CHAPTER 1

Introductory Concepts
1.0 INTRODUCTION

Hydroxamic acid having paired carbonyl and hydroxylamino (−NOH) functional groups (I) acquired phenomenal importance because of their tremendous applications in all sphere of life, including the fields of metal complexation and solvent extraction [1-20] biotechnology and nuclear chemistry [21-22], and iron-chelating therapy [23-35] etc.

They possess a great variety of biological and pharamaceutical activities [36-40]. The biological importance of hydroxamic acids has been proved beyond doubt and increased interest in their chemistry. Hydroxamic acid containing compounds are ubiquitous and are intimately associated with iron transport phenomena. The selectivity of this mechanism is critical since numerous other metal ions, which may not be essential or which may have a toxic effect on the organism, are present in the environment [41]. Hydroxamic acid is also known as constituent of growth factors, food additives, antibiotics, antibiotic antagonists, tumor inhibitors, antifungal agents and cell division factors. Several of them have been used as drugs [42-49]. Hydroxamic acids are also potent and specific inhibitors of urease activity [50-51], thermolysin [52-53], elastase [54] and aminopeptidase [55-56]. These enzymes are metalloproteinases and the mechanism of inhibition appears to involve chelation of
metals at their active sites. Inhibition of matrix metalloproteinase [57-58] or a unique deacetylase of lipid A biosynthesis [59] testify to the significance of this class of compounds.

Metal complexes are biologically important species that are crucial to a wide variety of processes including oxygen transport [60], digestion [61] and gene transcription [62]. For example, zinc-finger proteins regulate gene expression by complexing DNA [62] and some other metal complexes have been exploited as DNA cleaving medicinal agents [63].

Most of the work involving the interactions of metal complexes with DNA has focused on organic and inorganic substrates containing amines, carboxylates chelators and others, yielding systems that are highly useful for obtaining nucleic-acid structure and binding information [64-88]. In contrast, fewer studies have employed hydroxamic acid [89-94]. In an effort to develop hydroxamic acid metal complexes as new agents for the cleavage of DNA, we have begun to study acid-base equilibria and metal complexation of hydroxamic acids. It is necessary to develop simple but efficient artificial metallonuclease. This prospect is especially attractive and in need of exploitation in metal complexation.

Interaction of metal complexes with DNA is currently attracting considerable attention due to their potential use as drugs, in structure elucidation, gene manipulation, chemotherapeutics and tools for molecular biology. Chemist in the field of molecular design and synthesis are devoting extensive effort towards the development of DNA cleavage agents. Although many studies have been made on the chemical properties of hydroxamic acid-metal complexes [1-20], their interactions with DNA [89-94] have not been extensively investigated.

In spite of these important applications, hydroxamic acids remain one of the less well-characterized classes of organic compounds. Acid-base equilibria and overall stability of these compounds are known to have an important bearing on
their general usefulness in many of these applications. An accurate knowledge of the ionization (protonation and deprotonation) behaviour of hydroxamic acid is required both for structure-reactivity correlations and for the detailed kinetic analysis of acid and base catalysed reactions. A considerable amount of theoretical work [95-100] on the acidity and structure of hydroxamic acids have been conducted recently. Most of these have focused on their acid-base properties; thus many of these studies also presented computations on hydroxamic acid anions [97-100]. Some also included the protonated species [96-98]. On the contrary, only scanty data are available [101-103] for the experimental determination of the protonation constant.

Hydroxamic acids and their metal complexes have long been implicated in a wide spectrum of chemical, industrial and biological activities. Systematic studies of some metal-hydroxamates by solvent extraction technique have established the potentiality of some hydroxamic acids to be used in hydrometallurgy of certain metals for industrial uses [3]. Trace and ultra trace metals in environmental samples can be separated and quantitatively analysed as hydroxamic acid complexes.

Some naturally occurring hydroxamic acids serve as Fe(III) specific chelators known as siderophores [104-106] and are involved in microbial iron transport. One of the characteristics of hydroxamic acids is their ability to form stable metal complexes. Hydroxamic acid is a common structure found in naturally-occurring siderophores (low-molecular-weight iron carriers) secreted by microorganisms to sequester ferric ion in aqueous media [44]. A typical example of a trihydroxamic siderophore, desferrioxamine B (Desferal DFB), is now the

![Desferal](desferal.png)
most effective drug for the removal of toxic amounts of iron from thalassemia patients [23]. Recent studies in patients with thalassemia found that the magnitude of the body iron burden was the major determinant of the risk of clinical complications and of early death [107-108]. Target organs for iron-induced injury are the liver, pancreas and heart [109]. Desferrioxamine has recently been proposed in conjunction with recombinant α-2 interferon in the treatment of heptocellular carcinoma [110]. This is evident that desferrioxamine may be employed against certain solid tumors has been paralleled by reports on the use of desferal, in combination with ARA-C, in the treatment of acute leukemia [111]. Desferal is also useful for Malaria research [112]. This was shown to be able to arrest the growth of Plasmodium falciparum in vitro [113]. The treatment with desferal proved to be efficient even in children with cerebral malaria who recovered from deep coma [114].

Recently in HIV-1-infected Hg cells these reagents are reported to inhibit retrovirus replication [115] immunocompromised patients such as those with AIDS are very susceptible to opportunistic fungal infections with strains of Candida Cryptococcus, Aspergillus and related organisms. Iron transport mediated drug delivery offers a unique possibility to develop species-selective antimicrobial agents to address this problem. A number of reports suggest that hydroxamates are generated and utilized by Candida as siderophores [115-117]. The prospect of detailed studies of structure-reactivity relationships of hydroxamic acids and of siderophore drug conjugates, hold considerable promise for the development of new therapeutic agents.

A newly synthesized cyclic hydroxamic acid compound, BMD 188 [cis-1-hydroxy-4-(1-naphthyl)-6-octyl(piperidine-2-one], was found to inhibit the growth of androgen-independent prostate cancer cells (PCS), with an LD₅₀ at ~10 μM. The growth inhibition was due to apoptosis induction as evident by DNA ladder formation PARP [poly (ADP-ribose) polymerase] cleavage, and typical apoptotic morphology. Comparative studies indicated that BMD 188 induced a more potent
apoptotic response in PC3 cells than several conventional chemotherapeutic drugs [118].

