CHAPTER-I

INTRODUCTION AND REVIEW
1.1. GENERAL INTRODUCTION

The development of antiinflammatory drugs started at the end of 19th century when Buss in 1875 first used sodium salicylate in rheumatic fever and Nencki in 1886 introduced phenyl salicylate. Although antipyrine, a 5-pyrazolone derivative was synthesized in 1884 and within a few years it was followed by aminopyrine of the same class to be used in medicine but neither of them could provide lead for the search of better compounds in the series during that period. Prior to this, Gerhardt synthesized acetyl salicylic acid in 1853 but it remained out of focus until Hoffman in 1899 again synthesized it by an alternative method, while in search of a superior analogue of salicylic acid to be used by this arthritic father. In 1899, Dresser the Director of Bayer & Company where Hoffman was employed as a chemist, further tested the compound and introduced it in medicine under the trade name ‘aspirin’ derived by the combination of the letter ‘a’ from acetyl and ‘spirin’ from spireic acid, an old name for salicylic acid. The discovery of morphine early in the 19th century as a potent analgesic from opium and introduction of aspirin as an antipyretic and analgesic were the indispensable achievements of the century1-3.

The dawn of 20th century brought new hopes with these findings which remained the only contribution until the development of more advanced pharmacological methods. These permitted systematic study of the compounds in relation to their structures. The abuse of addiction associated with morphine proved to be a been as it always attracted the attention of various research groups to find an analogue free of addiction liability. Prior to 1929, attempts were mainly confined to small changes in the molecule with respect to groups or substituents. Another drawback was the non-availability of the advanced pharmacological methods. The continuity in efforts favoured the development of more advanced
pharmacological techniques but the state of affairs did not favour any substantial contribution of the therapeutic agents for about three decades of this century.

Two remarkable events, that occurred at the end of third decade of this century, may be considered as the turning point in the process of the development of medicinal agents including antiinflammatory drugs. First event was of 1929, when small, Eddy and coworkers⁴ for the first time attempted to depart from the practice of limiting the approach to small change in the molecule and synthesized an altogether new structure, of course, seeking essential parameters from the already existing active compound. This approach led Eisleb and Schaumann⁵ to discover meperidine with one-fifth analgesic activity of morphine in 1938. This discovery gave a momentum to the efforts of various research groups and soon a series of compounds belonging to meperidine, methadone etc. were discovered. The demonstration of superior activity and high potency in synthetic compounds related only distantly with morphine, stimulated the research groups to go either for the drastic changes in the existing molecule or find an altogether different structure. These developments affected other fields of medicine also and synthetic drugs started pouring in.

The other remarkable event was the observation of two American gynecologists, Kurzzrok and Leib in 1930 that strips of human uterus contract or relax in contact with human semen, followed by Goldblat in England and Euler in Sweeden, both independently within a few years that seminal fluid and accessory reproductive glands possess smooth muscle contracting and vasodepressor activity. Euler identified the active material as a lipid soluble acid and named it prostaglandin⁶. More than three decades had to pass when in 1962 structures of two prostaglandin were characterized and prostaglandin are at present accepted as a family of twenty carbon
unsaturated carboxylic acids. This development has given an insight to understand the inflammatory processes and the role of aspirin like antiinflammatory drugs with respect to inhibition of their synthesis in several tissues. However, inflammation is a complex process of cellular events caused by injury to the tissue by varying nature of stimuli including pathological injury, which may be recognized by erythema, edema, hyperalgesia and pain. Various chemical mediators are released or formed at the site of insult to the tissue from various cell sources including arachidonic acid, a precursor for the prostaglandin and related members of the family.

The discovery of eicosanoids, with the detailed and stepwise study of the biosynthesis of prostaglandin and related members, starting from the release of arachidonic acid from the phospholipid fraction of the cell allowed newer dimensions of understanding and elaborating non-steroidal antiinflammatory drugs. The inhibitory action of these drugs on cyclooxygenase preventing the formation of endoperoxides (PGG₂ and PGH₂) not only explains the decrease and control of inflammation but also their inhibitory effect on platelet aggregation by obstructing the formation of thromboxane-A₂, the endoperoxides being the precursors of the latter. The endoperoxides (PGG₂ and PGH₂) have been referred to be labile aggregation stimulating substances (LASS). The pain is not always pure with respect to its type or the pathways and thus attempts have been made to correlate narcotics and non-narcotics analgesics. It is interesting to note that cyclic adenosine monophosphate (CAMP) levels have been recorded in relation to different classes of compounds including some prostaglandin. Both prostaglandin- E₁ and dopamine stimulate cyclic adenosine monophosphate (CAMP) synthesis, where as opioids and enkephaline inhibit stimulated and unstimulated synthesis of cyclic adenosine monophosphate. The decreased
levels of cyclic adenosine monophosphate (CAMP) are considered as the biochemical basis for analgesia. It may be mentioned here that cyclic adenosine monophosphate activates specific dependent kinases to form protein phosphate complexes that chelate Ca$^{++}$. Thus increased levels of cyclic adenosine monophosphate and lowered levels of Ca$^{++}$ inhibit platelet aggregation. Aspirin, sulfinpyrazone and indomethacin have an inhibitory effect on platelet aggregation as they inhibit cyclooxygenase required to convert arachidonic acid to endoperoxides. Aspirin also inhibits platelet release reaction and antagonizes epinephrine, collagen and thrombin induced aggregation$^{10-12}$.

As noted that the aspirin like drugs do not inhibit the release of arachidonic acid, whereas glucocorticoids induce a protein that inhibits phospholipase-A$_2$ responsible for the release of arachidonic acid from phospholipids. Therefore, glucocorticoids could limit the substrate availability and decrease the formation of HPTE, HETE and leukotrienes including prostaglandins, endoperoxides and thromboxane – A$_2$, which may play an important role in chemotaxis and inflammation$^{13,14}$. Besides the steroidal drugs block collagenase production by rheumatoid arthritic synovial cell, while indomethacin stimulates its production. This partly explains the limitations of non-steroidal antiinflammatory drugs with respect to prevention of tissue damage in arthritis. Cortisone was the first corticoteroid used for its antiinflammatory effect followed by a number of modified hormones with greater antiinflammatory and sodium retention potency, such that in most of the compounds electrolytic effect are of no serious consequence. However, the therapeutic value parallels the metabolism of proteins and carbohydrates in almost all the cases. The superior therapeutic value of these compounds when considered with reference to two catagories of toxic effects: those arising from withdrawal,
including various other unavoidable side effects, have always directed the efforts of the medicinal chemist to find superior non-steroidal alternatives\textsuperscript{15,16}.

It is evident from the foregoing account that the basis for approach for the growth of the non-steroidal antiinflammatory drugs in the early time was to cure fever until the realization that fever was only an outward symptom of some more fundamental oilment. It is to be accepted beyond doubt that a few of them are still in use for the alleviation of minor pains or symptoms associated with rheumatoid or osteoarthritis. Even the development of corticosteroids could not decrease the value of aspirin and other aspirin-like drugs. It is important to add here that steroidal agents are in no way more effective then salicylates in inhibiting cardiac complications associated with rheumatic fever\textsuperscript{17}. Since the initial stages of development of these agents when salicylates started gaining importance, some pyrazolones had already been included therapy prior to the appearance of acetylsalicylic acid in the field which entered therapy as aspirin and is accepted as a prototype. It will not be out of the way to mention here that gold, in its elemental form, has been used for centuries as an antiprurite, which in 1890 was tried in arthritis. In 1929 some favorable observations created newer interests and at present gold compounds are used in rheumatoid arthritis especially when aspirin-like drugs fail to give results. As such, structurally diverse but biochemically similar compounds from salicylates, pyrazolones and pyrazolinediones, aryl and heteroaryls, weakly antiinflammatory p-aminophenols, fenamates and disease modifying anti-rheumatic drugs, form a unified group of clinically useful agents known as antiinflammatory drugs\textsuperscript{18}.

The entry of some fenamates with reluctance and restricted use in therapy, due to their associated toxic and other side effects is well known.
Although the biological activity of N-phenyl anthranilic acid derivatives was discovered in 1950, mephenamic acid was indicated in the management of pain and treatment of dysmenorrhea for the first time in 1967. This was followed by meclofenamate in 1980 which was allowed to be used in the treatment of rheumatoid arthritis and osteoarthritis but not to be prescribed for initial therapy. Theoretically these drugs have shown no superiority over other aspirin-like drugs\textsuperscript{19}.

The early references of anthranilic acid and its derivatives have been reviewed by Joseph Augustine in 1935. The use of this acid in the synthesis of ascridines, indigo, thioindizoid, methylred etc. including its use in the analytical laboratories is well known\textsuperscript{20}.

Anthranilic acid is a nitrogen analogue of salicylic acid and like the latter occurs in nature as its methylester. The role of anthranilic acid in biosynthesis of tryptophanevia shikimic acid, which is derived from glucose by microbial action, involving E. Coli, is an important biochemical aspect of the acid\textsuperscript{21}. The acid is reported to be an alkaloid precursor and its direct incorporation in the biosynthesis of peganin in Adhatodavasica is recorded\textsuperscript{22,23}. It is interesting to note that anthranilic acid stimulates the biosynthesis of chlorotetracycline by s. aureofaciens and also nourseothricin mixture\textsuperscript{24,25}. Besides its involvement in the biosynthesis of products from varying groups. It has been reported that some of its derivatives, mainly fenamates inhibit one cycle growth of RNA viruses to the extent of about 90% in the cultures of mouse and chick embryo cell. Further, anthranilic acid itself checks teratogeny in chick embryos if administered along with chloramphenicol\textsuperscript{26,27}. It has also been reported that 4-and 5-methyl anthranilic acids inhibit the growth of Ebethelia typhi, which is reversed by anthranilic acid, tryptophane and indole\textsuperscript{28}. This observation was supported by the report of Volcani et al.\textsuperscript{29} that some substituted anthranilic acids
interfere with the biosynthetic path of the acid in E. Coli. During this period an important observation was made by Goldberg et al.\textsuperscript{30} that phenyl anthranilic acids inhibits the growth of certain strain of M. tuberculosis. Anthranilic acid is well tolerated by some bacterial species and against a few species it shows weak inhibiting effect. Yeasts are not inhibited by the acid but some fungi do not grow in its presence even at a concentration of about 0.5%\textsuperscript{31}. A few esters have shown some bactericidal and fungicidal activities and some of its N-substituted phytopathogenic fungi\textsuperscript{32,33}.

In an early record, anthranilic acid has been shown to possess greater toxicity to mice in subcutaneous doses of 50mg/Kg than the other two aminobenzoic acid\textsuperscript{34}. When injected intravenously to rabbit, anthranilic acid causes marked swelling in the kidney along with diuresis. The acid exhibits a peculiar behaviour with respect to Lactobacillus arabinosus. While it is toxic at a concentration of 1 mg/ml it promotes growth of Lactobacillus arabinosus and Lactobacillus casci in a tryptophane free media\textsuperscript{35,36}. Another interesting observation was ascaricidal activity of cadmium salt of anthranilic acid in mice and sivine along with the distribution of cadmium in kidney, liver, spleen and in some cases also in lungs\textsuperscript{37,38}. Some N-substituted anthranilic acids have been found to possess good anthelmintic activity\textsuperscript{39-41}.

