in rats, rheumatoid arthritis in human, monocyte superoxide production in vitro, and the respiratory burst of human peripheral blood leucocytes with no effect on phagocytosis.

1.2 - PREVIOUS WORK IN THE FIELD

Synthesis and study of metalloelement complexes with anti-inflammatory drugs as ligand is a research area of considerable interest by a number of workers (133-148).

The synthesis and study of complexes, which exhibit synergistic activity between metalloelement and drug, has concentrated much attention as an approach of new drugs development.

The surveys of the metalldrug complexes are given in the following paragraphs in order to throw light on the type of work already done in the field.

Non-steroidal anti-inflammatory drugs (NSAIDs) are used for their analgesic, anti-inflammatory and antipyretic properties. Some common indication for these agents are for rheumatoid and osteoarthritis, musculoskeletal problems such as tendonitis, fibromyalgia, connective tissue diseases, spondarthropathies, gout, primary dysmenorrhea and systemic lupus erythematosus. The analgesic property has a ceiling effect, meaning that higher doses do not result in enhanced pain control. At lower doses NSAIDs are good for mild to moderate pain and higher doses have an anti-inflammatory effect.
NSAIDs produce their therapeutic activities through inhibition of COX, the enzyme that makes prostaglandin’s (PGs). They share to a greater or lesser degree, the same side effect, including gastric and renal toxicity. Recent research has shown that there are at least two COX isoenzymes. COX-1 is constitutive and makes PGs that protect the stomach and kidney from damage. COX-2 is induced by inflammatory stimuli such as cytokinase and produces PGs that contribute to the pain and swelling of inflammation. Thus, selective COX-2 inhibitors should be anti-inflammatory without side effect on the kidney and stomach. Of course, selective COX-2 inhibitors may have other side effects and perhaps other therapeutic potential. For instance, COX-2 (and not Cox-1) is thought to be involved in ovulation and in labor. In addition, the well-known protection action of aspirin on colon cancer may be through an action on COX-2, which is expressed in this disease. Moreover, NSAIDs delay the progress disease. Thus the selective COX-2 inhibitors may demonstrate new important therapeutic benefits as anticancer agents, as well as in preventing premature labor and perhaps even retarding the progression of Alzheimer disease.150

Salicylates are synthetic derivatives of salicylic acid. Salicylic acid provides analgesic and anti-inflammatory effect by the inhibition of prostaglandin synthesis much like other NSAIDs. Unlike other NSAIDs salicylates appear to inhibit COX but not lipoxygenase, which is an enzyme involved in forming leukotrienes from arachidonic acid, excess leukotrienes may be formed when the COX is inhibited. Excess leukotrienes may exacerbate asthma in some patients.150

Levy151 has demonstrated with the use of labeled iron that bleeding does occur following administration of aspirin. The effect varied with
formulation. It is suggested by Davenport \textsuperscript{152} that back diffusion of acid from stomach is responsible for capillary damage.

Because of these characteristics of aspirin, it has been extensively studied as an antithrombotic agent in the treatment and prevention of clinical thrombosis \textsuperscript{153}. It is thought to act by its selective action on the synthesis of the prostaglandin related thromboxane and prostacyclins, which are counterbalancing factors involved in platelet aggregation and are releases when tissue is injured. Although aspirin has now approved for the prevention of transient ischemic attacks, indicators of an impending stroke, it is not recommended for patients who have suffered heart attacks \textsuperscript{154}.