Some hydroxamic acids such as 2,4-dihydroxy-7-methoxy-1,4-benzoxari-3-one (I), play a defensive role in wheat and maize plants against insects [119]. In addition, it is toxic towards bacteria and fungi [120].

![Image](image.png)

(I)

2,4-dihydroxy-7-methoxy-1,4-benzoxari-3-one

The toxicity of these acids is associated with their interference with key metabolic processes such as energy transduction in mitochondria [121].

In the course of searching for new hydroxamic acid iron chelators to replace DFB, N-hydroxyamide-containing diazines [42] (II) have received much attention because of their potential for clinical use [113-114].

![Image](image.png)

N-hydroxyamide-containing diazine

Understanding how metal complexes interact with DNA has become a central question in some of the most active research areas at the interface between chemistry and molecular biology. Three kinds of metal DNA systems currently are of particular interest: metal complexes that are used as tools for molecular biology, metalloproteins that regulate gene expression by binding to DNA and metal
complexes that act as drugs. These systems span a wide range in complexity from simple transition metal chelate complexes, known to the inorganic chemists for many years [such as iron(II) EDTA and the bis- and tris- (phenanthroline complexes)], to more elaborate complexes specifically designed to bind to DNA [such as methidiumpropyl EDTA Fe(II)], to metals coordinated within proteins. All of these systems have the common feature that the chemistry of the metal is essential to the ability of the complex to interact with DNA [124].

The literature of recent years abounds in many diverse studies and uses of hydroxamic acids and its analogues and their metal DNA chemistry [89-94]. The metal ion-nucleic acid interactions are described in considerable detail in reviews, monographs and text books [125-133].

The interest of the bioinorganic community in the field of metal/nucleic-acid interactions has burgeoned in the last decade. These interest and the resulting progress have come about primarily because of the tremendous advances that have occurred in nucleic acid technology. Chemists have used their knowledge to synthesize a large variety of hydroxamic acids according to the structural requisites for their introduction in clinical and technical practice.

In general no target research work specially on acid-base equilibria and ion-coordinating properties of hydroxamic acids carried at India, although similar knowledge on other carboxylic acid derivatives have been generated from various laboratories of the World. The systematic and careful collection of data on physico-chemical properties of simple, cyclic and trihydroxmaic acids is prerequisite to lend novel idea into DNA cleavage. We hope that the dialogue among chemists and biochemists will be able to achieve the common target of development of elegant nucleases. This is almost a virgin field awaiting its exploration.
1.1 REVIEW OF THE EARLIER WORK

Before beginning a discussion of specific objectives of the present investigation, it may be useful to have some idea of the earlier work, i.e. acid-base equilibria and interaction of metal complexes with DNA. Research into the chemistry of nucleic acid has seen a rapid increase in activity over the last decade.

1.11 Acid–Base Equilibria

The chemical, physical and biological properties of hydroxamic acids are usually dictated by acidic and/or basic groups that are present in the molecules. The basicity and solvation are intrinsically important parameters for understanding the behaviour of biochemical systems.

To rationalize the chemical reactivity [134-138] many have been devoted to the search for relationships between structure and acidity or basicity. In this regard it is of prime importance to quantitatively characterize the protonation and deprotonation propensity. The overall picture (without regard for cis-trans isomers etc.) of the various acid-base and tautomeric equilibria of hydroxamic acid is given in Scheme-I.

The protonation equilibria in concentrated solutions of mineral acids of typical carbonyl compounds such as aromatic ketones [139-141] amides [142-144], anilides [145-147], esters [148] and other carboxylic acid derivatives have been the subject of a number of studies. But surprisingly very little work has been done on the protonation of hydroxamic acid. A few workers [98, 101-103] only have studied the protonation equilibria (by theoretically and experimentally). The $pK_{\text{bh}^+}$ studied so far are given in Table 1.01.

Tillett et al. [103] studied protonation equilibria of some para-substituted benzohydroxamic acids in sulphuric acid. The protonation equilibria in perchloric acid could not be studied because of insufficient differences between the spectra of the substrate and its conjugate acid. Also, data for the protonation behaviour of
Introductory Concepts

Scheme-I
TABLE 1.01 $pK_{bH^+}$ (PROTONATION CONSTANT) OF SOME HYDROXAMIC ACIDS.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Hydroxamic Acid</th>
<th>Condition</th>
<th>Method</th>
<th>$pK_{bH^+}$</th>
<th>Ref.</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>$C_6H_5CONHOH$</td>
<td>Aqueous $H_2SO_4$, 25°C</td>
<td>UV</td>
<td>- 1.93</td>
<td>103</td>
<td>1971</td>
</tr>
<tr>
<td></td>
<td>$H$--$N$--OH</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2.</td>
<td>$X.C_6H_4(C=O)N(OH).C_6H_4.CH_3$</td>
<td>HCl</td>
<td>Solvent Extraction</td>
<td>- 2.84</td>
<td>156</td>
<td>1998</td>
</tr>
<tr>
<td></td>
<td>$H_3C$--$\text{\includegraphics[width=0.3cm]{hydroxyl.png}}$--$N$--OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$X\text{\includegraphics[width=0.3cm]{carbonyl.png}}$--C=O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$X = Br$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>$X-C_6H_4CON(OH)C_6H_5$</td>
<td>$H_2SO_4$, 10% (v/v) 1,4-dioxane, 25°C</td>
<td>UV</td>
<td>- 2.24</td>
<td>101</td>
<td>1996</td>
</tr>
<tr>
<td></td>
<td>$\text{\includegraphics[width=0.3cm]{hydroxyl.png}}$--$\text{\includegraphics[width=0.3cm]{carbonyl.png}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$X = H$</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>$X = 4-CH_3$</td>
<td></td>
<td></td>
<td>- 1.78</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>$X = 4-Cl$</td>
<td></td>
<td></td>
<td>- 2.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>$OH-C_6H_4CONHOH$</td>
<td>$H_2SO_4$, 10% (v/v) 1,4-dioxane, 25°C</td>
<td>UV</td>
<td>- 1.27</td>
<td>102</td>
<td>1996</td>
</tr>
<tr>
<td></td>
<td>$H$--$N$--OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{\includegraphics[width=0.3cm]{hydroxyl.png}}$--C=O</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>$OH$</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>5.</td>
<td>$CH_3CONHOH$</td>
<td>Aqueous $H_2SO_4$, 25°C</td>
<td>NMR</td>
<td>-1.15</td>
<td>98</td>
<td>1994</td>
</tr>
<tr>
<td></td>
<td>$\text{\includegraphics[width=0.3cm]{carbonyl.png}}$--$\text{\includegraphics[width=0.3cm]{hydroxyl.png}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the parent compound are rather confusing ($pK_{\text{BH}^+}$ of BHA = -2.45, -1.93 and -0.45) and do not even provide clear conclusions about the protonation site.