Furosemide, an anthranilic acid derivative, from the group of 4-chloro-3-sulfamybenzoic acid series of diuretic compounds, is a well known nonthaizide potent high ceiling saluretic agent with a rapid diuretic response of eight hours\textsuperscript{42}. The high ceiling diuretics are effective for the treatment of edema of cardiac, hepatic or renal origin\textsuperscript{43,44}. The compounds as a group have a desirable effect in edema and hepatic cirrhosis. Some of them have been employed in therapy.
The search for non-narcotic analgesics with antiinflammatory properties promoted the research group to investigate compound from the N-arylanthranilic acid series because some of them had already been shown to have the desired activity. As noted above mefenamic acid was introduced in therapy in 1967, may be for a short term of only 7-days due to suspected blood disorders. This entry acted as an opening for other compounds of the series. It may be stated here that mefenamic acid represents an effective anti-phlogistic analgesic, discovered after aminopyrine. It is evident from the investigations that combination of both effects, antirecipientive (analgesic) and antiinflammatory, is a rarity among these compounds. The mechanism of analgesic action is likely to be the inhibition of prostaglandin synthetase\textsuperscript{45,46}. The potency of mefenamic acid with respect to aspirin has been studied in relation to dose response including gastrointestinal bleeding. In both the observation mefenamic acid is reported to be better than aspirin\textsuperscript{47,48}. Both mefenamic acid, N-(2,3-xylyl)-anthranilic acid and meclophenamate sodium, N-(2,6-dichloro-m-tolyl)-anthranilate sodium are clinically accepted fenamates. The latter is used in acute and chronic rheumatoid arthritis but the gastrointestinal disorders including diarrhoea limit its clinical applications.

It prompted by the lower incidence of gastrointestinal bleeding compared to aspirin, the study of anthranilic acid derivatives attracted more attention of the research group. As a result a number of aluminium salts of mephenamic, meclofenamic and other N-phenylanthranilic acids have appeared as patents from either professional companies or other research organisation\textsuperscript{49}. Another category of aminobenzoic acid derivatives, synthesized involving some selected pentose or hexose sugars has come up under the name aminobenzoic acid glycosides. These synthetic glycosides involve either o-or p-aminobenzoic acids as an active moiety and exhibit
antiinflammatory analgesic antirheumatic, antipyretic and antitumor activities, depending upon the sugar and the acid chosen. Almost all of them have appeared as patents\textsuperscript{50}.

During more than two decades, the aminobenzoic acids reported with antiinflammatory activity have either a free carboxyl group or an ester group. A number of esters of anthranilic acid derivatives including the esters of N-(3-trifluoromethyl phenyl) anthranilic acid, have been reported to have better analgesic and antiinflammatory activities\textsuperscript{51}. A series of compounds in which the carboxyl group of anthranilic acid or its derivatives form amide with substituted anilines has been reported to show improved activities with low toxicity\textsuperscript{52}.

Although a number of heteroaryl anthranilic acid derivatives have been reported to exhibit the desired activity, as yet only a limited heteroaryl rings have been incorporated into the aminobenzoic acid series of compounds and tested for the activity. In an attempt to compare the activity of 2-(2,3-dimethyl anilino), 2-(m-trifluoromethyl aniline) and 4-(m-trifluoromethyl aniline) nicotinic acid with that of mefenamic acid and flufenamic acid, the nicotinic acid derivatives exhibited the same order of activity\textsuperscript{53}. A few N-substituted thiophenyl and 4-pyrimidyl derivatives have been shown to have antiinflammmtory and analgesic activities\textsuperscript{54,55}. The low order of activity in N-pyrimidyl anthranilic acids found in 1967 was improved in 1970 when 4-pyrimidyl moiety with halogens and carboxyl groups at 5-and 6-positions gave improved derivatives. Departing from anthranilic acid structure but derived from the same, some acridines bearing a carboxyl group at the desired or analogous positions have shown activity against protein denaturation, hemolysis and carrageenan induced edema\textsuperscript{56}. The inclusion of 4-aminoquinolines and 5-pyrazoles in the anthranilic acid
series gave encouraging antiinflammatory activity by both the series where as latter failed to give analgesic effect.\textsuperscript{57}

The large number and diversity of structure of compounds that exhibit antiinflammatory activity are such that classification into groups is meaningless. Structure-activity relationships, though helpful in one series of active compounds, has not been transferable to other series. A common receptor site for non-steroidal antiinflammatory agents has been proposed and some of the more potent drugs, such as indomethacin, aminopyrine, the anthranilic acids, mefenamic acid, flufenamic acid, ibuprofen, phenacetin, phenylbutazone, piroxicam and diclofenac sodium, “fit” this site whereas many others do not.\textsuperscript{58, 59} Hundreds of new compounds are claimed to be antiinflammatory, many in the patent literature. Only those that are clinically active or have undergone extensive biological testing are reviewed here.

1.2. CHARACTERISTICS AND IMPORTANCE OF COORDINATION CHEMISTRY

The last two decades have witnessed spectacular development of coordination chemistry. Several factors have contributed to this. The chemistry of complex compounds has become a bridge to combine closely and logically inorganic, organic, physical and theoretical chemistry, in this way expressing the unity of chemistry as the science dealing with the structure of matter. The countless theories and methods used have been published which itself proved the popularity of the field among the scientists. The theory of co-ordination embraces a wide range of inter-atomic, inter-ionic and inter-molecular reactions in the solid phase as well as in solution. The chemistry of complex compounds has thus become most suitable ground on which to develop and at the same time to verify a number of theories and above all, the theory of chemical bond and structure
of compounds, thereby attracting considerable attention from the chemists, physicists and even mathematicians. The gradual development of valence bond theory\textsuperscript{60-65} via the crystal field theory\textsuperscript{66-74} and further the ligand field and molecular orbital theories\textsuperscript{75-86} developed in recent years on the basis of quantum chemistry\textsuperscript{87-91}, allow to relate the electronic structure and configuration of complex compounds to their physical and chemical properties.

The chemistry of complex compounds paved the way to the preparation of new compounds, which may possess some definite predetermined properties and thus show promising prospects for the future.

The usefulness of the complex compounds in various branches of theoretical and applied chemistry and allied fields is now generally recognized. In quantitative analysis, co-ordination compounds are widely used in gravimetric, volumetric and colorimetric determinations as well as in polarimetry and microscopy. It is generally said that 'the chemistry of solution is the chemistry of complexes'. Complex compounds are widely used in electrodeposition; deposits obtained from the simple salt solutions are sometimes loose, nonadherent, coarsely crystalline and generally undesirable, while metal deposits from appropriate complex salt solutions are often smooth, adherent and of high protective and decorative value. As a consequence of the ability of co-ordinated metal ions to influence many of the complex reactions upon which the vital processes of living organisms depend, co-ordination compounds of many varieties are found widely distributed in nature; chlorophyll, blood pigments, metalloproteins, metallo-enzymes, many vitamins, cytochrome etc. are the few examples of this series. In the past few decades the importance of co-ordination in dyeing has been recognized. Most dyestuffs are synthetic organic compounds; and of these, the large class of metal-dye compounds called 'dye lakes' are of great
interest to the co-ordination chemists. Co-ordination phenomenon is used in water softening by ‘tying up’ alkaline earth ions in soluble complex ions and thus preventing the formation of precipitates. The acetyl acetonates of metals are used in the purification of metals.

The chemistry of complexation has also get great importance in the field of pharmaceutical science. If the ligand forms a stable, water soluble metal chelate, it is said to be a sequestering agent. Sequestration (Latin: to remove) is the suppression of a property or reaction of a metal without removal of that 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, salt of EDTA form a stable lead chelate which is inert, nontoxic and rapidly eliminated. EDTA also increase the absorption of iron in the gastrointestinal tract. An iron 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. The complex of Fe(II) ion with o-phenanthroline is used as an 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 processes of carbohydrates and nucleoproteins, the iron in heme, magnesium in chlorophyll and cobalt in vitamin B₁₂ are examples of systems in which metal chelate complexes are essential for biological activity. Many enzymes contain metals which are essential for the activity of the enzyme system. Removal of metal or lack of the metal can inactivate the enzyme and therefore trace amount of copper, zinc, manganese, cobalt etc. are required for biological processes⁹²,⁹³. Cobalt is
found associated in vitamin B_{12} copper with enzyme tyrosinase, zinc in carbonic anhydrase, molybdenum occurs in Xanthine dehydrogenase.

1.3. SURVEY OF LITERATURE OF METAL COMPLEXES OF DRUGS

An exhaustive survey of literature on metal complexes of anti-inflammatory drugs reveal that their coordination chemistry is mainly due to the complexes by Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo and Cd etc. A general account of their chemistry is available in several texts. Developments of their coordination chemistry have been reviewed from time to time.

A colorimetric method was established by Sane et al. for the estimation of diclofenac in pharmaceutical dosage forms. The colour was developed using potassium ferricyanide in a basic medium and the absorbance was measured at 450 nm. Agarwal et al. determined diclofenac sodium by colour produced with sodium nitrate in presence of hydrochloric acid. The maximum absorbance of the developed colour was noted at 390 nm. Chen et al. have reported an ultraviolet spectrophotometric method for the estimation of diclofenac sodium in injection. The absorbance was measured at 276 nm. Shastri et al. determined some antiinflammatory agents by extractive spectrophotometric method with methylene violet. The absorbance of the extract was measured at 540 nm. Agrawal et al. have reported two spectrophotometric methods for determination of diclofenac sodium in tablet dosage form. The first method is based on reaction of diclofenac sodium with Fe³⁺ in aqueous ethanol to give Fe²⁺ which reacts with bipyridyl to form a coloured complex with an absorbance maxima at 520 nm. The second method is based on formation of colour complex with methylene blue at pH 6.8 (phosphate buffer). The complex is extracted with chloroform. The absorbance is measured at 640 nm.
An experimental design for spectrophotometric study of diclofenac sodium-copper(II) complex have been reported\textsuperscript{118}. The absorbance of complex (in organic phase) was measured at 680 nm. An extraction method with spectrophotometric detection for the determination of diclofenac sodium, using copper(II) acetate as the analytical reagent has been established, with the aid of two statistical optimization procedures. Cu and Cu compounds catalyzed the reaction of 2-ClC\textsubscript{6}H\textsubscript{4}CH\textsubscript{2}CO\textsubscript{2}H (or its salts with 2, 6-Cl\textsubscript{2}C\textsubscript{6}H\textsubscript{3}NH\textsubscript{2}. A mixture of 2-ClC\textsubscript{6}H\textsubscript{4}CH\textsubscript{2}CO\textsubscript{2}H, 2, 6-Cl\textsubscript{2}C\textsubscript{6}H\textsubscript{3}NH\textsubscript{2} K\textsubscript{2}CO\textsubscript{3}, KI and CuI in DMF was refluxed and worked upto give 2-(2,6-Cl\textsubscript{2}C\textsubscript{6}H\textsubscript{3}NH) C\textsubscript{6}H\textsubscript{4}CH\textsubscript{2}CO\textsubscript{2}Na useful as an antiinflammatory, antipyretic and analgesic\textsuperscript{119}. Studies on the potential interaction of diclofenac sodium with an antacid and digitoxin have been carried out\textsuperscript{120}. Isotopically labelled derivative of diclofenac terbutaline and orciprenaline were prepared\textsuperscript{121}.