Recently a growing body of evidence has accumulated on the beneficial effects of copper compound towards various models of inflammation and copper complexes of non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to be more effective in this respect than the parent agents. Brumas et al. \textsuperscript{33} have studied Cu(II) complex equilibria with salicylic acid and acetylsalicylic acids and benzoic acid used as a reference as well as the mixed ligand complex equilibria generated by these binary systems and L-histidine (main low molar mass ligand of Cu(II) in blood plasma) have been investigated under physiological conditions (37 °C, 0.15M NaCl), confirming previous observations by others, resulting simulated plasma copper distributions, virtually rule out any quantitative influence of salicylate on copper tissue diffusion at therapeutic levels. Even though, as is presently shown, both salicylate and acetylsalicylate may favour the gastro intestinal (GI) absorption of Cu, it seems unlikely that salicylate can exert its anti-inflammatory activity predominantly through copper complexation.
In 1971, Vane proved that aspirin and other NSAIDs inhibit the activity of enzyme now called COX, which leads to the formation of PGs that cause inflammation, swelling, pain and fever. However, by inhibiting this key enzyme in PG synthesis, the aspirin like drug also prevented the production of physiologically important PGs, which protect the stomach mucosa from damage by hydrochloric acid, maintain kidney function and aggregate platelets when required. This conclusion provided a unifying explanation for the therapeutic actions and shared side effects of the aspirin-like drugs. Twenty year later, with the discovery of second COX gene, it became clear that there are two isoforms of the COX enzyme. The constitutive isoform, COX-1 supports the beneficial homeostatic functions, whereas the inducible isoform COX-2, becomes upregulated by inflammatory mediators and its products cause many of the symptoms of inflammatory disease such as rheumatoid and osteoarthritis.

A recent report from Chandrasekharan et al. describes a third cyclooxygenase (COX-3) selective inhibited not only by paracetamol but also by low concentration of some NSAIDs including aspirin. COX-3 is a variant of COX-1, which has retained intron-1 during translation and which is found in human tissues in a polyadenylated form. Selective inhibition of COX-3 will discover potent and valuable new drugs for controlling pain and fever.

Several papers on COX-1 and COX-2 gene deficient mice have now been published. Mice that lack the gene for production of COX-1 appear to be perfectly healthy and do not show significant signs of pathological changes in the kidney. This is in accord with the finding that inhibition of COX-1 by NSAIDs does not alter renal function under normal physiological conditions. However, in COX-2 (-/-) null mice, the
kidneys failed to develop fully after birth, with the result that animals died before they were 8 week old\textsuperscript{158}.

Prostaglandin's synthesized by COX-1 are apparently essential for the survival of fetuses during parturition, since the majority of offspring born to homozygous COX-1 knockout mice do not survive\textsuperscript{155}. This high mortality of the pups may be due to premature closure of the ductus arteriosus. Female COX-2 knockout mice are mostly infertile, producing very few offspring due to a reduction in ovulation\textsuperscript{159}.

The importance of the discovery of the inducible COX-2 is highlighted by the difference in pharmacology of the two enzymes\textsuperscript{160}. Aspirin, indomethacin and ibuprofen are much less active against COX-2 than against COX-1\textsuperscript{161}. Indeed the strongest inhibitor of COX-1, such as aspirin, indomethacin and piroxicam are the NSAIDs that cause the most damage of the stomach\textsuperscript{162}. The spectrum of activities of some of standard NSAIDs against the 2-enzymes ranges from a high selectivity towards COX-1 (166 fold from aspirin) through to equal activity on both\textsuperscript{163}.

The range of activities of NSAIDs against COX-1, as compared to COX-2, nicely explain the variations in the side effects of NSAIDs at their anti-inflammatory doses. Drugs that have a highest potency on COX-2 and a better COX-2/COX-1 activity ratio will have potent anti-inflammatory activity with fewer side effects and the stomach and kidney. Rodriguez and Jick\textsuperscript{164} have published a comparison of epidemiologic data on the side effect of NSAIDs. Piroxicam and indomethacin in anti-inflammatory doses were found to produce high gastrointestinal toxicity. These drugs have much higher potency against COX-1 than COX-2\textsuperscript{165}. Thus when epidemiological results are compared
with COX-2/COX-1 ratios, a parallel relationship is seen between gastrointestinal side effects and COX-2/COX-1 ratios.