In 1994, Bagno et al. [98] investigated protonation and deprotonation behaviour of some hydroxamic acids (RCONHOH) by heteronuclear ($^{14}\text{N}$, $^{15}\text{N}$, $^{17}\text{O}$) NMR relaxation and NOE experiments ($R = \text{CH}_3$, $\text{C}_6\text{H}_5$) and ab initio theoretical methods. The resulting values (acetohydroxamic acid) were processed according to excess acidity method, thus obtaining $pK_{\text{BH}^+} = -1.15$. An analysis of the distribution curves B and BH$^+$ showed that quantitative protonation is achieved only in strong acids; therefore, the protonated form was generated in trifluoromethanesulfonic acid. Studies in super acids require drastic conditions that may affect the response if the solvation energies of the various ions differ greatly. It is therefore apparent that none of the techniques employed so far can answer the question of $pK_{\text{BH}^+}$ unambiguously. Furthermore, no attempt has been made to eliminate medium effect from protonation data. Recently our group at Raipur initiated to investigate protonation equilibria of some para substituted N-phenylbenzohydroxamic acid and salicylhydroxamic acid in mineral acids using UV method [101-102]. The deprotonation ($pK_a$) equilibria have been extensively investigated [149-155] both in solution and in the gas phase [138]. The question concerning the actual site of ionization has been long debated. However, contrasting evidence has accumulated over the year, and to date no definitive conclusion has been reached.

More recently the protonation behaviour of some N-substituted hydroxamic acids have been determined by solvent extraction method [156] using vanadium(V) by visible spectroscopy. Due to the hydrophobicity of aromatic ring of these reagents, the solubility of the neutral species is low in water, but remarkably increases in organic solvents and in acidic mixtures. An obvious limitation of this method is encountered for compounds which show solubility increases in acid solutions due to other effects apart from protonation.

A well established technique for the quantitative study of protonation
equilibrium involves monitoring some spectral change (e.g., UV and IR absorbance, intensities of Raman lines, NMR chemical shift) as a function of some measure of the acid or base strength of the solution (pH, acidity functions, excess acidity etc.). This technique, however, is not guaranteed to furnish information about the structure of the ions being formed. As their name implies, hydroxamic acids behave as weak acids; pK_a's are in the range of 8 - 9; in fact, their acidity is stronger than corresponding amides by some 6 pK_a units [157], and like most carbonyl compounds they are also weak bases. The ionisation equilibria (pK_a and pK_bH+) of hydroxamic acids is a challenging problem due to its extreme sensitivity to structure, solvent and thermodynamics and kinetics of chemical reactions.

A knowledge of the protonation constants (pK_bH+) is very useful for a proper understanding of the various equilibria which the hydroxamic acid undergoes in strongly acidic solutions. Needless to mention that with a knowledge of this constant the analytical chemist can judiciously and intelligently plan out the analytical schemes. These data can be utilised to throw light on substituent effects, structures and stabilities of metal complexes. Besides, the knowledge of pK_bH+ is likely to be useful to the physical organic chemist for explaining the rates and mechanism of proton catalysed hydrolysis of hydroxamic acid. From the literature survey it is clear that no comprehensive treatment of protonation behaviour of hydroxamic acid has been appeared.

Apart from the direct relevance of pK_bH+ measurements to studies of the kinetics of reactions, studies of acid/base strength provide information on molecular structure which is of fundamental importance in chemistry in general. The cultivation of the field is thus important but detailed fundamental studies of protonation behaviour are not yet adequate. Accordingly, we herein pursue the double aim of fulfilling an evident gap in the knowledge of protonation equilibria of different unsubstituted, N-substituted, cyclic and dihydroxamic acids and the study of electronic environment. We hope that comparative and quantitative description of the pK_bH+ of hydroxamic acids of diverse characters will reveal the
scope and limitations of the different theories and provide a useful guidance in the
design and selection of ligand for DNA-cleavage.

1.12 Metal Complexation of Hydroxamic Acids

The phenomenon of complex formation is really a general one but it is
markedly noted among the transition metal ions. The formation of a complex is the
result of a Lewis-acid base type of interaction, in which one atom with a vacant
orbital (generally the metal) attracts the electron pair on another atom (generally a
non-metal). For bonding to occur as a result of such an interaction, the metal must
possess vacant orbitals and these orbitals must be symmetrically correct sterically
available and of reasonably low energy since transition metal ions generally meet
these requirements best, they form complexes so readily.

Hydroxamic acids by virtue of the reactive hydroxamic acid functional
grouping, -N(OH).C(=O) fulfill the following two basic requirements for acting as
chelating agents : (a) the existence of two appropriate functional groups (i) the
acidic group, -N-OH and (ii) the purely co-ordinating group, -C==O (b) the
favourable disposition of the two groups for a ring formation. In hydroxamic acids
the acidic and co-ordinating groups are so situated that they allow the formation of
a five membered ring with the metal ion as the closing member.

Chelating agents and chelation have long been utilised in analytical chemistry,
but it has only been fully appreciated as a prominent chemical principle within the
last few decades. Metal chelates represents a type of coordination compound in
which a metal ion combines with a polyfunctional base, capable of occupying two
or more positions of the co-ordination sphere of the metal ion, to form a cyclic
compound.
Hydroxamic acids of the type I and II possessing the bidentate

\[
\begin{align*}
\text{I} & \quad \text{II} \\
H-N-OH & \quad R'-N-OH \\
R-C=O & \quad R-C=O
\end{align*}
\]

functional grouping fulfill the

\[
\begin{align*}
-R-N-OH & \quad =N-OH \\
\quad & \quad =C=O
\end{align*}
\]

fundamental requirements of forming metal complexes. Generally the complex formation takes place by replacement of the hydroxylamino hydrogen by the cation and ring closure through the carbonyl oxygen.

\[
n \left( \frac{R'-N-OH}{R-C=O} \right) + M^{n+} \rightleftharpoons \left( \frac{R-N-O}{R-C=O} \right)_{n} M + nH^{+}
\]

Bivalent metal ions form solid inner complexes with hydroxamic acids [158-160]. A large number of hydroxamic acid metal complexes have been synthesised in Professor Tandon's Laboratory during last two decades at Raipur [162-163]. The metal-ligand ratio in these complexes is 1:2 showing a co-ordination number 4 for the metal ion. The maximum complexation involves the four coordinated species, and no penta or hexa coordinated species is formed [162-163].

