CHAPTER I

Introduction
CHAPTER I

INTRODUCTION

Hydroxamic acids have attracted considerable attention over the past decade, since they were first reported by H. Lossen (1) in 1869. The chemistry and biochemistry of hydroxamic acids offers a fruitful area of research awaiting its exploration. The outstanding features of hydroxamic acids which prompted to pursue an investigation on them are,

- Hydroxamic acids are neutral molecules.
- These are polyfunctional (multifunctional) solutes.
- These have unique pharmacological, toxicological and pathological properties.

Inspite of these important propensities, hydroxamic acids remain one of the less well-characterised classes of organic compounds as far as their physico-chemical properties are concerned. Although the description of hydroxamic acids and their chemistry is beyond the scope of present work, yet the following section will throw a brief focus on them, followed by discussion on drug designing.

HYDROXAMIC ACIDS

In general, hydroxamic acids are the N-acylderivatives of hydroxylamine, I or phenylhydroxylamine, II (2,3).

\[
\begin{array}{c}
\text{H} & \text{-N-OH} & \text{H} \\
\text{H} & \text{-N-OH} & \text{H} \\
\end{array}
\]

(I)  (II)
N-arylhydroxamic acids, used in the present investigation, are the derivatives of structure, II, Bamberger in 1919 (4) laid the foundation of N-arylhydroxamic acids by synthesizing the parent compound of this series- N-PHENYLBENZOHYDOXAMIC ACID, trivially named as PBHA, III.

\[
\begin{align*}
&\begin{array}{c}
\text{N} \\
\text{OH} \\
\text{C}=\text{O}
\end{array} \\
\end{align*}
\]

(III)

After the recognition of its analytical potentialities by Shome (5), a large number of analogs of PBHA have been synthesized (6–12).

The key feature of these molecules is the presence of hydroxamic acid functional group, IV.

\[
\begin{align*}
&\begin{array}{c}
\text{N} \\
\text{OH} \\
\text{C}=\text{O}
\end{array} \\
\end{align*}
\]

(IV)

This functional group is an important pharmocophore with tremendous biological potentiality. Drug designing with N-arylhydroxamic acids is still at fairly primitive stage and major challenges are yet to be resolved. Hence, in the present investigation an attempt has also been made to study the parameters, essential for drug designing, of some hydroxamic acids.
HYDROXAMIC ACIDS IN NATURE

A substantial number of natural products also contain hydroxamic acids. These are mainly found in microbial sources. Variety of superior plant species comprise of hydroxamic acids (13-14). Their presence in graminee of economical importance, such as maize, wheat and rye (17-18), has been associated to a protection factor of these plants against the attack of different pathogens. The first naturally occurring hydroxamic acid is Aspergilllic acid (19), V which is closely related to amino acid anhydrides. Most of the natural hydroxamic acids are, from fungi and actinomycetes. Their presence in yeast,

\[
\begin{align*}
\text{V}
\end{align*}
\]

bacteria and green plants are also reported (16,20).

CLASSIFICATION OF NATURAL HYDROXAMIC ACIDS

According to the number of \(-\text{CO.N(OH)}-\) group, which they contain, the natural hydroxamic acids are classified into three groups.

MONOHYDROXAMIC ACIDS

Monohydroxamic acids contain one \(-\text{CO.N(OH)}-\) group and occur mainly in uncomplexed form. These are present in both aliphatic and cyclic structures. Most of them like Hadacidin, VI and Fusarinine, VII are related to conventional amino acids.
DIHYDROXAMIC ACIDS

Dihydroxamic acids are one of the derivatives of hydroxamate, which has two hydroxamate, \(-\text{CO.N(OH)}-\), subunits linked with a spacer like a methylene chain VIII.

\[
\text{CH}_3\quad \text{N} \quad \text{C} \quad \text{O} \quad \text{N} \quad \text{C} \quad \text{O} \quad \text{N} \quad \text{C} \quad \text{O} \quad \text{N} \quad \text{C} \\
\text{H} \quad \text{O} \quad \text{H} \quad \text{O} \\
\text{(VIII)}
\]

Dihydroxamic acid has two chelating sites (20). It is a biomimetic ligand analogous to siderophore (21).