Acetaminophen is antipyretic and analgesic, as are NSAIDs but it lacks the anti-inflammatory and anticoagulatory properties of these drugs. This had led to the speculation that a COX variant exists that is inhibitable by acetaminophen. An acetaminophen inhibitable enzyme is inducible in the mouse J774.2 monocyte cell line. Induction of acetaminophen- inhibitable prostaglandin E2 synthesis parallels induction of COX-2. Thus, inhibition of pharmacologically distinct COX-2 enzyme activity by acetaminophen may be the mechanism of action of this important antipyretic drug.\(^{166}\)

Michel Helene et al.\(^{167}\) have demonstrated that salicylate inactivates copper induced OH radicals through its bulk scavenging properties whereas OH inactivation by anthranilate under the same conditions is a direct function of the copper-anthranilate binding. Anthranilate thus seems to the recently defined notion of OH-inactivating ligand \((0.12)\). More generally, these results provide a beginning of rational for the anti-inflammatory properties of copper complexes with substance that are active or inactive against inflammation by themselves. The extra anti-inflammatory activity induced by copper on NSAIDs appears to be independent of any Cu(II)-NSAIDs association in vivo. On the contrary, the binding of inactive substances with Cu(II) at inflammatory sites seems to be essential to their activation by copper.

Hoffmann et al.\(^{168}\) have been developed improved analytical methodology for the structural characterization of covalently bound drug-protein adduct and has been applied to an investigation of the conjugates
formed in vivo and in vitro between (14C) acetaminophen and mouse liver proteins. The major adduct released by acid hydrolysis of hepatic protein samples, which accounted for approximately 70 % of the bound radioactivity in vivo and in vitro was identified as 3-cystein-S-yl-4-hydroxyaniline, a derivative whose structure reflects the predominance of acetaminophen thioether adducts in drug-modified proteins. It is concluded that the reactive, electrophilic metabolite of acetaminophen, which most likely is N-acetyl-p-benzoquinoneimine, binds with a high degree of selectivity to cysteiny1 thiol groups on protein, formally in a Michael-type addition reaction. Cysteine residues thus represent primary target sites for arylation by the reactive metabolite of acetaminophen, and proteins rich in free thiols may be especially vulnerable to damage by this toxic intermediate.

Two mononuclear Cu(II) ibuprofenate adducts with imidazol or 2-methylimidazol and two binuclear Cu(II) ibuprofenate adducts with metronidazol or caffeine have been prepared and characterized. The data for metronidazol or caffeine adducts are consistent with a binuclear structure as found for Cu(II) acetate monohydrate and other Cu(II) carboxylate dimers. In these complexes four carboxylate groups are bridging two Cu(II) atoms and two added bases coordinated at axial positions to form CuO$_2$N chromophore around each copper. The catalytic activities of the mononuclear complexes are lower than those of binuclear Cu(II) ibuprofenate or its metronidazole or caffeine mono-adducts.$^{169}$

The copper based NSAIDs have led to the development of numerous Cu(II) complexes of NSAIDs with enhanced anti-inflammatory activity. The spectroscopic data support the formation of dimeric [Cu(2)L(4)H(2)O(2)] complexes in which the COO$^{-}$ group behave as a
bridging bidentate ligand. The low wave number region of the Raman spectrum provided information on Cu-O and Cu-Cu bonds in the complexes. Thermogravimetric results gave further support to the vibrational data.

Complexes of Zn(II), Cd(II) and Pt(II) metal ions with the anti-inflammatory drugs, 1-methyl-5-(p-toluoyl)-1-H-pyrrole-2-acetic acid (Tolmetin), alpha-methyl-4-(2-methyl propyl) benzeneacetic acid (Ibuprofen), 6-methoxy-alpha-methyl naphthalene 2-acetic acid (Naproxen) and 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-H-indol-3-acetic acid (Indomethacin) have been synthesized and characterized. In the structurally characterized Cd(naproxen)₂ complex the anti-inflammatory drugs act as bidentate chelate ligand coordinatively bound to the metal ions through the deprotonated carboxylate group. Antibacterial and growth inhibitory activity is higher than that of the parent ligands or the Pt(II) diamine compounds.

Mononuclear and binuclear transition metals [Co(II), Cu(II), Ni(II) and Zn(II)] acetyl salicylates of the type [M(L)₂], [M(L)₂Cl₂] and [M₂L₃] have been prepared and characterized on the basis of their physical, spectral and analytical data. The complexes have been investigated in an in vivo animal model for anti-inflammatory activity and also show a better effect and a more potent action than acetyl salicylates.