The coordination chemistry of hydroxamic acid has received much attention due to its diverse coordinating behaviour and the role it plays in biological process. From results [164] of X-ray crystallographic studies it is known that hydroxamate group behaves as a bidentate (O, O as donors) ligand forming a five-membered
chelate ring. Review of the literature reveals that there is insufficient information about the redox and thermal properties of hydroxamic acid metal complexes. For the rational application of hydroxamic acids in analytical chemistry it is desirable to have a quantitative knowledge of the stabilities of their chelates with various metal ions. The stabilities of the complexes mostly follow the order of basicity of the ligands and the electron affinities of the metal ions as measured by their second ionization potential. To understand the nature and implications of metal binding to ligand, a comprehensive study on the metal complexation of mono, di and tri hydroxamic acids has been carried out.

1.13 Interaction of Metal Complexes with DNA

The design and investigation of artificial DNA-cleaving molecules in an area of exciting research that has numerous important biochemical and biomedical applications. In addition to the exploration of novel mechanistic strategies for DNA cleavage, new DNA-cleaving agents are of practical interest as potential antitumor agents, as prosthetic groups for antisense oligonucleotides, as artificial nucleases or photonucleases, as well as for designing new gene-selective drugs. For more than a decade, it has been a dream for organic and inorganic chemists to design sequence specific DNA cleavers that bind to DNA at any desired sequence and cleave DNA efficiently at the binding site. Many metal complexes capable of promoting DNA strand scission. Metal complexes are very suitable, because the geometry of a metal complex and the structure of the ligand can be readily manipulated to build in recognition features, and because the metal offers reactivity for cleaving DNA. The shape and the chemical structure of DNA provide a number of opportunities for interaction with metal complexes. The negative charge of the phosphates that are regularly spaced along the DNA backbone mediate electrostatic interaction with metal complexes. DNA has two grooves, major and minor, in which covalent, hydrophobic and hydrogen bonding interactions can occur. The DNA base pairs, stacked perpendicular to the axis of the double helix, offer sites of intercalation for aromatic groups.
The first artificial nuclease or metal complex capable of cleaving DNA was bis (1, 10-phenanthroline) Copper (I) discovered by Sigman and his coworkers [165-167]. A number of metal chelates have been used as agents for DNA strand scission. Table 1.02 summarizes different redox and photoinduced reactions of metal complexes bound to DNA studied very recently. The presentation of all the results would have made the thesis very bulky and hence in order to economize the space, only the structure and chemistry of DNA cleavage have been presented in Table 1.01. In most cases the DNA cleaving principle of these DNA cleavers involves (i) oxidative destruction of DNA deoxyribose back-bone by hydrogen abstraction reactions or (ii) alkylation of DNA bases, and in fewer cases (iii) metal activated hydrolytic cleavage of phosphodiester linkage of DNA. Many of these reagents have already provided useful footprinting reagents in vitro. The chemistry must be refined so that the reagents are membrane permeable and will not be inhibited by normal cellular defence mechanisms. Both redox and hydrolytic reactions of metal complexes with nucleic acids have been exploited with much success in the development of tools for molecular biology. The photoinduced DNA cleavage of some compounds have also been examined.

Despite of considerable efforts devoted to understanding the nuclease activity of different metal complexes, only a few attempts have been made to understand DNA cleavage activities of hydroxamic acid metal complexes. The hydroxamic acid and metal ions so far used for DNA cleavage are given in Table 1.03.

The review illustrates that only two groups of the World have been actively engaged in DNA strand scissions by hydroxamic acids. Dr. K. N. Ganesh of National Chemical Laboratory, Pune, India has contributed much in understanding specificity, mechanism and targeting of DNA scission reactions using hydroxamic acids [89, 93]. Professor Yushin Nakamura [90-92] of Science University, Tokyo, Japan first published indicating how hydroxamic acids in the absence or presence of metal ion interact with DNA in vitro or in vivo. He indicated that the hydroxamic acid (CH$_3$CONHOH, C$_6$H$_5$CONHOH, carbazoloylxyacitohydroxamic acid) in the
<table>
<thead>
<tr>
<th>S. No.</th>
<th>METAL COMPLEX</th>
<th>DNA</th>
<th>TARGET</th>
<th>CHEMISTRY</th>
<th>REF.</th>
<th>YEAR</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(Ligand &amp; Metal Ions)</td>
<td></td>
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</tr>
<tr>
<td>1.</td>
<td><img src="image" alt="Co(III) complex" /></td>
<td>Co(III)</td>
<td>pUC19</td>
<td>Photochemical</td>
<td>64</td>
<td>1999</td>
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<td>Sugar</td>
<td>Oxidative</td>
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<td>Co(III) pBR 322 DNA</td>
<td>Bipyridine, Phenanthroline, 1,4,8,9-tetraaza-tripheylene</td>
<td>68</td>
<td>1998</td>
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<td>1998</td>
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<td>PUC 19 plasmid Sugar</td>
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<td>70</td>
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<td>8.</td>
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<td>Calf-Thymus DNA</td>
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<td>71</td>
<td>1998</td>
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<th>METAL COMPLEX (Ligand &amp; Metal Ions)</th>
<th>CHEMISTRY</th>
<th>DNA TARGET</th>
<th>REF. YEAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.</td>
<td><img src="image" alt="Ochatoxine" /></td>
<td>Cu(II), Fe(III)</td>
<td>Supercoiled φ X 174 DNA</td>
<td>72 1998</td>
</tr>
<tr>
<td>10.</td>
<td><img src="image" alt="3-Clip-phen" /></td>
<td>Cu(II)</td>
<td>plasmid pTZ19R</td>
<td>73 1998</td>
</tr>
<tr>
<td>11.</td>
<td><img src="image" alt="2-Clip-phen" /></td>
<td>Co(II)</td>
<td>Photoinduced Cleavage (visible)</td>
<td>74 1996</td>
</tr>
</tbody>
</table>