TRIHYDROXAMIC ACIDS

Trihydroxamic acids contain three \(-\text{CO.N(OH)}-\) groups. These hydroxamic acids mainly occur as ferric derivatives and are divided into two groups.

- Ferrioxamines: These are made up of repeating units of \(1-\text{amino}-\omega\)-hydroxyaminoalkane and succinic or acetic acid.

\[
\text{Fe}^{2+} \\
\text{(IX)}
\]
Desferrioxamine B is currently being used for the treatment of iron overload disease (20).

- Ferrichromes: In this series the basic structure is cyclic hexapeptide with the hydroxamic acid linkages provided by acyl—N—hydroxyornithine.

![Ferrichrome structure](image)

\[
\text{Ferrichrome (XV): } R = R' = H; R'' = CH_3
\]

\[
\text{Ferrichrysin (XVI): } R = R' = \text{HOCH}_2; R'' = \text{CH}_3
\]

Ferrichrome completely reverses the toxicity of albomycin for organisms such as Escherichia coli (22) or Bacillus subtilis (23). Ferrichrome and Ferrichrysin are found in Japanese sake and account for part of its yellow colour (24).

All natural hydroxamic acids with a ferric trihydroxamate center act as iron transfer agents and are designated as siderochromes (19). These have a power to antagonize the toxicity of the related antibiotics.

APPLICATIONS OF HYDROXAMIC ACIDS

Hydroxamic acids are widespread in the tissues of plants, in metabolites of bacteria and fungi, including complex compounds. Hydroxamic acids and their derivatives fulfill a variety of important roles in several spheres.

A brief concerted attempt has been made here to describe the role of hydroxamic acids in various fields.
ANALYTICAL APPLICATIONS

• Hydroxamic acids and their derivatives serve as bidentate ligand towards many metal ions. The resultant complexes are highly coloured and therefore, useful in colorimetric analysis of metal ions (25–27) or hydroxamic acids (28).

• The extraction and separation of Germanium with hydroxamic acid have been investigated in detail (29).

• Crown hydroxamic acids (29,30) are used for the estimation of Lanthnum (III) and Copper (II).

AGRICULTURAL APPLICATIONS

• Allelopathy, the chemical interaction of plants within the same species or between plants of different species, is important in the plant competition for water, nutrients and light. Hydroxamic acids have been ascribed a role in this interaction in many reports (31–33).

• Due to the ability to modify auxin action, hydroxamic acids exert growth inhibitory effect on plant species (34,35).

• Hydroxamic acids have been shown to have a negative impact on the survival and reproduction of aphids. Inverse correlations between hydroxamic acid content in different varieties of rye and wheat and the growth rate of the aphid Metopolophium dirhodum have been reported (36).

• Hydroxamic acids have been shown to be induced by infestation with insects (37,38).
NUCLEAR APPLICATIONS

• Hydroxamic acids are used for the retention of fission products especially Zirconium (39).

• Polyhydroxamic acid chelating ion exchange resin is used in the separation of Yttrium and Uranium (40).

• Hydroxamic acid enables the survival for cells exposed to histone deacetylase inhibitor (41,42).

TECHNICAL APPLICATIONS

• The use of hydroxamic acids in photoelectric converters, photoelectrochemical cells and thermal energy technology of metal complexes is reported (43).

• These are good corrosion inhibitors (44–46) and applied successfully in flotation techniques (47,48).

• Methacryloyl hydroxamic acid when copolymerized with N–isopropylacrylamide yielded thermotropic polymers capable of complexing with metal ions (49).

MEDICINAL AND PHARMACOLOGICAL APPLICATIONS

Hydroxamic acids have been employed not only in analytical, nuclear or technical field but also in biological and medicinal field. The most basic medicinal and pharmacological applications of hydroxamic acids are listed here.

• Hydroxamic acid moieties are widespread in microbial and plant kingdoms as key functional groups of siderophores. The siderophore is a low molecular weight compound with remarkably high affinities scavenging of Fe(III) from the environment (50).
• Hydroxamic acids act as alternatives to aspirin and as nitric oxide donors (51).