Spectrophotometric method for determination of composite diclofenac sodium injection solution has also been reported by Huang et al.\textsuperscript{122}. Diclofenac sodium and paracetamol in injectable solution were determined by dual wavelength spectrophotometry and U.V. difference spectroscopy. Spectrophotometric determination of diclofenac sodium in pharmaceutical preparation was reported by Shakya et al.\textsuperscript{123}. Colorimetric estimation of diclofenac sodium in dosage form was reported by Mathur et al.\textsuperscript{124}. The method is based on formation of nitroso derivative followed by alkalisation to yield a stable yellow coloured chromophore, which exhibits absorption maxima at about 378 nm. A simple colorimetric method for estimation of diclofenac sodium from tablets was reported by Bhatia et al.\textsuperscript{125}

Lovrecich et al. have studied the dissolution enhancement of drug by adsorption on polymers or inorganic compounds\textsuperscript{126}. Succinimide esters and glycine amides of non-steroidal antiinflammatory drug, viz, d-naproxen, ibuprofen, ketoprofen, aspirin, diclofence and indomethacin have been
synthesized. Antiinflammatory and ulcerogenic properties have been compared with parent drug molecules. Enzymatic hydrolysis studies in vitro has also been undertaken\textsuperscript{127}. Yang et al.\textsuperscript{128} have reported an ultraviolet method for the determination of diclofenac sodium in suppository. The drug was extracted with ethanol and absorbance was measured at 276 nm. Kamath et al.\textsuperscript{129} have reported an extractive spectrophotometric determination of diclofenac sodium form pharmaceutical preparation. The method is based on formation of ion pair complexes of drug with dyes like acridine orange, basic fuchsin, methylene blue, safranine and toluidine blue in the presence of phosphate buffer. The complex formed are extracted into chloroform and absorbance was measured at 470, 556, 640, 515 and 630 nm respectively.

Leis and coworkers\textsuperscript{130} have reported a femtomole analysis of diclofenac in human plasma by G C negative ion chemical ionization mass spectrometry using 18 labelled diclofenac as internal standard. The application of proton magnetic resonance spectroscopy in the analysis of diclofenac sodium in tablets have also been studied\textsuperscript{131}. A method for determination of diclofenac sodium through the formation of charge transfer complex with chloranil has been reported\textsuperscript{132}. Inclusion of antiinflammatory drugs fenamates with in the cavity of β- cyclodextrin in aqueous solution was confirmed by C.D., U.V. absorption and N.M.R. Soly and spectral changes were quantitatively investigated and the stoichiometric ratio, which was 1:1, formation constants and thermodynamic parameters were obtained for complex\textsuperscript{133}.

A method for determination of diclofenac in human plasma by selected ion monitoring has been reported by Del Puppo and coworkers\textsuperscript{134}. The application of radio telemetric technique in evaluation of diclofenac sodium absorption after oral administration of various dosage forms in
healthy volunteers have been carried out. Estimation of diclofenac sodium by densitometry is reported by Ionescu and cowokers. The drug is separated by TLC on silica gel GF254 with benzene–methanol–anhydrous acetic acid (85:10:1) as mobile phase and the density is measured at 284 nm. Detection and quantification of NSAID agents by gas chromatography mass spectrometry (GCMS) have been reported. A column coated with 0.33 μm of methylsilicone and EIMS detection at m/e 214 and 209 was used.

Giachetti et al. have reported a method for determination of diclofenac in plasma samples and comparison between high resolution gas chromatography (HRGC) and HPLC. For HRGC the drug was extracted from acidified plasma into hexane-methylene chloride (1:1) and tetrabutyl ammonium derivative was prepared and extracted in to hexane containing methyl meclofenamate as internal standard. The gas capillary column coated with ov-1 and 63Ni electron capture detector was used as internal standard. The drug was detected at 280 nm. Gieger et al. have reported a gas liquid chromatographic method for quantitative estimation of diclofenac sodium in biological fluids. The internal standard was 0-(2,6-dichloro-4-methoxy phenyl ) acetic acid. The detector used was electron capture detector. Improved gas chromatographic method for determination of diclofenac sodium in plasma was reported by Ikeda and cowarkers. The column ov-17 and nitrogen as carrier gas were used.

A method for simultaneous determination of diclofenac sodium and its metabolites by capillary column gas chromatography using electron capture detector has been reported by Schneider et al. Helium was used as carrier gas. Brumbasher et al. have reported a GLC method for quantitative determination of diclofenac sodium in human blood serum. Shastri et al. reported a GLC method for determination of diclofenac sodium in tablets using electron capture detector. Fused silica capillary
column gas chromatographic method for the determination of diclofenac in human plasma and urine electron capture detector has been reported. Zecca et al. have reported a method for determination of diclofenac sodium and its metabolites in plasma and cerebrospinal fluid by electrochemical detector.

A method for determination of diclofenac sodium in plasma by HPLC have been reported. For HPLC a column shimpack CLS-ODH was used. The drug was detected at 280 nm. Plavsic and Culig developed an HPLC method for the determination of serum diclofenac concentration. In this method the plasma was extracted with benzene and benzone phase (extract) was used for HPLC using a column of lichrosorb NH2 (2.5 mμ) with mobile phase acetonitrile and perchloric acid speed of mobile phase was adjusted to 0.9 ml/min and eluted chemical was detected at +900 mv. The calibration graph was rectilinear for 200μg/L of diclofenac and the limit of determination was 5μg/L in serum or plasma. Another quantitative method was developed by Chan and Vyas using HPLC. Here diclofenac can be estimated in the synovial fluid and plasma. They used column of supelcosil LC-18 and mobile phase methanol, acetonitrile and sodium acetate. The speed of the phase was 2ml/min and detection was at 215 nm.

Hydrophilic colloids interact with metallic ions to yield crosslinked insoluble salts. Such a principle was utilized in the preparation of diclofenac sodium beads from sodium alginate and sodium CM – cellulose. Hard spherical beads of aluminium alginate and aluminum CM – cellulose with a narrow particle size distribution and low friability respectively were obtained with high yield (80-90%) and a drug content approaching 70-80%. The type and concentration of the polymers as well as the pH of the dissolution medium affected the rate of drug release. Beads prepared from Na – alginate showed a non significantly faster rate of drug release than that
prepared from NaCMC. The higher the polymer concentration, the slower was the rate of drug release. Diclofenac sodium was not released in 0.1N HCl (pH 1.2) for 2h, but was released in pH 6.8 phosphate buffer solution from the 2 formulations studied and from the com. voltaren retard tablet. The 2 formulations of the beads resulted in a sustained – release action of diclofenac sodium for 24h. They showed mean residence time (MRT) values of 9.56 and 7.86h, respectively. The relative bioavailability of the 2 formulation were 59.01 and 47.96% respectively relative to that of the com. voltaren retard tablets have been studied by several workers\textsuperscript{149}.

The reactions of ZnCl\textsubscript{2}, CdCl\textsubscript{2} and Hg(NO\textsubscript{3})\textsubscript{2}.H\textsubscript{2}O with deprotonated diclofenac (L) were studied in aqueous solutions. Complexes of formulae [Zn(L)\textsubscript{2}(H\textsubscript{2}O)], [Cd(L)\textsubscript{2}(H\textsubscript{2}O)], [HgL\textsubscript{2}] and [Cd\textsubscript{2}(H\textsubscript{2}O) (C\textsubscript{2}H\textsubscript{5}OH)\textsubscript{2} (L)\textsubscript{4}]\textsubscript{n} were isolated and characterized as solid products by elemental analysis and spectral (IR, H-1 NMR) and thermal studies. The crystal structure of the complex [Cd\textsubscript{2}(H\textsubscript{2}O)(C\textsubscript{2}H\textsubscript{5}OH)L\textsubscript{4}]\textsubscript{n} was also solved. The crystal structure of the diclofenac acid is also reported\textsuperscript{150}. The complexes ML\textsubscript{2}2H\textsubscript{2}O[M=Co, Ni, Cu; 2HL= diclofenac (2-(2,6-dichloroanilino) phenyl acetic acid)] were prepared from diclofenac sodium and the respective metal salts. The complexes have an octahedral structure as indicated by electronic and IR spectra studied\textsuperscript{151}. Antiinflammatory effects of Co(II), Ni(II) and Cu(II) complexes of diclofenac sodium have been studied\textsuperscript{152}.

The hydroxyl radicals (\textsuperscript{–}OH) are thought to be generated at sites of inflammation and to contribute to tissue damage. All antiinflammatory drugs tested were able to scavenge \textsuperscript{–}OH generated in free solution at almost diffusion – controlled rates. Much \textsuperscript{–}OH generation in vivo occurs at specific sites, where bound metal ions (such as Fe\textsuperscript{2+}) react with H\textsubscript{2}O\textsubscript{2} to produce–OH that immediately attacks the site. Only \textsuperscript{–}OH scavengers that have sufficient metal – binding ability to with draw metal ions from this site can protect
against site-specific damage. All antiinflammatory drugs tested were able
to protect against site-specific damage by -OH radicals in a simple model
system in vitro. Penicillamine, diclofenac sodium, piroxicam, azathioprine,
primaquine, chloroquine and hydroxychloroquine were especially effective.
The ability of an antiinflammatory drug to protect against -OH formation in
vivo depends not only on its rate constant for reaction with -OH, but also on
its metal-binding ability and on the geometry and redox potential of any
metal complex formed\textsuperscript{153}.

Recent work has shown that several non-steroidal antiinflammatory
drug (NSAIDS) prevent free-radical mediated biochemical changes in rat
liver after acute intoxicification with ethanol. The precise mechanism by
which these agents induce such effects is not known. Thus, the influence of
NSAIDS on hydroxyl and hydroxyethyl free radical production in a Fenton
type reaction system was tested in vitro using spin-trapping techniques.
The drugs chosen were considered as representatives of each of the
NSAIDS families and included acetylsalicylic acid, naproxen, diclofenac,
phenylbutazone, mefenamic acid, piroxicam and nimesulide. Among these
drugs, only piroxicam showed the ability to reduce the EPR (ESR) signal
intensity of oxygen- and carbon-centered radical adducts in a dose
dependent fashion and also to induce the formation of different adduct
species. Possible interaction with reaction media metals was ruled out using
divalent cation chelators during the assay. Other NSAIDS showed no
meaningful effect in the range of concentrations tested. It is proposed that
piroxicam is a hydroxyl free-radical scavenger that may form less-
reactive radical species to explain those effects found under in-vivo
conditions. Other NSAIDS failed to reproduce such effects in vitro at least
with the radical species tested. Thus, these drugs may have a different
mechanism of action than piroxicam or a different spectrum for scavenging free-radical species other than hydroxyl radicals\textsuperscript{154}.