Technetium-aspirin like complexes were prepared. The structure of N-acetyl-anthranilic acid (NAA) has been decided through CNDO calculations. The ionization potential (IP) and electron affinity (Ea) of the NAA molecule as well as the charge densities were calculated. Comparative studies of the electronic absorption spectrum of acetylthio-
salicylic acid (ATS) and aspirin (ASP) reveal the structure resemblance in which the acetyl carbonyl group is perpendicular to the plane of the corresponding organic acid. The studies of the electronic absorption spectra of NAA and anthranilic acid reveal the planarity of the NAA molecule. The electronic absorption spectra of Tc(V)-ASP and Tc(V)-ATS complexes have two characteristic absorption bands at 450 and 600 nm, but the Tc(V)-NAA spectrum has one characteristic band at 450 nm. As a comparative study, Mo-ATS complex was prepared and its electronic absorption spectrum is comparable with the Tc-ATS complex spectrum.

Wendel and Heidinger treated benzo(alpha) pyrene induced male mice with 200 mg/kg paracetamol. The ethane exhalation of the animals was taken as an in-vivo index of lipid peroxydation, it amounted to 42098 n moles of ethane/Kg body wt. after 4 hours. A dose of 10 mg/Kg of bovine sulpheroxide dismutase had no effect on lipid peroxydation. After 4 daily repeated i.p. injections of 5mg/Kg copper tyrosine or copper-aspinate an inhibition of the ethane exhalation was observed by 97 % control experiments indicated that CuSO₄ and tyrosine alone inhibited this drug induced lipid peroxydation by 21 % and 24 % respectively. However, the copper-ethylenediamine tetra-acetic acid (Cu-EDTA) complex was also effective. The experiment shows that in vivo various copper compounds effectively depress this type of drug induced lipid peroxydation at low concentrations.

\[ [\text{M} (\text{H}_2\text{L})_2 (\text{A})_2 \cdot y \text{H}_2\text{O}] \text{ (Where } \text{H}_2\text{L: neutral piroxicam, } (\text{A})_2: \text{Cl}^- \text{ in case of Ni(II) or acetate in case of Cu(II) and Zn(II) ions and } y = 0 - 2.5) \text{ and } [\text{M} (\text{H}_2\text{L})_3 (\text{A})_z \cdot y \text{H}_2\text{O}] \text{ (A= } \text{SO}_4^{2-} \text{ in case of Fe(II) ion (z=1) or Cl}^- \text{ in} \]
case of Fe(III) (z=3) and Co(II) ions (z=2) and y=1-4) Cheletes are prepared and characterized by Zayed et al. \(^{175}\).

Polymorphic and pseudopolymorphic forms of drugs belonging to the oxicam class of NSAIDs whose X-ray structures have been reported include the crystal forms of piroxicam. \(^{176, 177}\) Piroxicam monohydrate \(^{178}\) and tenoxicam \(^{179}\). The influence of the solvent on the crystallization of piroxicam polymorphs has been discussed in terms of solvation equilibrium including polar and apolar solvents \(^{180}\).

![Piroxicam structure](image)

Piroxicam

![Piroxicam-pivalate structure](image)

Piroxicam-pivalate (Pir-Piv)

**Fig.- 1.6**

The ester of 4-hydroxy-2-methyl-N(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide [(pioxicam), Fig 1.6a] with
pivalic acid (2,2-dimethylpropanoic acid) [(Pir-Piv), Fig 1.6b] is an effective prodrug with activity comparable to the parent compound, while possessing fewer ulcerogenic effects than the latter, owing to the esterification of the free acidic function of piroxicam.

Pir-Piv has been reported to exist in two polymorphic forms showing an enantiotropy relationship. Both forms were isolated by crystallization from solvents and exhibited unique properties when examined by thermal analysis (DSC), infrared spectroscopy (FTIR), and X-ray diffraction on powder.