• Hydroxamic acid is found to be an essential pharmacophore in hydroxyurea, XI, a clinically useful inhibitor of ribonucleotide reductase (52). It is a well known anticancer drug (53–55).

\[
\text{H}_{2}\text{N} \equiv \text{C} \equiv \text{NH.OH}
\]

(XI)

• The γ-lactum hydroxamic acid compounds, XII, are highly selective inhibitors of the metalloprotease responsible for producing active tumor necrosis factor α, TNF α (56–58) and helps in the treatment of rheumatoid arthritis (59).

\[
\text{H}-\text{N}-\text{O} \equiv \text{N}
\]

(XII)

• Benzohydroxamic acid, XIII, has significant antitumor activity (60). Substituted benzohydroxamic acid and its complexes with copper metal ions (Cu–benzohydroxamic acid) are used as potential antitumor drug (61,62).

\[
\text{H}-\text{N}-\text{OH}
\]

(XIII)

• Salicylhydroxamic acid, XIV, an oral chelator, is found to have promising advantages in the clinical treatment of thalassaemia major (63), as a trypanocidal drug (64) and used as inhibitors of viral growth (65).
Introduction

Desferrioxamine, IX, a chelator of iron, aluminium and other metals, is used therapeutically for the treatment of iron-overloaded-patients i.e., Cooley's anaemia (66-67) or β-thalassaemia major (68-72). It also decreases NF-κB activation of HIV-1 (73).

The ability of the hydroxamic acid functionality to form a bidentate chelate with the zinc and nickel atoms in the enzyme's active site is an important functional feature i.e., inhibition of matrix metalloproteinases (MMPs) (74-77).

Their use as agonists (78, 79) and antagonists (80, 81) is also important.

Hydroxamic acids are the key pharmacophore in many important antibacterial (82), antifungal (83), antitumor (84), anti-inflammatory (85) and antiviral agents (86).

The hydroxamic acid pharmacophore shows wide spectrum of activities in various fields of pharmacology, toxicology and medicinal chemistry. This inspired to work on N-arylhydroxamic acids, and to determine their physico-chemical parameters applicable in QSAR (Quantitative Structure Activity Relationship) analysis, a part of drug designing.

DRUG DESIGNING

Medicinal chemistry involves the discovery of new chemical entities for the treatment of disease and the systematic study of the structure activity relationships of these molecules. Such studies provide the basis for the development of better medicinal agents from "lead compounds" found via random screening or rational design.
WHAT IS A DRUG?

Medicines are substances used to treat disease whereas drugs are molecules used as medicines or as components in medicines to diagnose, cure, mitigate, treat, or prevent disease (87).

WHAT IS DRUG DESIGNING?

The term drug design represents mainly the effects to develop new drugs on a rational basis. Drug design increasingly is based on modern computational chemical techniques, it also utilizes sophisticated knowledge of disease mechanisms and receptor properties. Drug design means the application of previously recognized correlations of biological activity with physico-chemical characteristics, in the hope that the pharmacological success of a not yet synthesised compound can be predicted. But without a thorough understanding of the physico-chemical properties of the organic functional groups that comprise any given structure, the task would be impossible (88).

STEPS INVOLVED IN DRUG DESIGN

The various approaches involved in drug design include (89) –

• The first step is the detection of some biological action in a group of compounds so as to serve as a LEAD. The lead is a prototype compound that has the desired biological or pharmacological activity but may have many undesirable characteristics, e.g., high toxicity, other biological activities, insolubility or metabolic problems.

• This is followed by MOLECULAR MANIPULATIONS to increase or modify the activity.

• Next step involves, QSAR methods which are used with an assumption that the biological properties of organic compounds are a direct
consequence of their physico-chemical properties. The ultimate objective of such studies is to understand the forces governing the activity of a particular compound or a class of compounds. QSAR is thus a scientific achievement and an economic necessity to reduce an empiricism in drug design to ensure that every drug synthesized and pharmacologically tested should be as meaningful as possible.

- On the basis of conclusions drawn from QSAR studies novel compounds with desired biological activity are SYNTHESIZED and tested experimentally.

- The results gained in this way are incorporated into the data base, which helps in making a confident decision about whether the "BEST ANALOGUE" has been prepared or whether the series should be abandoned (90).