Salem et al.\textsuperscript{155} have reported atomic absorption spectrometry of acidic pharmaceutical constituents through precipitation by metal ions (Ag(I), Cu(II) and Fe(III)) determination of mefenamic acid. Mefenamic acid-metal complexes, prepared by mixing equivalent quantities of Na-mefenamate and inorganic metal salt (at pH 7-8) in water, had low toxicity when tested in mice (oral single administration). Prophylactic activities of these salts were examined after oral intoxication with lindane (I), DDT, polychlorocamphene and polychloropine. The highest detoxifying activity was seen following acute intoxication with I; the Zn-mefenamate had the most pronounced activity, where as Fe-mefenamate showed low activity. Prophylactic activities of the metal complexes varied during intoxication with other chloro compounds. The degree of hydrolysis of the metal complexes at physiologically pH affected the activity of these compounds have been carried out\textsuperscript{156}.

Derivatives of arylalkanoic acids and thiazolidine 4-carboxylic acid having analgesic, antipyretic, antiinflammatory and antiarrhythmic activity and their preparation and pharmaceutical compositions have been studied\textsuperscript{157}. β-glycyrrhizic acid-drug complexes as new delivery systems have been reported\textsuperscript{158}. The aluminium N-(3-trifluoromethyl phenyl) anthranilate is prepared and can be used a antiinflammatory agent\textsuperscript{159}. The aluminium N-substituted anthranilate compounds (I) are prepared by reaction of N-(m-trifluoromethyl phenyl) anthranilic acid (II) with Al(OR)\textsubscript{3} in which R is a lower alkyl groups. Thus, 70 ml dry toluene containing 2.5g Al(Ob-r-iso)\textsubscript{3} and 7g II was refluxed for 3 hrs and 1.5 hrs after addition of 10 ml H\textsubscript{2}O. The reaction mixture was distilled in vacuo and the residue was dissolved in 50
ml. EtOH, the 50ml. H₂O was added to the solution to give 7.5 g I have been prepared¹⁶⁰.

The aluminium–flufenamate (AF) showed the same inhibitory effects as those of flufenamic acid on the uv-induced erythema in guinea pigs (ED₅₀~5mg/Kg) and on the carrageenan induced edema (ED₅₀~13mg/Kg), croton oil-stimuated exudation and cotton pellet – granuloma in rats. But the maximum inhibition of carrageenan-induced edema by aluminium flufenamate occurred slower then F. The antiinflammatory activity of aluminium flufenamate was 4-6 times higher than that of phenylbutazone. When orally administered in rats, nearly 94% of aluminium flufenamate was excreted in feces ; none was found in urine, blood and tissues. Urinary excretion of F was 14-15% for 48 hr and biliary excretion was minimal. In rats the max. blood conc. of F was obtained in 4 hr after oral administration of 100 mg/Kg aluminium-flufenamate and within 1 hr after administration of 10 mg/Kg aluminium-flufenamate. In dogs the blood level pattern was the same as in rats, but in rabbits a low level was noted. In intact rats a relatively large amount of F was concd. in the liver, spleen, kidney and in croton oil-treated rats a marked accumulation was noted in the granuloma pouch¹⁶¹.

According to Savoia¹⁶² the p-ethoxyacetanilide (I) and o-ethoxybenzamide (II) were determined simultaneously in mixtures by UV spectrophotometry by measuring the absorbance at 245, 293 and 277 μ (the max. of I and II and their isobestic point ). Separation of acetylsalicylic acid (I), aminopyrine (II), phenacetin (III) and caffeine(IV) mixtures was achieved by thin layer chromatography on silica gel G or H plates under the conditions. Mobile phases cyclohexane - CH₃Cl - AcOH (50:40:10) and cyclohexane-CH₃Cl-Et₃NH (50:40:10) respectively, developing time 35 min; visualization for II,III and IV by spraying with the FeCl₃-I solution and
for I with the $K_4[Fe(CN)_6]_3$ solution. $R_f$ values for I is 36 and 0, for II 0 and 63, for III 16 and 20 and IV 10 and 30, respectively$^{163}$. 

Antipyretics were separated by TLC on 1:5 polyamide (Nylon 6)-Kieselguhr G plates. The $R_f$ values obtained after a 60 – min development in 40:60:1:10 CHCl$_3$-cyclohexane-HOAC-dioxane or in 40:60:1 CHCl$_3$-cyclohexane-HOAC were: salicylic acid (I), 0.14, 0.12 ; salicylamide, 0.31, 0.21; acetylsalicylic acid, 0.48, 0.37; quinine-HCl, 0.58, 0.53; phenacetin, 0.68, 0.59; antipyrine 0.84, 0.85; aminopyrine, 0.94, 0.93. The spots were detected with a 0.07% Rhodamine-B(in alc.) spray and UV irradiation. After immersing the plates in water, the visualized adsorbent layers can be pelled from the glass plates and filed as records. The method can be used to detect I contamination in aspirin$^{164}$. TLC data ($R_f$ values and approx. detection limits) are given for caffeine and the antipyretics, sulpyrin, quinine-HCl, Na salicylate, salicylic acid, antipyrine, aminopyrine, aspirin, phenacetin and salicylamide developed by 4 solvent systems on 10:52 nylon 6-silica gel G mixed have also been studied$^{165}$. 

Goenechea$^{166}$ have reported a TLC detection of acetanilide, phenacetin and some chemically related analgesics. Bachrata et al.$^{167}$ have reported a chromatographic method on 0.4mm layers of silica gel CH (Lachema), developed for the separation of the components of analgesics containing amidopyrine, antipyrine, phenacetin, phenobarbital, caffeine, citric acid, codeine phosphate, belladonna dry extract and ethylmorphine. Qualitative analysis of analgesic mixtures containing acetyl salicylic acid, aminopyrine, antipyrine, caffeine, codeine phosphate and phenacetin by thin-layer chromatography was carried out. All components were distinctly separated with silicic acid and silica gel G as adsorbents. Acetone, 0.5N NaOH, CHCl$_3$ and H$_2$O were used as solvents. The solvent system for
partition chromatography were: BuOH-HOAC-H₂O (4:1:5) for the first and BuOH-H₂O(1:1) for the second run.  

Thin layer chromatographic separation of the components of antirheumatic drugs has been described by some workers. The following substances could be distinguished on a single chromatogram on a silica gel layer: phenacetin, phenazone, aminophenazone, noraminophenazone, phenylbutazone, salicylamide. The solvent was BuOAc-CHCl₃-85% HCO₂H (6:4:2). A 5% solution of FeCl₃ in acetone, over which 1% alcoholic o-phenanthroline was sprayed, was used as the developer. The various pharmaceuticals, such as alkaloids, phenothiazine derivatives, local anesthetics, analgesics, antihistamines, sedatives and hypotics were separated by paper and TLC.  

An infrared spectrophotometric method is described for the rapid determination of small amount of isopropamide (I) in pharmaceutical preparations containing aminopyrine (II), phenacetin(III), caffeine and I, by the use of a compensation method. Me₂CO was chosen as a solvent, the key band used for I was 702 cm⁻¹ and a solution of a mixture of II and III was used as a compensation solution. The method 0.1% 4-chloroacetanilide and 0.005% p-phenetidine can be detected by TLC have also studied. D.T.A. was used to study 28 systems of acetanilide, phenacetin and benzanilide.  

David et al. prepared Iron (III) complexes with antiinflammatory drugs such as: Fe (aspirinate)₃. 2H₂O, Fe(indomethacin)₃. 2H₂O and Fe(piroxicam)₃. 2DMF and investigated by IR, EPR and Mossbauer spectroscopies. The complexes appear to have an octahedral stereochemistry involving three non-steroidal ligand molecules in the process of coordination. Powder EPR spectra of these complexes are characteristic to the
dimeric species with the metallic ion in the high spin state \((S=5/2)\). The moppbaer parameters are typical for the covalent iron complexes.

Cini\(^\text{175}\) have reported the synthesis and structural characterization of \([\text{PtCl}_2(\text{dmso})(\text{HL})]\) (\text{dmso} = \text{dimethyl sulfoxide}) which is the first complex of piroxicam with any of the third series of d-block element to be analysed via X-ray diffraction. The synthesis and characterization of \(\text{Fe(II)}, \text{Co(II)}, \text{Ni(II)}, \text{Cu(II)}, \text{Zn(II)}\) and \(\text{Cd(II)}\) complexes with a potent antiinflammatory drug piroxicam are described. The crystal and molecular structures of \(M(\text{Pir}_2)(\text{DMF})_2(M = \text{Cu, Cd}; \text{Pir} = \text{piroxicam}; \text{DMF} = \text{N, N-dimethyl formamide})\) are also reported\(^\text{176}\).

Piromatic has been found to form yellow coloured complex with \(\text{Cu(II)}\) in neutral medium which is soluble in chloroform and shows maximum absorbance at 404 nm. Job’s method gave the metal to ligand ratio as 1:2. The complex solution obeyed Beer’s law within the range of 1.50 meg to 20 meg/ml of piroxicam added\(^\text{177}\). Piroxicam was found to exhibit keto-enol tautomeric equilibrium between the keto and enol forms in aqueous media with the enol form as the predominant species (about 80%). Making use of the electroanalytical methods, differential pulse polarography and cyclic voltammetry, the keto-enol tautomeric equilibrium constant, \(kt\), was evaluated\(^\text{178}\).