- [N,N-Bis (2-picolyl) amine]
TABLE 1.03 Examples of Hydroxamic Acid Metal Complexes That Cleave DNA

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Hydroxamic Acid</th>
<th>Metal Ions</th>
<th>DNA</th>
<th>Chemistry</th>
<th>Target</th>
<th>Ref.</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>6,6-bis(allyl)-1-hydroxy-piperazini-2,5-dione</td>
<td>Fe\textsuperscript{II}, Cu\textsuperscript{II}</td>
<td>pBR 322</td>
<td>OH\textsuperscript{-}, Fenton</td>
<td>Sugar</td>
<td>89</td>
<td>1998</td>
</tr>
<tr>
<td>2.</td>
<td>Distamycin-Linked hydroxamic Acid</td>
<td>Fe\textsuperscript{II}, Vo\textsuperscript{II}, Cu\textsuperscript{II}</td>
<td>Co\textsubscript{1E1} plasmid</td>
<td>Hydrolytic</td>
<td>Phosphate</td>
<td>91</td>
<td>1996</td>
</tr>
<tr>
<td>3.</td>
<td>N-[5-(hydroxylaminocarbonyl) penty1] phenanthridium chloride</td>
<td>Lu\textsuperscript{III}, Tm\textsuperscript{III}, Eu\textsuperscript{III}</td>
<td>Co\textsubscript{1E1}</td>
<td>Hydrolytic</td>
<td>Phosphate</td>
<td>92</td>
<td>1995</td>
</tr>
<tr>
<td>4.</td>
<td>Desferal</td>
<td>Fe\textsuperscript{II}, Cu\textsuperscript{II}, Ni\textsuperscript{II}, Co\textsuperscript{III}</td>
<td>pBR 322</td>
<td>Oxidative</td>
<td>Sugar</td>
<td>93</td>
<td>1994</td>
</tr>
</tbody>
</table>
### Table 1: Hydroxamic Acids and their Targets

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Hydroxamic Acid</th>
<th>Metal Ions</th>
<th>DNA</th>
<th>Chemistry</th>
<th>Target</th>
<th>Ref.</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>Acetohydroxamic Acid</td>
<td>Fe&quot; , Vo&quot; , Fe&quot;&quot;</td>
<td>Co1E1 plasmid</td>
<td>Hydrolytic</td>
<td>Phosphate</td>
<td>94</td>
<td>1992</td>
</tr>
<tr>
<td>6.</td>
<td>Benzhydroxamic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Corbazolyloxyaceto hydroxamic Acid</td>
<td>Cu&quot;</td>
<td>Co1E1 plasmid</td>
<td></td>
<td>Phosphate</td>
<td>94</td>
<td>1992</td>
</tr>
</tbody>
</table>
presence of Cu(II) (without any reducing agent) causes the DNA strand scissions. Inhibition experiments indicated that hydrogen peroxide and superoxide participated in the reactions, but hydroxyl radical or singlet oxygen did not. Most likely Ganesh and Joshi [89, 93] were the first who described detailed mechanistic studies on DNA cleavage by Cu(II), Co(II) and Ni(II) complexes of a trihydroxamic acid siderophore, Desferal. The DNA nicking ability of metallodesferals was investigated by plasmid cleavage assay. The general oxidative mechanisms (Scheme II B ) proposed to account for DNA cleavage by hydroxy radicals via abstraction of a 'H' atom from sugar units predict the release of specific chemical residues arising from transformed sugar, depending on the position from which the 'H' atom is removed.

This is similar to the Fenton reaction (Scheme II A) which indirectly promotes DNA strand scission through radical reactions on the sugar ring. As do other redox-active divalent metal ions, ferrous ion, in the presence of H₂O₂, generates hydroxyl radicals, and in the presence of a reductant such as mercaptoethanol, the 'OH' radical production can be made catalytic.

The use of N-arylalkyl-N-phenyl hydroxylamines (Ph-N-OH-Ph) to react with O₂ for the production of HO* by photolysis provides an efficient way to cleave DNA [168]. This new method allows the use of 312 nm UV light as the trigger to initiate the DNA strand scission, yet it does not require external photosensitizer, H₂O₂ or metal ions.

Nakamura et al. [90] advocated hydrolytic cleavage (Scheme III). Hydrolysis reactions of the phosphodiester linkage of polynucleotides appear preferable to redox-mediated cleavage reactions, since in the hydrolytic reaction all information is preserved. In redox cleavage by sugar oxidation, for example, both a sugar fragment and free nucleic acid bases are released from the polymer, and, in contrast to hydrolytic chemistry, the direct religation of the fragments become practically impossible.
(A) \[ \text{Fe(II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)} + \text{OH}^* + \text{OH}^- \]

\[ \text{Fe(III)} + e^- \rightarrow \text{Fe(II)} \]

(B)

**Scheme II**

Oxidative Cleavage

**Scheme III**

(Hydrolytic Cleavage)
Phenanthridine-linked hydroxamic acid (Table 1.02, S. No. 03) effectively cleaves ColE1 DNA in the presence of transition (ferrous ferric, and vanadyl) or lanthanide (III) (lutenium, thulium and europium) metal ions [92]. Similar novel distamycin derivatives [91] possessing hydroxamic acid moiety can induce effective plasmid DNA cleavage in the presence of transition or lanthanide metal ions. According to Nakamura et al. [90] lanthanide cations behave as typical hard acids and interact preferentially with have bases such as nitrogen [169].

Hence, the lanthanide-hydroxamic acid complex should interact preferably with the hard oxygen sites of the phosphate diester backbone of DNA. A suitable molecular design for a hydroxamic acid-lanthanide system would aid in the development of an artificial hydrolytic metallonuclease which could, ultimately, access the enormous catalytic activity of enzymes. Currently the basic biochemical and spectroscopic characterization of the enzyme is being carried out.

Recently Ganesh et al. [89] synthesized peptide-related hydroxamic acids for DNA cleavage. The Cu(II) and Fe(III) complexes of 6, 6-bis (allyl)-1-hydroxypiperazine-2,5 dione (cyclic hydroxamic acid) were able to convert supercoiled DNA into open circular DNA. The metal complex may be directly reduced by the peroxide to produce the corresponding DNA-Cu-hydroperoxy species thereby leading to DNA damage.