Thus, the process of developing drugs with specific activities goes through the following cycle,

After following such a tedious process, only fewer drugs can reach to the level of clinical applicability. Such compounds have to be given extensive trials before they are tried on humans. This adds to the cost of research for new
drugs. Broadly, this means that if the development of new drugs is to remain economically feasible, the ratio of output to input must be increased.

**QSAR PARAMETERS**

Physical organic chemistry deals with characterisation of the structure and prediction of the properties, the descriptors for which, are found experimentally. If some property depends on the set of selected descriptors, the ordering of the structure will parallel the ordering of the properties, (91). In other words, the structural information is coded in these properties. Therefore, good correlation of physico-chemical parameters with a particular set of indices...
may help in understanding the contribution of these invariants in determining the property.

For this investigation, the following physico-chemical parameters used in QSAR studies were determined.

**TABLE 1. PHYSICO-CHEMICAL PARAMETERS USED IN QSAR STUDIES**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>PHYSICO-CHEMICAL PARAMETERS</th>
<th>SYMBOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>HYDROPHOBIC PARAMETERS:</td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Parachor</td>
<td>P</td>
</tr>
<tr>
<td>(ii)</td>
<td>Partition coefficient</td>
<td>logP</td>
</tr>
<tr>
<td>(iii)</td>
<td>Hydrogen–bond strength</td>
<td>εa, εβ</td>
</tr>
<tr>
<td>(iv)</td>
<td>Hydrophobic constant</td>
<td>πx</td>
</tr>
<tr>
<td>II.</td>
<td>ELECTRONIC PARAMETER:</td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Dipole moment</td>
<td>μ</td>
</tr>
<tr>
<td>III.</td>
<td>STERIC PARAMETERS:</td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td>Molar volume</td>
<td>V</td>
</tr>
<tr>
<td>(ii)</td>
<td>Molar refraction</td>
<td>Rm</td>
</tr>
<tr>
<td>(iii)</td>
<td>van der Waals volume</td>
<td>vbb</td>
</tr>
</tbody>
</table>

These physico-chemical parameters are needed to describe the interaction and the transport and distribution of drugs in a quantitative manner and to correlate them with biological activities. These parameters will be discussed in the subsequent chapters.

Moreover, the pharmacokinetic and pharmacodynamic properties are governed by hydrophobic, electronic and steric features of the drug molecule. Some drugs exert their physiological effect by interacting with a particular cellular binding site called RECEPTOR. These binding interactions may be,
Introduction

- van der Waals Interactions.
- Electrostatic Interactions.

In life sciences, hydrogen–bond is the most important kind of specific molecular interaction which influences the drug solubility, bodily transport and docking to active sites. The greater will be the affinity of a drug for its binding site, the higher will be its potential biological activity. Thus, the knowledge of hydrogen–bond parameters is applicable for the recent developments of scales useful for drug design.

Besides the use of these parameters in drug design, the concentration and temperature dependence of these fundamental physico-chemical parameters are also worked out and discussed to gain valuable information about solute–solute and solute–solvent interactions.

SUMMARY OF THE PRESENT INVESTIGATION

In order to meet the above mentioned objectives the present investigation has been divided into SIX CHAPTERS. A summary of each subsequent chapter has been presented here.

CHAPTER I: This part of the investigation covers an INTRODUCTION of the hydroxamic acids and the principles of drug design. Its sub–chapter, IA, describes the procedure of SYNTHESIS of five hydroxamic acids by the reported method.

CHAPTER II: This chapter reports the determination of DENSITY and related parameters to throw light on intermolecular interactions.

CHAPTER III: Hydroxamic acids are biologically active molecules. The study of SURFACE CHEMISTRY of hydroxamic acids in terms of excess surface properties and surface thermodynamic parameters is very essential, from both, physico–chemical and biological view points. Therefore, these parameters are discussed in this chapter.
CHAPTER IV: REFRACTOMETRIC PARAMETERS of hydroxamic acids are reported and discussed in chapter IV. Molar refraction is an important physico-chemical parameter in drug design. Moreover, other refractometric parameters are also obtained to throw light on specific interactions between the solute and the solvent.

CHAPTER V: In this chapter, the VISCOMETRIC PARAMETERS of hydroxamic acids are studied. These parameters are obtained at two temperatures and at different concentrations. It gives information on the existence of specific molecular interactions. Viscosity also affects the permeation of bio-active molecules through biological membranes.