The Infrared and Raman spectra of the antiinflammatory drug piroxicam and of its \(\text{Cu(II)}\) and \(\text{Ni(II)}\) complexes with stoichiometry \(M(\text{Pir}_2)(\text{H}_2\text{O})_2\) are reported and briefly discussed. Detailed information on the metal-ligand vibrations has been obtained\(^\text{179}\). As part of a program to evaluate the possible role of metal ions in the phototoxicity of piroxicam in biological systems, the interactions of various transition metal ions with piroxicam have been studied by spectroscopic methods. The absorption band of piroxicam disappears in the presence of \(\text{Zn}^{2+}\) and \(\text{Cu}^{2+}\) by 1st-order
kinetics, indicating that the structural change of piroxicam is catalyzed by metal ions. The metal-catalyzed reaction product has been isolated and its structure has been identified by IR, NMR and mass spectroscopic methods. These results indicate that Zn$^{2+}$ and Cu$^{2+}$ ions are highly effective in promoting a rearrangement of the piroxicam molecule.$^{180}$

The preparation, spectroscopic and magnetic properties are reported for complexes of Mn(II),Fe(II), Co(II), Ni(II), Cu(II) and Zn(II) with the antiinflammatory drug piroxicam. In all the complexes, studied, the piroxicam acts as a chelate monoanionic ligand with co-ordination involving the enolate oxygen atom and the carbonyl oxygen atom of the amide group. The complexes appear to have an octahedral stereochemistry involving two chelate piroxicam ligands in the case of divalent central metal ions and three chelate piroxicam ligands in the iron (III) complex. The manganese (II) and copper (II) complexes exhibit marked superoxide dismutase activity in the nitroblue tetrazolium assay have been reviewed by Pipe et al.$^{181}$ The interaction of various metal ions (Co(II),Cu(II), La(III), Ce(III), Y(III) and Al(III) with piroxicam in aqueous solution was studied by spectroscopic methods. The interaction of the metal ions with HMBDC (4-hydroxy -2- methyl-1, 2 lambda -benzothiazine -1, 1-dioxide -3-methyl - carboxylate ) was also studied for comparison. Al(III) and Y(III) ions interact significantly with both piroxicam and HMBDC to form 1:1 complexes in aqueous solution. The stability constants for the complexes were determined at mu. foward o. The thermodynamic parameters for the complex formations were also calculated for the Gibbs-Helmholtz plot. On the basis of the spectroscopic and thermodynamic evidence, the binding sites of piroxicam and HMBDC to the metal ions are suggested.$^{182}$

Four possible pyridine, monohydrated metabolites of the antiinflammatory agent piroxicam have been synthesized for comparison.
with a natural pyridine - hydroxylated metabolite of this compound. In addition, another metabolite of piroxicam, derived from dehydration of the parent drug has been made and characterized. The antiinflammatory activity of these compounds and four other known metabolites of piroxicam has been measured in the carrageenan induced rat paw edema model and all are found to be less active than piroxicam itself. Different analytical methods like HPLC, colorimetry and UV spectrophotometry have been used for estimation of piroxicam.

Piroxicam, can prevent free radical mediated changes during acute alc. intoxication in rats. The precise mechanism of such effects is not known. Other drugs have shown ability to interfere with metals in the reaction media or to scavenge free radicals in vitro studies. For this aim, spin-trapping studies were carried out during the auto-oxidation process of phenylhydrazine and fenton reaction sep.,in the presence of piroxicam. The spin adducts were measured by ESR. Piroxicam increased free radical production during the auto – oxidation of phenylhydrazine and decreased it in the Fenton reaction system at high concentrations. The possible interaction of piroxicam with metals in the reaction media is discarded as well as a ph and hydroxyethyl radicals scavenger effect. Fifteen patients consulted for photoallergy to piroxicam and all had a positive test to thiosalicylic acid which is the compound most frequently responsible for sensitization to thiomersal and for cross-reaction between thiomersal and piroxicam have been reported.

The preparation of Cu(II) complexes of aminopyrine (amp=1-phenyl-2,3-dimethyl-4-dimethylaminopyrazol-5-one), viz. Cu (amp)_2 X_2.n H_2O [X = ClO_4^- (N = 1) NO_3^- or CuBr_2^- (n=0)], Cu(amp) X_2 (X = NO_3^- and (ampH_2) [CuBr_4] have been investigated. Souchay prepared a large number of aminopyrine complexes of transition metal ions. The compounds
Cu(amp)(NO₃)₂ and Cu(amp)(N₃)₂ have been prepared for the first time. The CuX₂ (Apy) (X = AcO, BzO, salicylate and o-, m- or p-cresotate, Apy = antipyrene) were prepared from CuX₂ and antipyrene in anhydrous MeOH or BuOH. The complexes showed low magnetic moments (1.39-1.44 μB)¹⁹¹.

Dick et al.¹⁹² have reported the complexing properties of Pt(II), Pt(IV) and Pd(II) with pyrazolinone ligands and anions. The following compounds were prepared [PtX₆] (Hpyr)₂ (X = Cl or Br; Pyr = 4 -dimethylamino -1 -phenyl -2, 3 -dimethyl-2-pyrazoline-5-one), [ptX₄] (Hpyr)₂ and [Pd PyrX₂] The compounds were characterized by UV and IR spectra and also by thermogravimetric method. The complexes (Hpyr) [TlCl₄], where pyr = pyramidine; (H antp₂) – [TlCl₄], where antp = antipyrene ; (HL) [TlL₄], where L = pyr, methylaminoantipyrine (mam) or aminoantipyrine (am); [Tl(antp)₂X₃], where X = Cl, Br, I; [Tl(pyr)X₃], where X = Cl, Br, I ; [In (antp)₆] I₃ ; [In(antp)₃] I₃ ; [In(pyr)I₃] ; [Ge(pyr)₃] Cl₄ and (HL)₄[GeO₄.12MoO₃], where L = pyr, mam, am or antp were prepared. The ir spectra for [Tl(pyr)Br₃], [In(pyr)I₃], [Tl(antp)₂Cl₃] and (Hpyr) [TlL₄] have been studied¹⁹³.

The complex compounds of metals with antipyrene and its derivatives of the M(R)ₙ - Xₘ and (R.H)ₙ-m (MeₙXₙ) type ,where R is the ligand and X is either Cl, Br, I, SCN' behave as acid and as base both in nonaqueous media. Their acidic properties depend on the nature of the ligand. Complex compounds of Zn, Cd, Hg, Co and Mn with 1-2 molecules of the ligand in M₂Co behave as bases. Compounds of the M(R)ₙ Xₘ type are ampholytes. Complex compounds of Bi, Pt, Os, Zn and Sb with antipyrene and derivatives of the 2nd type behave as acids in Me₂CO¹⁹⁴. Complex formation in the system NH₄VO₃-4-(2-pyridylazo) resorcinol (PAR) was studied by UV spectroscopy and by the method of isomolar series¹⁹⁵.
The IR spectra were reported for complexes of pyrididine with ZnCl₂, Zn(SCN)₂, CdBr₂, Co(SCN)₂, Co(SCN)₄H₂, HgCl₂ and HgBr₂ in acid, neutral and weakly acid media, in the solid state and in MeCN solutions. Aqueous extracts of Cochlospermum planchonii Hook. f. (Cochlospermacese) rhizomes are used by native medical practitioners in northern Nigeria to treat jaundice. Adaptation of their method was hepatoprotective in carbon tetrachloride – treated rats (CCl₄) and it inhibited cytochrome P 450 enzymes, which constitutes a plausible hepatoprotective mechanism. A cryst. inhibitor was isolated using inhibition of two rat cytochrome P 450 enzymes, aminopyrine – N-demethylase and aniline hydroxylase as bioassays to guide fractionation by solvent partitioning, polyamide column chromatography, preparative thin layer chromatography and fractional crystalline. The inhibitor was identified as zinc formate by inductively coupled plasma at. emission spectroscopy, NMR spectroscopy and comparison with synthetic material by power X-ray diffraction crystallography.

According to Dwivedi the drug metabolic activity and the homeostasis of essential metal ions in the lenticular system of adult rats exposed to long term low level lead (lead acetate 0.1% w/v). The results of this investigation demonstrate that long term low level lead exposure impaired the phase I & phase II metabolic activity of the lenticular system when assessed by aminopyrine demethylase, benzopyrene hydroxylase, aniline hydroxylase and UDP glucuronyl transferase (UDPGT), glutathione s-transferase (GST) respectively. A more pronounced decrease (55%) in GST was noticed compared to UDPGT, aminopyrine demethylase, benzopyrene hydroxylase and aniline hydroxylase (20-30%). Increased lead concentration in the lenticular system of the rats as monitored by atomic absorption spectroscopy resulted in a significant decrease (15-32%) in the
levels of Ca, Cu, Zn and Fe, along with a progressive loss in body weight. Respective increase in blood lead level was also monitored parallel to increase in lenticular lead concentration at different time points in lead treated rats.

The alkylamines are oxidized by a number of different type of enzymes and the low oxidation potentials favor 1-electron transfer processes and aminium radicals. The mechanism of N-dealkylation by hemoproteins using the prototypic substrate N, N - dimethylaniline (with isotopic substitution on the methyl groups). Since there were considerable data available from kinetic deuterium isotope studies suggesting alternative mechanisms for different hemoproteins\textsuperscript{199,200}. Cytochrome P 450 2B1, chloroperoxidase and several biomimetic metalloporphyrin system showed low kinetic hydrogen isotope effects and hemoglobin, horseradish peroxidase and prostaglandin – H synthase yielded high isotope effects in agreement with previous studies\textsuperscript{201}. Dinnocenzo and Banach\textsuperscript{202} have provided evidence that the pKa for an alphahydrogen of the N, N-dimethylaniline aminium radical is approximately 9 and also estimated that the pKa for the 4-hydrogen of a 1, 4-dihydropyridine aminium radical is approximately 3.5. The oxidations of two model 1, 4-dihydropyridines showed only low kinetic hydrogen isotope effects with all of the enzymes examined. Aminium radicals derived from aminopyrine and N, N-dimethylthioanisole accumulated only with those hemoprotein systems showing the high isotope effects with N, N-dimethylaniline. Its concluded that specific base catalysis of alkyl hydrogen removal from aminium radicals by the (Fe.O)\textsuperscript{2+} complex is a feature of some hemoproteins, including P 450 s and that the lack of such catalysis in other hemoproteins is the basis of their high kinetic hydrogen isotope effects.
A useful NMR technique for the detection, estimation and characterization of N-methyl groups has been reported\textsuperscript{203}. The separation of aminopyrine, chinophen and salicylic acid by ion-exchange chromatography has been investigated\textsuperscript{204}. The TLC was reported for the separation of phenazone (I; Antipyrine), 4-aminophenazone, 4-methylaminophenazone, 4-dimethylaminophenazole (Pyramidon), 1-phenyl-3-methyl-pyrazolone, 4-hydroxyphenazone, phenylbutazone (Butazolidin) and novamine sulfone (Novalgin), in conjunction with the discovery of decomposition products of the pyrazolone derivatives upon their storage with the systems CHCl\textsubscript{3}/ether(1:1), CHCl\textsubscript{3}/methylal (7:3), CHCl\textsubscript{3}/methylal/Meo (7:3:0.5), up to 6 substances could be separated. An essential result of the investigation was the easy demethylation of the C-4 amino group of pyramidon\textsuperscript{205}.