Cu-based NSAIDs have led to the development of numerous Cu(II) complexes of NSAIDs with enhanced anti-inflammatory activity and reduced gastrointestinal (GI) toxicity compared with their uncomplexed parent drug. These low toxicity Cu drugs have yet to reach an extended human market, but are of enormous interest, because many of today's anti-inflammatory drug therapies, including those based on the NSAIDs, remains either largely inadequate and/or are associated with problematic renal, GI and cardiovascular side effects.

Monique Roch-Arveiller et al. compare the effects of aspirin, 3,5-diisopropysalicylic acid (3,5-DIPS) and indomethacin with those of their copper complexes: Cu(II)₂ (aspirinate)₄, Cu(II)₂ (3,5-DIPS)₄, and Cu(II)₂ (indomethacinate)₄ as well as Cu(II)₂ (acetate)₄ on polymorphonuclear leukocyte (PMNL) random and directional migration in addition to their anti-inflammatory activities. Copper complexes of NSAIDs were found to be more effective in decreasing random migration and chemotaxis of PMNIs than their parent drugs or Cu(II)₂ (acetate)₄ in vitro studies. Only chemotaxis was found to be reduced significantly for
PMNLs obtained from Pleuritic rats after in vivo treatment and the order of copper complex effectiveness was: Cu(II)2 (indomethacinate)₄ > Cu(II)₂ (3,5-DIPS)₄ > Cu(II)₂ (aspirinate)₄. All doses of Cu(II)₂ (acetate)₄ administered in vivo failed to affect chemotactic activity. Copper complexes of NSAIDs were also more effective than their parent drugs as anti-inflammatory agents, and Cu(II)₂ (acetate)₄ had no anti-inflammatory activity in this model of activity was: Cu(II)₂ (indomethacinate)₄ > Cu(II)₂ (3,5-DIPS)₄ > Cu(II)₂ (aspirinate)₄.

The inhibition of prostaglandin synthesis by NSAIDs can alleviate the pain and inflammation associated with a variety of disorders. NSAIDs have a role, therefore, in the treatment of non-rheumatic conditions as well as in the treatment of rheumatic diseases, an area in which these agents have been used and studied more extensively.

A variety of drugs inhibit the conversion of arachidonic acid to prostaglandin-G₂ by the COX activity of prostaglandin endoperoxide synthetase. Several modes of inhibitor binding in the COX active site have been described including ion pairing of carboxylic acid containing inhibitors with Arg-120 of COX-1 and COX-2 and insertion of arylsulfonamides and sulfones into the COX-2 side pocket. Recent crystallographic evidence suggests that Tyr-385 and Ser-530 chelate polar or negatively charged group of arachidonic acid and aspirin.

Cini Renzo prepared the complex [Pt Cl₂ (dmso) H₂] of the widely used anti-inflammatory drug piroxicam (HL=4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazime-3-carboxamide-1,1-dioxide, dmso=dimethyl sulfoxide) was obtained from K₂[PtCl₄]. Its crystal structure reveals that the metal atom is linked to the pyridyl nitrogen atom.
Di Leo Daniela et al.\(^{189}\) prepared crystalline trans – Pt \(\text{Cl}_2(\eta^2\text{-C}_2\text{H}_4)\) H pir)]0.5 \(\text{C}_2\text{H}_3\text{OH}\) with the reaction of Zeise’s salt \(\text{K}[\text{PtCl}_3(\eta^2\text{-C}_2\text{H}_4)].\text{H}_2\text{O}\) and piroxicam [4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiaizine-3-carboxamide 1,1-dioxide, Hpir] in ethanol at room temperature.

Preparation, spectroscopic and magnetic properties were reported for complexes of Mn(II), Fe(II), Co(II), Ni(II) and Cu(II) with the anti-inflammatory drug tenoxicam. In all the complexes studied the tenoxicam acts as a chelate monoanionic ligand with coordination involving the enolate oxygen atom and the carbony oxygen atom of the amide group. The complexes appears to have an octahedral stereochemistry involving two chelate tenoxicam ligands in the case of divalent central metal ions and three chelate tenoxicam ligands in the Fe(II) complex. The Mn(II) and Cu(II) complexes exhibit marked superoxide dismutase (SOD) activity in the nitroblue tetrazolium assay\(^{190}\).