In conclusion, both hydrolytic and oxidative cleavage of DNA have been accomplished by use of the hydroxamic acid-metal ion system.

It has become apparent that design must be preceded by a thorough understanding of cleavage mechanisms. Understanding this very novel interaction of a metal center and DNA will require some new ideas, and certainly represents one new challenge for the bioinorganic chemists. To date, our knowledge of DNA cleavage is still insufficient. In this thesis we have attempted to use some simple N-substituted hydroxamic acids for DNA cleavage. The Cu(II), Ni(II), Co(II), Zn(II),
Fe(III) metal ions have been used. It is too early to propose definite guidelines for
the design and mechanism of artificial nucleases. Many studies, many compounds,
and hence much time will be necessary before we can perfectly control the
targeting to define DNA sequences by the use of designed synthetic ligands.

1.14 Kinetics and Mechanism of Hydrolysis Reaction

A hydrolysis reaction is one in which a σ bond is cleaved by the addition of
the elements of water to the fragments formed in the cleavage. The hydrolysis in
which 'lysis' (meaning to loosen) takes place contrast with hydration in which water
is added to a multiple bond, but no fragmentation of the molecule occurs. Chemists
recognize the importance of hydrolysis reactions and have studied them in detail.

The hydrolysis of hydroxamic acids to hydroxylamine is of first step in the
quantitative analysis of hydroxamate siderophores. The hydrolysis of hydroxamic
acids is similar to the hydrolysis of amides. It is slow process even when catalysed
by acid or base. A large number of research papers in this context have come up
from this laboratory in the last fifteen years [170-183], which usually describe the
hydrolysis of simple monohydroxamic acid. But surprisingly, one of the important
trihydroxamic acid siderophore, i.e. Desferal has been neglected. Till now none of
the hydroxamic acids used for pharmaceutical and analytical work has been
supported by its kinetic properties. This was thought to be essential because the
faster a hydroxamic acid hydrolyses the less efficient. It is a reagent for
spectrophotometry, solvent extraction and other important biomedical applications.

1.2 OBJECTIVES AND PRESENT INVESTIGATION

As reviewed in the preceding sections, hydroxamic acids are now used
extensively as analytical reagents, as drugs, and as elegant artificial nucleases.
Interpretation of data based on DNA cleavage by hydroxamic acid-metal complex
and design of efficient artificial nucleases requires a knowledge of the binding and
Introductory Concepts

kinetic mechanisms. So, we became interested in defining and evaluating many key questions related to hydroxamic acid and DNA chemistry. Acid-base equilibria and ion-coordinating properties of hydroxamic acids would also provide us an opportunity to obtain additional structural insight into the binding event. Electrochemical studies on metal complexes reveal the significance of electrostatic interactions in dictating the binding phenomenon. The main objectives of the present investigation are as follows:

1. To synthesize and characterize some mono, di and polyhydroxamic acids.
2. To determine the protonation and deprotonation equilibria of hydroxamic acids by UV and NMR methods.
3. To synthesize and characterize hydroxamic acid-metal complexes.
4. To study the nuclease activity of hydroxamic acid-metal complexes (specificity, mechanism and targeting of DNA-cleavage).

Against this background it was planned to carry out investigations on some hydroxamic acids which detailed studies have not been made so far and this lead to the work presented in the thesis. An accurate knowledge of the protonation behaviour of weak bases or acids is required both for structure-reactivity correlations and for the detailed kinetic analysis of acid catalysed reactions. Evaluation of the site of ionization (protonation and deprotonation) of polyfunctional bases and acids has attracted considerable interest both as a source of information about electronic structures and as a means of interpreting the reactivities of acid-catalysed reactions.

The important role of metal ions in the structure, stability and reactivity of the double helical DNA and the fact that nucleic acids do need metal ions to exert their important biological functions such as in replications, transcription and translation process are now well known.

To achieve this specific goal and above objectives the present investigation has been briefed in the following manner:

Some C- and N- substituted hydroxamic acids of type (III) and its derivatives of type (IV) have been synthesized.

\[
\begin{align*}
\text{(III)} & \quad \text{R'-N-OH} \\
& \quad \text{R-C=O}
\end{align*}
\]

\[
\begin{align*}
\text{(IV)} & \quad \text{H-N-OH} \\
& \quad \text{X-C=O}
\end{align*}
\]

\(\text{R} = \text{C}_6\text{H}_5; \text{R}' = \text{H}, \text{C}_6\text{H}_5\)

The purities of these hydroxamic acids were established by elemental analysis, melting point determination and analysis by ultraviolet and infrared spectroscopies.

[B] Determination of Protonation Constant (pK\text{BH}_+\text{)} of Hydroxamic acids

The acid-base properties of organic molecules are among the most relevant factors affecting their reactivity and therefore studies leading to the definition of such properties through equilibrium measurements yield extremely valuable data, e.g., for the elucidation of reaction mechanisms and are also important in organic chemistry and biochemistry. The Hammett-Deyrup [134], the Bunnett-Olsen [184], the Cox-Yates Excess Acidity [185] and Marziano-Cimino-Passerini [186] methods have been compared in order to rationalize the differences observed between pK\text{BH}_+. 
values estimated by each classical methods. An attempt has been made to apply characteristic vector analysis (multivariable analysis) to separate the effect of protonation from the medium effect.

In the present study the protonation constant of N-substituted hydroxamic acids (e.g. N-Phenylbenzohydroxamic acid), Unsubstituted hydroxamic acids (e.g. Benzohydroxamic acids and its derivatives 4-OCH₃, 4-CH₃, 4-F, 4-Cl, 4-Br, 4-NO₂) have been determined UV spectrophotometrically in acidic medium.