Metal complexes of antipyrine, 4-aminooantipyrine and pyramidon have been studied earlier. In all the above cases the ligand was found to act as a monodentate ligand bonding through its carbonyl oxygen which was further confirmed by spectral studies. Antipyrine can be converted to an effective chelating ligand by substituting dimethylaminomethyl group by Mannich reaction and the product obtained is dimethylaminomethylantipyrine (DMAMA)\textsuperscript{206-208}. The metal complexes of 4-dimethylaminomethylantipyrine with Fe(III), Co(II), Cu(II), and Zn(II) have been studied\textsuperscript{209}. DMAMA acts as a bidentate ligand and forms sixteen-membered chelate on complex formation. The laser raman spectrum of phenylbutazone was recorded in the region 4000-200 cm\textsuperscript{-1} and its fourier transform infrared spectrum in the region 4000-400 cm\textsuperscript{-1}. The spectra have been analysed assuming C\textsubscript{1} point group symmetry. The probable assignments to the observed bands have been made with the help of magnitude and intensities of the recorded spectra\textsuperscript{210}. 
According to Iscan and coworkers\textsuperscript{211} when Cd (3.58 mg CdCl\textsubscript{2}.H\textsubscript{2}O/Kg, ip) was administered to male guinea pigs 72h prior to sacrifice, the metal significantly inhibited the aniline 4-hydroxylase (AH) (16%), ethylmorphine N-demethylase (EMND) (26%) and aminopyrine N-demethylase (AMND) (18%) activities and cytochrome P-450 (12%) and cytochrome b5 (10%) levels. Cd did not alter the hepatic microsomal heme level. Cd, however, significantly increased the hepatic microsomal p-nitroanisole o-demethylase (p-NAOD) (53%) activity. When Ni (59.5 mg NiCl\textsubscript{2}.6H\textsubscript{2}O /Kg, SC) was administered to the guinea pigs 16h prior to sacrifice, the metal significantly depressed AH (49%), p-NAOD (66%), EMND (47%) and AMND (37%) activities and cytochrome P-450 (15%), cytochrome b5 (24%) and microsomal heme (28%) levels. For the combined treatment, animal received the single dose of Ni 56h after the single dose of cd and then were killed 16h later. In these animals, significant inhibitions were noted in AH (51%), EMND (47%) and AMND (30%) activities and cytochrome P-450 (15%), cytochrome b5 (26%) and microsomal heme (30%) compared to those of controls. In the case of P-NAOD activity, the influence was in favour of Ni, i.e., inhibition was about 61% by the combined treatment.

A rapid, sensitive and specific procedure for determination of ibuprofen has been developed. The drug solution in chloroform is treated with Cu(II) solution at pH 5.5, forming a blue compound which is extractable in the organic phase. This complex is spectrophotometrically measured at lambda 675 nm obeying Beer’s law in a concentration range of 0.5 – 3.2 mg.ml\textsuperscript{-1}. The obtained complex has an apparent molar absorptivity of 0.48 × 10\textsuperscript{2} and beer’s no interference from other metal ions. The stoichiometry of the reaction was studied and the reaction product was isolated for further studies\textsuperscript{212}. Manganese catalysts promote the selective
and rapid autooxidation of aldehyde I to give ibuprofen (II) in high yields. Thus, the autooxidation of I with oxygen at 120 psi in decane in the presence of manganese (II) stearate at 0 degree for 2h gave 72% II and 9% ketone III, 1% alco. IV and 1% formate V\textsuperscript{213}.

The reaction yield of the esterification of (R,S)- ibuprofen with n-propanol, catalyzed by phys. adsorbed candida antarctica lipase-B on anionic resin, is improved by the addition of benzo-[18]-crown-6-or meso-tetraphenylporphyrin but reduced by the presence of metal-porphyrins. The interaction of benzo-[18]-crown-6 or meso-tetraphenylporphyrin with the lid of pure lipase –B on candida antarctica, would produce the activation of the lipase increasing the reaction rate but not the enantioselectivity\textsuperscript{214}. A one-pot reaction sequence consisting of three consecutive metalation and electrophilic substitution stages starting with p-xylene leads 2-(4-isobutylphenyl) propanoic acid with a 52% overall yield. A crucial step is the alkylation of deprotonated p-ethyltoluene with isopropyl bromide. In general terms, sec-alkyl halides and benzyl – or allyl – type alkali metal reagents undergo coupling reactions with surprising case has been concluded\textsuperscript{215}.

The biochemical isomerization of ibuprofen led us to the successful purification of “2-arylpropionyl – Co A epimerase” from rat liver cytosol and mitochondria have been investigated\textsuperscript{216}. Synthesis and pharmacological evaluation of poly(oxyethylene) derivatives of 4-isobutylphenyl-2-propionic acid (ibuprofen) have been studied by several workers\textsuperscript{217}. The N-hydroxy methyl derivative of 2(4-isobutyl phenyl) propionamide was synthesized and condensed with seven different active hydrogen containing compounds (antipyrine, pyrrolidine, piperidine, phthalimide, morpholine, piperazine and hydrazine ). These compounds were characterised by their analytical and spectral data. The antiinflammatory activity of the synthesized compounds
was evaluated by carrageenan induced rat paw oedema method and the compounds with pyrrolidine, phthalimide and hydrazine showed potent antiinflammatory activity\textsuperscript{218}.

The degradative effects of interleukin-1 (IL-1) on the extracellular matrix of connective tissue are mediated primarily by metalloproteinases and prostaglandin. The clinical observations suggest that these effects can be prevented, to some extent by the use of non-steroidal antiinflammatory drugs. The role of prostaglandin E\textsubscript{2}(PGE\textsubscript{2}) in IL-1-induced gene expression by human skin fibroblasts in culture has also been examined. Incubation of confluent fibroblast cultures with varying concentration (0.01-1.0 micro gram/ml) of PGE\textsubscript{2} led to a dose -dependent elevation of collagenase mRNA steady-state levels, the promoter activity and the secretion of the protein, whereas relatively little effect was observed on stromelysin and TIMP gene expression. Exogenous PGE\textsubscript{2} had no additive or synergistic effect with IL-1 on collagenase gene expression. Furthermore, commonly used non-steroidal antiinflammatory drugs (indomethacin, acetyl salicylic acid and ibuprofen) at doses which block prostaglandin synthesis in cultured fibroblasts, failed to counteract IL-1-induced collagenase and stromelysin gene expression, nor did they affect TIMP expression. Although the effects of PGE\textsubscript{2} did not potentiate those of IL-1 on collagenase gene expression in vitro, one could speculate that massive production of PGE\textsubscript{2} by connective tissue cells in vivo in response to inflammatory mediators such as IL-1 or tumor necrosis factor-alpha, could lead to sustained expression of collagenase in connective tissue cells after clearance of the growth factors\textsuperscript{219}.

In vitro, 2 mols. of indomethacin chelate with 1 atom of Zn\textsuperscript{++} or Cd\textsuperscript{++} as shown by spectrophotometric and ir methods. As the Cd\textsuperscript{++} content of the blood vessels is high in collagen diseases, a beneficial effect of
indomethacin is postulated\textsuperscript{220}. We cultured calvarial bones from neonatal mice and exposed them to Cd to study the effects of the metal on calcium release and on the activity of some enzymes of importance for bone resorption and bone formation. Cd does-dependently stimulated calcium release from the bones. Maximal release was noted at Cd concentrations of 0.4-0.8 micro M, which was similar to the level of release in the presence of maximal stimulatory concentrations of parathyroid hormone (10 nm) and prostaglandin E\textsubscript{2} (10 micro M). Cycloheximide (1 micro M) inhibited calcium release elicited by Cd, prostaglandin E\textsubscript{2} and parathyroid hormone. Cd-induced calcium release was linearly increased from 24 to 72 hr of culture. Production of prostaglandin E\textsubscript{2} by the bone specimens was dose-dependently stimulated by Cd and inhibited by 1 micro M indomethacin. Cd-induced calcium release was inhibited by acetazolamide (100 micro M), indomethacin (1 micro M) and ibuprofen (10 micro M). Prostaglandin E\textsubscript{2}-stimulated calcium release was not inhibited by indomethacin. Exposure to 32 microM Cd, present during a 48hr incubation period, significantly decreased prostaglandin E\textsubscript{2}-stimulated calcium release from 38.9\% to 29.8\%. Calcium release induced by parathyroid hormone was more sensitive to inhibition by the metal (i.e., Cd concentrations of 0.2 and 32 micro M) decreased the release from (37.7\% to 31\% and 19\%) respectively. Cd present in the culture medium during a 48 hr incubation dose-dependently inhibited the activity of alkaline phosphatase and tartrate-resistant acid phosphatase in the bones but did not influence the activity of carbonic anhydrase. We conclude that Cd has a direct stimulatory effect on bone resorption and this effect is dependent on prostaglandin production and also on protein synthesis. On the other hand, Cd also has an inhibitory effect on bone resorption (i.e., resorption is inhibited by higher concentrations of the metal). Moreover, Cd may impair bone formation by impeding the activity of alkaline phosphatase\textsuperscript{221}. 
Complexes of 4-[(N-benzoyl)amino] antipyrine (BAAPY) with thorium (IV) and dioxouranium (VI) having the general formulae [ThX₄n BAAPY] (where (i) X=Cl, Br, NO₃ or NCS, n=2 and (ii) X=1 or ClO₄, n=3) and [UO₂X₂n BAAPY] (where (i) X=Cl, Br, NO₃ or NCS, n=1 and (ii) X=1 or ClO₄, n=2) have been synthesized and characterized. The IR spectra of the complexes suggest that the ligand BAAPY acts as 0-0 chalating agent. The probable coordination number of Th (IV) is 6,8 or 12 and that of U(VI) is 6 or 8 in the complexes²²².

Singh et al.²²³ have reported stable solid complexes of U (IV) and Th (IV) with α-picoline acid, nicotinic acid, anthranilic acid and phenylanthranilic acid. Complexes of salicylic, sulphosalicylic and picolinic acid with pyridine di-carboxylic acid were prepared in view to study their antiinflammatory and anti-ulcer properties in experimental animals²²⁴. The Tl (III) complex was determined with ≤ 0.62% relative error by titration with EDTA at pH 2.6 in the presence of Fe (III) – salicylate complex as the indicator²²⁵. The CuL₂.ROH (HL =2,3-cresotic acid, R = Me, Et, Pr, Bu, hexyl, nonyl) and CuL¹₂.ROH (HL¹ = Salicylic acid, R = Et, Pr) were prepared by treating CuL₂ or CuL¹₂, respectively with ROH. Magnetic susceptibility measurements indicate that the complexes are dimeric²²⁶.

The salicylatogroup in the salicylatotetraminecobalt coordinates as a bidentate chalate ligand and form a nonplaner –6- membered ring with the Co atom²²⁷. The green products of the reaction of HNO₃ with salts of the salicylatotetramine (III) (I) or salicylatobis (ethylenediamine) cobalt (III) cations are formulated as complexes of cobalt (III) containing the hydrogen-5- nitrosalicylate ion as a ligand. The evidence for the previous formulation of the product from I as 5-nitrosalicylate complex of cobalt (IV) is discussed²²⁸.
The unit cell dimensions and space group of cadmium salicylate dihydrate have been studied\textsuperscript{229}. The complex formation of Zn – salicylate (I) and Cd – salicylate (II) with EtNH\textsubscript{2} was studied. I adds more EtNH\textsubscript{2} then II under the same conditions. The complexes Zn(C\textsubscript{6}H\textsubscript{4}OHCO\textsubscript{2})\textsubscript{2} EtNH\textsubscript{2}, 2Zn (C\textsubscript{6}H\textsubscript{4}OHCO\textsubscript{2})\textsubscript{2}. 2EtNH\textsubscript{2} and Cd (C\textsubscript{6}H\textsubscript{4}OHCO\textsubscript{2}). EtNH\textsubscript{2} were obtained\textsuperscript{230}. The extraction of Be (II), Mn(II), Zn(II) and Cu(II) into Bu\textsubscript{3}PO\textsubscript{4}(TBP) by salicylic acid was examined. Be(II) was extracted as Be(OC\textsubscript{6}H\textsubscript{4}CO\textsubscript{2}).2H\textsubscript{2}O, Zn(II) and Mn(II) were extracted as species of formula M(HOC\textsubscript{6}H\textsubscript{4}CO\textsubscript{2})\textsubscript{2}TBP, while Cu(II) extraction was as species of both these types. The mode of extraction, which depends on the magnitude of the formation constant of the monosalicylato – metal (II) chelates, have been reported\textsuperscript{231}. The partition behaviour of Co(II) between aqueous solution and Bu\textsubscript{3}PO\textsubscript{4} (TBP) in the presence of salicylic acid was investigated\textsuperscript{232}. The species, extracted, is probably Co(C\textsubscript{7}H\textsubscript{5}O\textsubscript{3})\textsubscript{2}.2TBP.n H\textsubscript{2}O; under certain conditions the species Co(C\textsubscript{7}H\textsubscript{4}O\textsubscript{3}) is formed in the aqueous phase but is not extracted into TBP although it is uncharged.