CHAPTER VI: This chapter describes the HYDROGEN–BOND DONOR AND ACCEPTOR PARAMETERS of hydroxamic acids. The lipophilicity of hydroxamic acids has been determined in 1-octanol–water system, log P(o/w). It is compared with the logP, obtained by theoretical method and also using software programmes. The hydrogen–bond donor and acceptor strength is also evaluated. Applying these parameters, the substitution constant, pi(π), is obtained. Hydrogen–bond is an important parameter in drug design. In subchapter VIA, some of the molecular properties calculated online, for hydroxamic acids are briefly reported.
CHAPTER IA

Synthesis of Hydroxamic Acids
CHAPTER I

SYNTHESIS
OF
HYDROXAMIC ACIDS

Synthesis of five hydroxamic acids containing various substituents, at different positions of upper and lower phenyl rings, is presented in this chapter. It follows a three step process. The first step involves the preparation of acid chlorides. This is followed by the preparation of hydroxylamine, in the next step. The third step is the preparation of hydroxamic acids by coupling hydroxylamine with acid chloride at very low temperature in presence of aqueous sodium bicarbonate suspension.

The hydroxamic acids prepared were purified thrice with benzene for further use. The purity of these compounds was checked by determining their melting points, which tally with the reported data.
SYNTHESIS OF HYDROXAMIC ACIDS

SYNTHESIS OF ACID HYDROXYLAMINE CHLORIDE I

SYNTHESIS OF HYDROXYLAMINE II

COUPLING

HYDROXAMIC ACIDS III
SYNTHESIS
OF
HYDROXAMIC ACIDS

This part of investigation is a brief discussion on the synthesis of hydroxamic acids. Five hydroxamic acids containing different substituents in structure I, at different positions of upper and/or lower ring were synthesised.

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

(1)

The procedure followed for the preparation of these reagents in general is reported in the literature (92–96).

Synthesis of hydroxamic acids is a THREE STEP process.

STEP I – PREPARATION OF ACID CHLORIDES

All the acid chlorides were prepared by the action of excess of thionyl chloride on corresponding carboxylic acids (97). The reaction mixture was refluxed on oil bath for four to five hours. Purification was done by distillation under reduced pressure. Middle fraction was collected for further use.

\[
Y \quad \quad \text{COOH} + \text{SOCl}_2 \rightarrow \quad \text{COCl} + \text{HCl} + \text{SO}_2
\]

STEP II – PREPARATION OF HYDROXYLAMINES

Hydroxylamines were obtained by the reduction of corresponding nitro compounds with zinc dust and aqueous ammonium chloride below 65°C.
**Synthesis of Hydroxamic Acids**

\[ X \text{NO}_2 \xrightarrow{\text{Reduction}} X\text{NOH} \]

The products were purified by crystallisation with benzene and petroleum ether (60–80°C) and further used without delay.

**STEP III – PREPARATION OF HYDROXAMIC ACIDS**

For the preparation of hydroxamic acids, hydroxylamine and acid chloride were taken in an equimolar quantities.

\[ X\text{NOH} + \text{Cl} \xrightarrow{\text{NaHCO}_3} X\text{NOH} - \text{HCl} \]

The reaction was carried out in ether medium at 0°C or below, in presence of sodium bicarbonate suspension. The crude products were first crystallised with benzene and petroleum ether and then thrice with benzene alone to ascertain their purity. The melting points were determined and compared with the literature data (98).

All the hydroxamic acids were dried in vacuum over phosphorus pentoxide for 24 hours before use. Names, molecular and structural formulae, 3D optimised structures, molecular weights and melting points, both observed and reported, of the hydroxamic acids synthesised are given in the following pages.
COMPOUND - I

N-PHENYL-2-CHLOROBENZOHYDROXAMIC ACID

Molecular Formula

\[ \text{C}_7\text{H}_9\text{O}_2\text{NCl} \]

Mol. Structure

3D Optimized Structure*

Mol. Wt.