In addition to simple C– and N–substituted hydroxamic acids, the protonation parameter of some cyclic and dihydroxamic acid have also been determined UV spectrophotometrically by measuring the ionization ratios, (C⁺/[C] = 1), in concentrated aqueous acid solution. High field NMR technique have also been attempted. The summary of the present investigation is given in Table 1.04 in a Tabular form.

\[
\begin{align*}
\text{Benzohydroxamic acid} & \quad \text{N-Phenyl benzohydroxamic acid} & \quad \text{N-Hydroxyphthalamide} & \quad \text{Oxalodihydroxamic acid} \\
(X = H, CH₃, OCH₃, NO₂, Cl, Br, F)
\end{align*}
\]

[C] Structure–Reactivity Correlations

In order to extend the scope of linear free energy relationships in hydrolysis reaction and ionization equilibrium (protonation pK⁺ and deprotonation pK⁻), also in connection with recent discussions, concerning the reactivity selectivity principle — a systematic investigation have been made. The validity and direct application of Hammett and other equations have been discussed.
SUMMARY OF THE PRESENT INVESTIGATION

TABLE 1.04 ACID-BASE EQUILIBRIA & KINETICS OF HYDROXAMIC ACIDS

\[ A \] \( pK_{\text{BH}}^+ \)

Monohydroxamic Acid

Unsubstituted Hydroxamic Acid

\[ H-N-OH \]

Benzohydroxamic acid

\[ X = H, \text{CH}_3, \text{OCH}_3, \text{NO}_2, \text{Cl}, \text{Br}, \text{F} \]

N-Substituted Hydroxamic Acid

Cyclic Hydroxamic Acid

Phthalamohydroxamic acid

Succinohydroxamic acid

Dihydroxamic Acid

Trihydroxamic Acid

Oxalo dihydroxamic acid

Desferal

Mineral acids: HCl, H\(_2\)SO\(_4\), HClO\(_4\)
Aqueous
Temp. 25°C

Method: UV
Method: pH Titrimetric

1. Mono Hydroxamic Acid

\[
\text{Value} \quad \text{Condition}
\]

Benzo Hydroxamic Acid

Value: 9.8
Condition: Aqueous 35°C

2. Cyclic Hydroxamic Acid

Phthalamohydroxamic acid

Value: 5.31
Condition: Aqueous 35°C

Succinohydroxamic acid

Value: 6.74
Condition: Aqueous 35°C

[C] Kinetics

Acidic Hydrolysis

Desferal

Catalyst: HCl
Temp.: 45°, 55°, 65°C
[D] Reaction Kinetics and Effect of Solvent

The hydrolysis of hydroxamic acids to hydroxylamine is an important first step in the quantitative analysis of hydroxamate siderophores. The rate of acidic hydrolysis of Desferal have been investigated. Salt, Solvents, Isotopes and temperature effects have also been studied, (Table 1.04).

Kinetic solvent effects on hydrolysis of hydroxamic acids of the type $R.N(OH)=OR'$; $R=H$; $R'=CH_3$, $C_6H_5OH$; $R=CH_3-C_6H_4$, $R'=C_6H_5$ in aqueous mixtures of some polar, protic, polar aprotic and little polar basic solvents have been studied. The solvatochromic parameters $\alpha$ for the hydrogen bond donating ability, $\beta$ of the hydrogen-bond accepting ability and $\pi^*$ for the dipolarity/polarizability and linear solvation energy relationships have been used to quantify solvent effects. Kamlet and Taft's triparametric equation explain $> 70\%$ of the effect of solvent on the hydrolysis. The other parameters i.e. dielectric constant $D$, $E$, and $Z$ have also been used.

[E] Synthesis and Characterization of Hydroxamic Acid-Metal Complexes

Hydroxamic acid ($RCONCR'OH$), is a bidentate oxygen ligand possessing affinity for a variety of metal ions. The Cu(II), Ni(II), Fe(III), Co(II), Mn(II), Zn(II) complexes of some N-substituted hydroxamic acids ($R = C_6H_5$, $R' = C_6H_5$, 4-CH$_3$-C$_6H_4$ and C$_6H_5.CH_2$) have been synthesized and characterized, (Table 1.05).

All the complexes are coloured, non hygroscopic solids and stable in air. They are soluble in DMF, DMSO, etc. and sparingly soluble in CH$_3$CN and C$_2$H$_5$OH and insoluble in water. FTIR spectra of metal complexes were recorded. The complex formation is believed to take place by the replacement of the hydroxylamino, hydrogen by the metal ion and ring closure through coordination from oxygen of the carbonyl group. The electrochemical studies and thermal analysis of some metal complexes have also been done.
### TABLE 1.05 SYNTHESIS AND CHARACTERIZATION OF HYDROXAMIC ACID METAL COMPLEXES

<table>
<thead>
<tr>
<th>Type</th>
<th>Formula</th>
<th>Metal Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsubstituted Hydroxamic Acid</td>
<td>$\text{H-N-OH}$</td>
<td>Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$</td>
</tr>
<tr>
<td>Benzohydroxamic acid $X = \text{H, CH}_2$, OCH$_3$, NO$_2$, Cl, Br, F</td>
<td>$\text{H-N-OH}$</td>
<td>Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, Fe$^{3+}$</td>
</tr>
<tr>
<td>Monohydroxamic Acid</td>
<td>$\text{H-N-OH}$</td>
<td>Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$</td>
</tr>
<tr>
<td>N-Substituted Hydroxamic Acid</td>
<td>$\text{H-N-OH}$</td>
<td>Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$</td>
</tr>
<tr>
<td>N-Phenyl benzo-hydroxamic acid</td>
<td>$\text{H}<em>2\text{C-CH}</em>{2}\text{N-OH}$</td>
<td>Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$</td>
</tr>
<tr>
<td>N-p-Tolybenzo-hydroxamic acid</td>
<td>$\text{H}<em>{2}\text{C-CH}</em>{3}\text{N-OH}$</td>
<td>Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$</td>
</tr>
<tr>
<td>N-Benzyl benzo-hydroxamic acid</td>
<td>$\text{H}<em>{2}\text{C-CH}</em>{2}\text{N-OH}$</td>
<td>Cu$^{2+}$, Ni$^{2+}$, Co$^{2+}$</td>
</tr>
<tr>
<td>Dihydroxamic Acid</td>
<td>$\text{H}<em>2\text{C-CH}</em>{2}\text{N-OH}$</td>
<td>Cu$^{2+}$</td>
</tr>
<tr>
<td>Trihydroxamic Acid</td>
<td>$\text{H}<em>2\text{C-CH}</em>{2}\text{N-OH}$</td>
<td>Cu$^{2+}$</td>
</tr>
</tbody>
</table>

**Examples:**

- **Desferal**
- Oxalodihydroxamic acid
- N-Phenyl benzo-hydroxamic acid
- N-p-Tolybenzo-hydroxamic acid
- N-Benzyl benzo-hydroxamic acid
Nucleic acids provide exciting and difficult challenges for chemists and biochemists. There is a considerable interest in the development of artificial nuclease. A number of metal chelates have been used as probes of DNA structure in solution, as agents for mediation of strand scission. In the present study the DNA nicking ability of Cu(II) complexes of some hydroxamic acids (PBHA, p-TBHA, BBHA), (Table 1.06) was investigated by plasmid cleavage assay at National Chemical Laboratory, Pune under the supervision of Dr. K. N. Ganesh. The DNA cleavage reactions were carried out on plasmid pBr 322 DNA using agarose gel electrophoresis.