Garaj and Kratsmar – Smogrovic\textsuperscript{233} have studied the effect of the conditions of preparation on the structure of isomeric forms of [Cu(Sal)\textsubscript{2}(Py)\textsubscript{2}] sal = (salicylate ion by X-ray powder patterns. The β - modification of [Cu(Sal)\textsubscript{2}(Py)\textsubscript{2}] is formed by the reaction of pyridine vapor with the fine, crystal [Cu(Sal)\textsubscript{2}(H\textsubscript{2}O)\textsubscript{2}]. 2H\textsubscript{2}O complex or with anhydrous Cu(Sal)\textsubscript{2}. Similar powder patterns were obtained for the precipitate obtained from a solution of pyridine and [Cu(Sal)\textsubscript{2}(H\textsubscript{2}O)\textsubscript{2}].2H\textsubscript{2}O in EtOH. The α- modification of [Cu(Sal)\textsubscript{2}(Py)\textsubscript{2}] was formed in an alcoholic solution containing Cu : pyridine in the ratio 1:6. The α - modification can be distinguished from β - by the evaluation of the relative height of the diffraction maximum change in the conditions of preparation, i.e. different concentrations of pyridine or the solvent, cause the formation of
intermediate product between the α- and β-modifications which can be characterized by the intensity of the diffraction patterns. α - [Cu(Sal)₂(Py)₂] is very sensitive to pressure and in 3 min. at 13 atm. can be changed to the β-modification.

Carey and Martell²³⁴ have investigated the formation of mixed ligand complexes of U(IV) with ethylenediamine tetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) as primary ligands and salicylic acid (SA), 5-sulphosalicylic acid (SSA), 1,2-dihydroxybenzene-3, 5-disulphonate disodium salt (tiron), pyrocatechol (Py), 5-sulpho-8-hydroxyquinoline (HQS), 1,8-dihydroxynaphthalene-3, 6-disulphonate disodium salt (CS), phthalic acid (Ph) and iminodiacetic acid (IMDA) as secondary ligands. The relative magnitudes of equilibrium constants for combination of secondary ligand with the [U(IV)(EDTA)] chelate are CS>Tiron>Py>SSA>HQS>IMDA>Ph. Similar mixed ligand chelates with Th (IV) using NTA, HEDTA, EDTA and CDTA have also been reported²³⁵. The interaction of Cu²⁺ and C₆H₄(OH)CO₂⁻ in aqueous solution to form ([Cu(Sal)₂(H₂O)₂]₂H₂O) were studied by spectrophotometric and paper electrophoretic methods²³⁶.

Potentiometric studies of the interaction between Cu(II) and various bidentate chelating agents are described. Evidence is given for the formation of Cu(II) chelates having a 1:1 molar ratio of Cu(II) with the respective ligands: 3, 5-disulfopyrocatehol (Tiron), pyrocatechol, 3, 6-disulfo-1, 8-dihydroxynaphthalene, oxalic acid, 8-hydroxy-5-quinolinesulfonic acid, salicylic acid and 5-sulfo salicylic acid. A study of the behaviour of Cu(II) in the presence of equimolar concentrations of each of these ligands and α, α’-dipyridyl gave evidence for the formation of mixed ligand chelates containing 1:1:1 molar ratios of Cu(II) to dipyridyl to the secondary ligand²³⁷. The ternary complexes of the
system V-salicylate-organic base (aminopyrine (I), quinine (II), C₅H₅N (III). and Bu₃N(IV) were studied spectrophotometrically²³⁸.

According to a study carried out by Degawa and coworkers²³⁹ male F344 rats were pretreated with lead nitrate, nickel chloride, cobalt chloride or cadmium chloride and their effects on the induction of cytochrome P450(CYP) enzymes, mainly CYP1A2 enzyme, with 2-methoxy - 4-aminoazobenzene (2-MeO-AAB) in the livers were comparatively examined by enzymatical, immunochemical and molecular biological methods. When rats were pretreated with each ionic metal, the total CYP amount in the liver microsomes decreased, as compared with that of rats treated with 2-MeO-AAB alone. However, among the ionic metals used, only lead reduced the levels of the mRNA and protein of CYP1A2 induced with 2-MeO-AAB in the rat liver and decreased the microsomal activity (per CYP) for CYP1A2-mediated mutagenesis. Furthermore, ionic lead, but not other ionic metals, showed an ability to induce a placental form of glutathione s-transferase (GST-P). The level of CYP1A2, induced with 2MeO-AAB, was decreased along with increase in that of the induced GST-P. The drug inhibition of the macrophage response to metal wear particles (cobalt chrome alloy and titanium alloy) in vitro have been studied by several workers²⁴⁰.

The preparation and antiinflammatory activities of Cu-aspirin and Cu-salicylate complexes have been investigated²⁴¹. In rats after induction of edema with carrageenan, the Cu content was decreased in liver and brain; however, the concentration of Cu was increased in the serum and the edema site. Using this model to test antiinflammatory activity, the rate of edema inhibition by aspirin was higher than that of its Cu complex, where as the rate of edema inhibition by the Cu naproxen. The content of Cu of the serum and the edema site was decreased after administration of the antiinflammatory agents. Induction of hemorrhage in the stomach by Cu-
salicylate was found to be greater than that by Na-salicylate, where as induction of hemorrhage by Na-naproxen was found to be higher than that by the Cu-naproxen complex\(^\text{242}\).

According to Sorenson\(^\text{243}\) the copper chelates of compounds with no antiinflammatory activity such as anthranilic acid and compounds which are inflammation inhibitor such as aspirin were more active in tests for antiinflammatory activity than either cupric acetate or the chelating compounds. The Cu chelates appeared to be less toxic than the parent compounds. Thus, Cu chelates may be the active metabolites of the antiinflammatory compounds in vivo. The copper complexes, a unique class of potentially more therapeutically useful antiarthritic agents having both antiinflammatory and antiulcer activities, are presented. Points of interest with regard to their relatively low toxicities and mechanisms of action are discussed\(^\text{244}\). Copper complexes of a range of ligands have been prepared and evaluated for antiinflammatory activity and irritancy after oral, subcutaneous and local administration in rats and guinea pigs\(^\text{245}\).

Inspite of good bioavailability after oral administration contra-indicative manifestations are associated with diclofenac therapy. It is extensively metabolized in the liver and mainly excreted in urine. It has also a narrow therapeutic index. Because of its short biological half life the drug has to be given quite frequently\(^\text{246}\).

A search of the literature with regard to the biological requirements and toxicity of Cu led to the following conclusions. Copper is an essential element and is required for normal metabolism in man\(^\text{247-249}\). Copper, like the essential fats, amino acids and enzyme cofactors, is required for normal metabolism of all tissues. Since co-ordinated forms of Cu are always more stable forms, compared to ionized forms, it exists in biological systems as a variety of complexes\(^\text{250-253}\). A series of recent observation indicated that
copper complexes when administered in conjunction with antiinflammatory drug exhibit synergistic activity\textsuperscript{254}. It has also been found that the Cu – complexes of some antiarthritis drugs are themselves more active as antiinflammatory agents than their parent compounds\textsuperscript{255}.

Formation of the uncharged Cu-piroxicam species is of particular interest, since it has been shown\textsuperscript{256} that such neutral Cu – drug complexes are essential for effective distribution of the pharmacoactive agent and maintaining the copper balance in blood plasma. Recent reports\textsuperscript{257,258} on metal complexes with other therapeutic agents of the same class suggested that the drugs act as chelate ligands with co-ordination involving the enolate oxygen atom and the oxygen atom of the amido group. In contrast, we show here that piroxicam prefers a co-ordination mode employing the pyridyl group of the side chain and the amide oxygen atom.

1.4. IMPORTANCE OF THE PRESENT WORK

In recent years it has becomes increasingly apparent that the proper balance of the biologically available metals like V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo and Cd etc. is necessary for the efficient metabolism and growth of animal and plants. A slight change in the concentration of these metal ions brings about enormous changes causing various diseases. The drug changes its activity by combining with these metal ions. Binding with metal ions and proteins can markedly influence biological action of medicinal agents. Of particular importance in pharmacy and medicine is the relationship of complexation to the absorption and the way, that complexation influences the onset and duration of drug action\textsuperscript{259}. The process of formation of complexes with drugs and metal ions essential for life processes have drawn attention of a large number of chemists\textsuperscript{260-310}.
The metal also helps in the storage and transport of the drug in the body system through enzymes or amino acid. It has been reported that complexes of metallic salts are more potent and less toxic in many cases as compared to the parent drug\textsuperscript{311}. It has been suggested that during the action of drug complex formation takes place and this favours the attachment of chelate with tissues and a metal atom acts as a bridge between drug and nucleic acid of the tissue. Sometimes the traces of transition metal ions present in the body may change the behaviour of enzyme system by replacing the essential metals. Due to replacement of essential metal the structure and function of the nucleic acid may also be affected. In this way, it is most expected that the traces of metals present in the body can help to transport the drug to the site of its physiological action.

The chemistry of life involves, in an essential and indispensible way, many of the chemical elements, including metals\textsuperscript{312,313}. The importance of sodium, calcium and iron has long been recognized but many others, especially Cu, Zn, Mn, Mo and Co are necessary for life. Cu and Zn are known to from metalloenzymes. The metal ion does not merely participate during the time that the enzyme – substrate complex exits, but is a permanent part of the enzyme. The metal atom occurs at or very near to the active site and plays a very important role in the activity of enzyme\textsuperscript{314}.