247.5

M.P., °C

105 OBSERVED

106 (99) REPORTED

*Obtained using software, ACD Labs Freeware, Version 5.0
**COMPOUND - II**

**N-PHENYL-4-ETHOXYBENZOHYDROXAMIC ACID**

Molecular Formula: $C_{15}H_{15}O_3N$

Mol. Structure:

![Molecular Structure](image)

3D Optimized Structure:

![3D Optimized Structure](image)

Mol. Wt.: 256.999

M.P., °C: 145 OBSERVED

146 (100) REPORTED

*Obtained using software, ACD Labs Freeware, Version 5.0
COMPOUND - III

N-o-TOLYL-4-CHLOROBENZOHYDROXAMIC ACID

Molecular Formula

\[ C_{14}H_{12}O_2NCl \]

Mol. Structure

3D Optimized Structure

Mol. Wt.

261.5

M.P., °C

121 OBSERVED

120 (101) REPORTED

*Obtained using software, ACD Labs Freeware, Version 5.0
COMPOUND - IV

N-o-TOLYL-4-ETHOXYBENZOHYDROXAMIC ACID

Molecular Formula

\[ \text{C}_{16}\text{H}_{17}\text{O}_{3}\text{N} \]

Mol. Structure

\[
\begin{align*}
\text{C}_2\text{H}_5\text{O} & \quad \text{C} = \text{O} \\
\text{CH}_3 & \quad \text{N} - \text{OH}
\end{align*}
\]

3D Optimized Structure*

Mol. Wt. 270.999

M.P., °C

105 OBSERVED

104 (96) REPORTED

*Obtained using software, ACD Labs Freeware, Version 5.0
COMPOUND - V

N-p-TOYL-2-ETHOXYBENZOHYDROXAMIC ACID

Molecular Formula

\[ C_{16}H_{17}O_3N \]

Mol. Structure

3D Optimized Structure*

Mol. Wt.

270.999

M.P., °C

167 OBSERVED

167 (100) REPORTED

*Obtained using software, ACD Labs Freeware, Version 5.0
GENERAL PROPERTIES OF HYDROXAMIC ACIDS

COLOUR:

All the N–arylhydroxamic acids reported here are white crystalline solids.

SOLUBILITY:

Hydroxamic acids are very sparingly soluble in water but display enhanced solubility in mineral acid solutions. These are freely soluble in acetone, benzene, carbon tetrachloride, chloroform, 1,2–dichlorobenzene, ethyl acetate, toluene, and water immiscible higher alcohols.

ACIDIC CHARACTER:

Hydroxamic acids are weakly acidic compounds and do not liberate carbon dioxide from aqueous sodium bicarbonate solution. The proposed structure for anion is II, and derivative obtained by substitution in the hydroxyl group is an ester III,

\[
\begin{align*}
H-N-O^- & \quad \quad \quad \quad \quad \quad H-N-OH & \quad \quad \quad \quad \quad \quad H-N-OR' \\
\text{(II)} & \quad \quad \quad \quad \quad \quad \text{(III)} & \quad \quad \quad \quad \quad \quad \text{HYDROXAMIC ACID}
\end{align*}
\]

ACTION OF REDUCING / OXIDISING AGENTS:

Hydroxamic acid bond is reduced by Raney nickel or hydrogen gas and oxidised in presence of hydroiodic acid, performic acid and periodic acid. Periodic acid selectively cleaves the hydroxamic acid linkage while amide and even more sensitive bonds remain unaffected.
SYNTHEISIS of Hydroxamic Acids

COLOUR REACTION:

These reagents perform the characteristic colour reaction (102) with Vanadium(+5) and iron (+3).

A reddish-violet coloured complex is obtained with ferric chloride (103,104).

\[
\text{H-N-OH} + \text{Fe}^{3+} \rightarrow \left(\text{H-N-O}\right)\text{Fe}^{2+} + \text{H}^+
\]

Hydroxamic acid's solution in water immiscible solvents when treated with pentavalent Vanadium in presence of hydrochloric acid produce characteristic violet extracts.

\[
2 \text{R}_1\text{N-OH} + \text{V}^{(+5)} + \text{HCl} \rightarrow \text{R}_1\text{N-O} \overset{\text{V}}{\text{O\text{-C-R}_2}} \text{R}_2\text{C=O} \text{O-N-R}_1
\]

This reaction is used for the estimation of Vanadium and hydroxamic acids (105, 106) both.