Complexes of formulae \([\text{ZnL}_2(\text{H}_2\text{O})]\), \([\text{CdL}_2(\text{H}_2\text{O})]\) and \([\text{HgL}_2]\) were isolated and characterized by spectroscopy and thermal studies. The monomer \([\text{CdL}_2(\text{H}_2\text{O})]\) in ethanol was transformed to the polymer \([\text{CdL}_2(\text{H}_2\text{O})(\text{EtOH})_2]\). The crystal structure of this polymer was also solved by X-ray crystallography\(^{191}\).

### 1.3 - IMPORTANCE OF THE PRESENT WORK

Of late, study of drug complexes has assumed much importance. Particularly complexes of drugs with metal ions essential for life process have drawn much attention of a large number of chemists, only few of which are referred here\(^{192-201}\).
The chemistry of life involves, in an essential and indispensable way, many of the chemical elements including metals \(^2\). The importance of sodium, calcium and iron has long been recognized but many others especially Cu, Zn, Mn, Mo and Co are also necessary for life processes. Cu and Zn are known to form metalloenzymes. The metal ion does not mainly participate during the time that the enzyme substrate complex exists but is a permanent part of the enzyme. The metal atoms occur at or very near to the active site and plays a very important role in the activity of enzyme \(^2\).

Metal complex play important role in the biological activity of drugs, as the complex formation has been suggested as one of the important mechanism of the drug action. It is also well known that the expected coordination number for the metal ion is not attained in the metal enzyme. This may favour the attachment of the chelate with the tissue, thus resulting in the binding of these drugs with the nucleic acids via transition metal ions. The transition metal ions are present in the human body in traces and these may change the behaviour of enzyme system by replacing the essential metals. This may also affect the structure and functions of the nucleic acids by binding with them. In this way it is most expected that trace metals present in the body can help to transport the drug to the site of its physiological action.

Recently complexes of Zn(II), Cd(II) and Pt(II) metal ions with the anti-inflammatory drugs have been synthesized and characterized; and it was reported that the antibacterial and growth inhibitory activity of the complex is higher than that of the parent ligands\(^1\).
Z.H. Chohan et al.\textsuperscript{172} synthesized transition metal complexes of acetylsalicylates and reported that the complexes show better anti-inflammatory effect and are more potent than acetylsalicylic acid. Colburn and Mass \textsuperscript{(205, 206)} discover that quite an appreciable amount of Cu(II) and Zn(II) etc. are present in the subcellular fraction of the brain, lead these researches to speculate on the possible importance of metal co-ordination in the mechanism of metal binding, storage and transport \textsuperscript{(192, 193, 207)}.

Study of the coordination compounds of drugs led to the investigation directed towards establishing the site(s) of the metal binding in the drug \textsuperscript{208-213} Cu(II) has often been used as a paramagnetic ion probe of binding sites in molecules of biological interest \textsuperscript{214-217}.

The study of the stereochemistry and the chemical reactivity of the coordination compounds of the drugs will help to determine the relationship, which exists between chemical structure and biological activity of these drugs\textsuperscript{210}.

It is important to note that for all the sixteen metallodrug complexes undertaken in the present investigations describe the binding site(s) for PIROXICAM, ISOXICAM, ASPIRIN and DICLOFENAC with biologically important Fe(II), Co(II), Ni(II) and Cu(II) ions. The complexes have been characterized by different Physico-Chemical methods, such as elemental analysis, conductometric, magnetic moment, infrared and electronic spectroscopic methods. The anti-inflammatory activities of all the as synthesized complexes have also been studied on albino rats.

The aim of the present pain stroke research work is to investigate...
> Nature of bonding between metal and ligand (such as Fe-L, Co-L, Ni-L and Cu-L)

> Potentiality of sulphur, nitrogen and oxygen as a donor site.

> Position of ligand in spectrochemical series and their behavior and position in nephelauxetic series

> Stereochemistry and co-ordination number of metal complexes and metal ions

> Change in biochemical behavior of NSAIDs due to complexation with different biologically important metals.
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