The role played by metal ions in living system is now established and in recent years it has been recognized that inorganic biochemistry is a bridge between the academic disciplines of biochemistry and inorganic chemistry. In 1975 Wood\textsuperscript{315} made the salient point that “biochemistry is the co-ordination chemistry of living system.” This dependence is well exemplified by the observation that one third of all enzymes have a metal ion as an essential component\textsuperscript{316}. In addition, it has been reported that almost all globular proteins bind a wide range of metal ions\textsuperscript{317}. As metal
ions play such an important role in biological systems it is to be expected that the phenomenon of chelation will be studied with interest in the medical sciences. Iron has a vital functional role in living systems, i.e. oxygen transport and electron-transfer. The best known biological function of cobalt is its intimate involvement in the coenzymes related to vitamin B12$^{318}$ which is synthesized by bacteria$^{319}$. Nickel has some undisclosed function in living organism$^{320}$ which can activate a number of metals in vitro bound to ribonucleic acids and is found in blood, having a special affinity for bone and skin and plays important role in pigmentation$^{321}$. Copper compounds have limited therapeutic application. The relative case of reduction of the cupric ion to form coloured insoluble compounds finds important diagnostic uses, e.g. determination of glucose in blood and urine. Small quantity of copper may enhance physiological utilization of iron and is thus often present in haematopoietic preparations. Various copper compounds find commercial application as algaeicides, fungicides and insecticides.

Albert$^{322}$ has studied the metal complexes of a number of drugs. He divides metal binding agents showing chemotherapeutic activity into two classes: (1) those that can be used as antidotes in metal poisoning by removing the metal ions from the tissue and (2) those that can act as antibacterial agents by introducing metals in sufficiently large quantities to bring about a derangement to bacterial metabolism. Salicylic acid has been used in beryllium poisoning and ethylenediamine tetraacetic acid has been used as an antidote in lead poisoning. Metal cannot be injected directly into the blood stream to act as antibacterial agent because of its lack of selectivity and toxicity to the host. Furthermore, soluble metal ions, such as Fe$^{3+}$, cannot penetrate through the membranes of bacterial cells to exert their action. It has been found that 8-hydroxy-quinoline (oxine), however, can complex with iron normally present in the host and carry it across the
cell membranes of bacteria and fungi. The complex is thus capable of acting as an antibacterial and antifungal agent. Another explanation that has been offered for the antibacterial action of 8-hydroxyquinoline is that cobalt is essential to the metabolism of organisms which produce vitamin B\textsubscript{12} and 8-hydroxyquinoline complexes, this metal renders it unavailable to the organism. Albert has concluded, however, that the action of 8-hydroxyquinoline is based not on the removal of metallic ions but rather on supplying an excess of an ion which is toxic to the organism. The action is thus metallic poisoning rather than removal of essential trace metals from the metabolic scheme of the organism.

Foye and Duvall\textsuperscript{323}, investigated p-aminosalicylic acid for antitubercular activity. The binding by the tetracycline has been shown to markedly reduce their efficiency. Dithiozone (a drug) forms coloured complexes with many metals and is useful in the estimation of trace quantities of lead and zinc\textsuperscript{324}.

Investigations on the chelation reaction of the anticancer compounds\textsuperscript{325}, riboflavin\textsuperscript{326}, folic acid\textsuperscript{327}, thioguanine\textsuperscript{328} and adenine\textsuperscript{329} and the carcinogenic compounds 1-amino-2-naphthol\textsuperscript{330} have been reported and their chelating properties have been discussed in the light of their anticancer activities. Since in the cancer cell the abnormal cell growth may be due to the complexation of the abnormal or the carcinogenic metal ions with the enzyme of nucleic acid system, the anticancer drugs may check the carcinogenic binding of abnormal metal ions\textsuperscript{331}. Crim et al.\textsuperscript{332} have shown that in cancer treatment the active species is not the thiosemicarbazone itself but a metal (Zn and other) chelate of this drug. Some drugs have increased activity when administered as metal complexes and many drug complexes inhibit tuber growth\textsuperscript{333,334}. There are some other aspects of the metal drug chelation. Takaji et al.\textsuperscript{335} have used solutions of barbital-Cu complex mixed
with ammonium hydroxide and ammonium alginate as possible antidote for viper poison. Recently Colburn and Mass\textsuperscript{336,337} have reported that quite and appreciable amount of Cu(II), Ni(II) and Co(II) are present in the subcellular fractions of the brain. Complex forming property of analgin with Pb(II) ion, which is used for gravimetric estimation of analgin\textsuperscript{338}, led the researchers to speculate the possible importance of metal co-ordination in the mechanism of metal binding storage and transport\textsuperscript{339}.

In general these reviews have delth with the use of chelating agents as therapeutic countermeasures to either the uptake of toxic metal ions or the build-up and accumulation of essential metal ions to levels where they become toxic. The use of chelation therapy in the removal of calcium from artherosclerotic plaque has been advanced on and off for many years\textsuperscript{340}.

Copper complexes are the principal antiinflammatory agent under investigation at the moment\textsuperscript{341,342}. Although elsewhere in this review one or two other chelates are also discussed. The mechanisms by which copper complexes exert antiinflammatory action are no doubt numerous. The microbial properties of chelating agents were among the first of the biological effects studied and this has been a topic regularly updated by Albert\textsuperscript{343}. Investigations with oxine and analogue established that its site of action was within either the cell or cell membrane. The inhibition of the antibacterial action of oxine by cobaltous salts points to the catalytic degradation of hydrogen peroxide.

The potential for antimicrobial activity among the thiosemicabazones and thiosemicarbazides is very wide. Although the pharmaceutical industry is shifting away from exploiting fermentation products for antimicrobial properties, research is not yet in the direction of chelating agents. Few reviews of the exploitation of chelation in totally synthetic antimicrobial agents have appeared. In neutral complexes of
platinum(II) arises from the high anticancer activity of cis-[Pt(NH$_3$)$_2$Cl$_2$] (cisplatin) and related species. However, the antineoplastic activity of platinum(II) compounds is not restricted to cisplatin – type complexes and the preparation of new compounds is important to investigate potential activities$^{344,345}$.

Study of co-ordination compound of drugs led to the investigation directing towards establishing the site(s) of the metal binding in the drug$^{346-351}$. Cu(II) has often been used as a paramagnetic ion probe of binding sites in molecules of biological interest$^{352-355}$. The study of the stereochemistries and the chemical reactivity of the co-ordination compounds of the drug will help to determine the relationship which exists between chemical structure and biological activity of these drugs$^{348}$.

Diclofence sodium which is phenyl acetic acid derivative, has analgesic, antipyretic and antiinflammatory action and used mainly in the treatment of rheumatoid arthritis and other rheumatic disorders. It is used in painful post-operative and post-traumatic inflammation, swelling pain and following dental surgery, painful inflammatory conditions in gynaecology and symptomatic treatment for primary dysmenorrhea$^{356-359}$.

Diclofenac is well tolerated compared with other non-steroidal antiinflammatory drugs and no other agent of this class appears to have a safety profile which is clearly superior to diclofenac. Diclofenac is rarely or never associated with some other serious side effects, caused by other NSAIDS, e.g. pancreatitis, aseptic meningitis, severe cutaneous or phototoxic reactions. The drug diclofenac sodium inhibits leukotriene production by modulating uptake and release of arachidonic acid – a precursor of both prostaglandins and leukatrienes. Leukocytes accumulation apparently release an enzyme that destroys cartilage Dr. Brandt says researches expect diclofenac may cause less leukocyte accumulation.
Having a drug that prevents leukocyte build-up and the possible deleterious effect on cartilage could be very important\textsuperscript{360-363}. Diclofenac is a potent inhibitor of cyclo-oxygenase in vitro and in vivo, hereby decreasing the synthesis of prostaglandin, postacyclin and thromboxane product. Also diclofenac is a potent reversible inhibitor of the secondary phase of induced platelet aggregation. Like other NSAIDS, diclofenac is highly (> 95%) protein bound\textsuperscript{360}.

Mefenamic acid and flufenamic acid is anthranilic acid derivative, which is a analgesic and has antiinflammatory action. An analgesic drug indicated for the relief of mild to moderate pain when therapy will not exceed one week. Mefenamic acid and flufenamic acid is also indicated for the relief of pain resulting from dental extractions. It is contraindicated in patients with ulceration of the upper or lower intestinal tract, children under 14 years of age, women during pregnancy and patients known to be hypersensitive to the drug. Untoward effects include diarrhea which may be severe, autoimmune hemolytic anemia, thrombocytopenic purpura, leukopenia, pancytopenia, agranulocytosis and bone marrow hypoplasia. Minor reactions include drowsiness, gastrointestinal discomfort, dizziness, headache, vomiting, urticaria, rash, eosinophilia, blurred vision, insomnia and perspiration. Rarely, palpitations, facial edema, dysphea, eye pain, ear pain, dysuria, hematuria, reversible loss of colour vision and increased insulin need in diabetic patient. Mild renal and hepatic toxicity have also been reported. Since this drug is useful in mild to moderate pain, physicians would be well advised to consider its use only in cases which either can not tolerate or do not respond to less toxic agents\textsuperscript{364}. The commonly used acidic non-steroidal antiinflammatory agents (antiinflammatory, antinociceptive, antipyretic and ulcerogenic) is related to their ability to inhibit the
biosynthesis of prostaglandin from arachidonic acid (AA) by inhibiting the enzyme cyclo-oxygenase$^{365,366}$.

Piroxicam is a non-steroidal antiinflammatory drug belonging to the oxicam group. It is noted for its potent and long lasting analgesic and antiinflammatory effects enabling once daily dose. This drug is structurally different from other agents. It has been used to treat rheumatoid arthritis, ankylosing, spondylitis, osteoarthritis and acute gout$^{367}$. Piroxicam prevents the edema, erythema, tissue proliferation, fever and pain by its powerful antiinflammatory action. It not only inhibits the synthesis of prostaglandin due to inhibition of cyclo-oxygenase enzyme, but also inhibits 1 gm rheumatoid factor, superoxide anions formation and release of lysosomal enzymes from polymorphonuclear leucocytes. Due to powerful antiinflammatory action, it is effective regardless of the etiology of the inflammation. Piroxicam is well absorbed from the gastrointestinal tract. It is metabolised in the liver by hydroxylation and conjugated with glucuronic acid and excreted predominantly in the urine with smaller amounts in the faeces. Piroxicam is extensively bound to plasma proteins$^{368,369}$.

The purpose of the present work is two fold. It seeks to investigate the structure of the complexes of non-steroidal antiinflammatory drugs : diclofenac sodium, mefenamic acid, flufenamic acid and piroxicam with some 3-d transition metal ions like Cr(III), Mn(II), Fe(III), Co (II), Ni(II), Cu(II) and Zn(II). It also aims at determining the changes in the activities of drugs due to complexation. It is also to note that for all the drugs undertaken in the present investigation, the metal binding site(s) are either not reported or there is much controversy because solid complexes of these drugs were not prepared and their studies with modern techniques were still a matter of interest. In the present research work solid complexes of above drugs have been isolated and characterized on the basis of elemental analysis, molar
conductance, magnetic moment and spectral (electronic and infrared) studies. Biological activity of complexes have also been carried out on albino rats to determine the change in antiinflammatory activities of the drug after complexation. The antiinflammatory effects have been studied by carrageenan induced rat paw edema test.
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