1.3 IMPORTANCE OF THE STUDY

There is a serious need to develop efficient and practical microbial chelators as drug delivery agents and antimalarials. Artificial metallonucleases require ligands which effectively deliver the metal ion to the vicinity of the DNA strand, some of which mimic the metal chelation site DNA cleaving antibiotics or metallo-proteins.

Knowledge about the toxicity via hydroxyl radical production will be useful in designing biomedical applications. The hydrolytic and oxidative-cleavage of DNA may be examined by the use of the hydroxamic acid-metal ion system. The simple cleavage system would be useful for the development of artificial metallonucleases, especially, artificial hydrolytic nucleases. The binding modes of a metal complex with DNA play a key role in their anticancer activity. It seems all highly active anticancer metal drugs have the ability to bind with both phosphate groups and
SUMMARY OF THE PRESENT INVESTIGATION

TABLE 1.06 DNA CLEAVING ACTIVITY OF HYDROXAMIC ACID METAL COMPLEXES

<table>
<thead>
<tr>
<th>Hydroxamic Acid</th>
<th>Metal Ion</th>
<th>DNA Target</th>
<th>Target</th>
<th>Chemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure 1" /></td>
<td>Cu**</td>
<td>pBR322</td>
<td>Sugar</td>
<td>Oxidative Cleavage</td>
</tr>
<tr>
<td>N-Phenyl benzo-hydroxamic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image2" alt="Structure 2" /></td>
<td>Cu**</td>
<td>pBR322</td>
<td>Sugar</td>
<td>Oxidative Cleavage</td>
</tr>
<tr>
<td>N-p-Tolylbenzo-hydroxamic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><img src="image3" alt="Structure 3" /></td>
<td>Cu**</td>
<td>pBR322</td>
<td>Sugar</td>
<td>Oxidative Cleavage</td>
</tr>
<tr>
<td>N-Benzylbenzo-hydroxamic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
nitrogen sites on bases of DNA. Dihydroxamic acids (\(-\text{N}–\text{C}–\text{C}–\text{N}\) ) are recognized as very useful and efficient absorbing agent for toxic air pollutant like \(\text{SO}_2\) [187-189]. N-Hydroxyphthalamide used in the present study, has recently been recognized as a valuable catalyst for the oxidation of various organic compounds [190-191]. Ishii et al. [192-193] have used this compound, generally in association with transition-metal complexes for the aerobic oxidation of various organic substrates.

This study will also offer an understanding of quantitative structure reactivity relationships. This will help in establishing the relationship of physico-chemical properties of hydroxamic acids with biological potency and analytical reagents.

The mechanism of hydroxamic acid is of a fundamental importance in the context of its applicability in the nuclear fuel reprocessing, pharmaceuticals, solvent extraction and spectrophotometric determination of metals particularly using concentrated acid solutions. Particular attention has been devoted to the biological and chemical aspects of hydroxamic acids as well as to their potential clinical use as novel therapeutic agents. Information about hydrolysis of reaction could be useful for understanding the biochemical production and degradation of hydroxamic acids. Further, this investigation may open a relatively unexplored but promising research field for further fruitful investigations. From analytical considerations it will be very rewarding to kinetically investigate the hydrolytic stabilities of metal-hydroxamic acid complexes. At present, an analytical chemist normally examines the effect of time on coloured systems, empirically; the effort being tedious, time consuming and often irrational. Kinetic data for such systems will provide a rational basis for the choice of optimum range of acid concentration for maximum colour development.

The chemical data of hydrolysis reactions provides a judicious and rational basis for the search for the new analytical reagents.
In conclusion, the combined use of protonation behaviour and metal-DNA chemistry of hydroxamic acids provide a better picture of applications of hydroxamic acids. The insight gained from such attempt enable us to design more effective hydroxamic acid for analytical applications leading eventually to the design of more selective and sensitive reagents.

1.4 ORDER OF PRESENTATION

The following order of presentation has been adopted:

The opening chapter of the thesis provides a brief review of the work already done in the relevant field. An important section of this chapter outlines the objectives and statement of problem. The emphasis is given to metal-DNA chemistry and acid-base equilibria of hydroxamic acids.

Chapter 2 of this thesis includes the discussion on the site of protonation and protonation equilibrium of hydroxamic acids determined by UV spectrophotometrically in aqueous mineral acids (H$_2$SO$_4$, HCl and HClO$_4$). In addition to simple unsubstituted hydroxamic acids, the protonation parameter of some cyclic, N-substituted and dihydroxamic acid have also been evaluated. Characteristic vector analysis has also been discussed to separate protonation from medium effect.

Chapter 3 describes the structure reactivity correlations and linear free energy relationships (LFER) of hydroxamic acids. The analysis of structural effects on the relative acidities have been accomplished from linear and multilinear regression analysis, in which substituent, steric, polarizability and field/inductive effects are considered.

Chapter 4 of this thesis reports detailed investigation of acidic hydrolysis of hydroxamate siderophore i.e. desferal.
Chapter 5 describes a detailed study of the solvent effect on the hydrolysis of hydroxamic acid. The solvatochromic parameters have also been discussed.

Chapter 6 of the thesis is divided into two parts i.e. synthesis and characterization of hydroxamic acid-metal complexes and mechanism and targeting of DNA cleavage reactions.

Chapter 7 is the concluding part of this work. It also discussed few aspects which needs to be taken in future to obtain a better understanding of artificial metallonucleases. Hopefully, these investigations will make the achievement of the goal close at hand. Furthermore extensive efforts were made at each phase of this research to understand the basic chemical principles involved in acid-base equilibria and DNA cleavage. The list of possibilities is endless and limited only by imagination. The coming years promise to provide additional exciting and challenging opportunities for the study of DNA cleavage activets of hydroxamic acid-metal complex.
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