Experimental
EXPERIMENTAL

3.0 CHEMICALS

In the present study, the following chemicals were used, whose details are given below as follows:

3.01 Acids

The acids used in the experimental investigations were hydrochloric, sulphuric and perchloric acid.

[A] Hydrochloric acid : [Qualigens ExcelaR]
Sp. gr. 1.18 gm ml⁻¹, 11.6M

[B] Sulphuric acid : [Qualigens ExcelaR]
Sp. gr. 1.83 gm ml⁻¹, 18.0M

[C] Perchloric acid : [Qualigens ExcelaR]
Sp. gr. 1.70 gm ml⁻¹, 10.0M

All the acids were standardized with sodium hydroxide, which in turn was standardized with potassium hydrogen phthalate (BDH, AnalR).

3.02 Solvents

[A] 1,4 - Dioxane (BDH, AnalR) : was used as a solvent in the entire kinetic study.

[B] Water : Double distilled water was used for preparation of solutions. It was prepared by redistilling deionised water in distillation glass assembly over alkaline potassium permangnate.

[C] Other organic solvents : Other organic solvents used for the kinetic studies were : DMSO (Merck, GR), Acetone (Qualigens ExcelaR) Acetonitrile (Merck, GR), Ethanol (Qualigen, ExcelaR); organic solvents used for synthetic studies were : Diethyl ether (Qualigens ExcelaR),
Petroleum benzene (Merck, boiling range 60-80°C) Benzene Extrapure (Merck), Nitrobenzene (Merck,AR), Methanol (Qualigens ExcelaR), Acetic Acid (Qualigens ExcelaR), Ethyl Acetate (Merck, AR). The above solvents were used as obtained.

3.03 Solutions


[B] Ferric chloride solution

(i) Acidic : The ferric chloride solution used in the colorimetric procedure for acidic hydrolysis was prepared by dissolution of 44gm of anhydrous ferric salt (Mol. wt- 162.21, Qualigens ExcelaR) in 1 litre of distilled water containing 10ml of concentrated hydrochloric acid to prevent ferric hydroxide formation. Solution, so prepared was filtered through ordinary filter paper.

3.04 Salts

Some anhydrous salts such as lithium chloride (Loba, GR), sodium chloride (BDH, AR) and potassium chloride (Qualigens ExcelaR) were used in the measurement of salt effect. Each of these salts were dehydrated before use.

3.05 Surfactants

The following surfactants has been used in kinetic study.

(i) Cetyl trimethyl ammonium bromide (CTAB) (E. Merck, AR)

\[\text{M.W. 364. 5}\]

(ii) Sodium dodecyl sulphate (SDS) : (Loba Chemie)

\[\text{M.W. 364. 5}\]
Polyoxyethylene (23) lauryl ether (Brij 35) (Loba Chemie)

M.W. 1198

3.06 Acid chloride

Following acid chlorides were used in the synthesis of heterocyclic hydroxamic acids.

[A] Hydroxylamine hydrochloride: (Merck, AR)

F.W. 69.49, m.p. - 159°C

[B] Furoyl chloride: (Merck, AR)

F.W. 130.53, b.p. - 173 - 174°C

3.1 APPARATUS

Following apparatus were used in experimental investigation.

3.11 Thermostat

Kinetic studies were carried out in thermostat set at 45-65°C with automatic temperature control ±0.1°C precision, thermometers 0-50°C and 0-100°C of least count 0.1°C were used. The temperature setting remained undisturbed throughout the course of investigation.

3.12 Mechanical stirrer

(Remi motors 220/230V, 50~AC), magnetic stirrer (Remi equipments, 2MLH) and vacuum pump (Wiswo, RPM 5400) used for synthesis purpose. Melting point apparatus (Tempo S.No. A 233) was used to determine melting points.

3.13 Spectrophotometer

Kinetic measurements were made by the use of spectrophotometric method using a systronics UV-VIS 106 spectrophotometer and it was used to study complexation with Vanadium.
3.14 pH meter

Digital pH-meter (Systronics model 331) was used for pH measurements to observe the effect of pH on metal complexation.

3.15 Respirable dust sampler

Respirable Dust Sampler (Instrumex, Bombay) was used to collect air sample, for spectrophotometric determination of Vanadium using heterocyclic hydroxamic acid in polluted air.

3.16 Glasswares

[A] Reaction vessels: Tubes 6 x 1" with stoppers were used as flat bottom reaction vessels.

[B] Burettes: Corning burettes of 5 x 0.02 ml and 50 x 0.1 ml were used.

[C] Pipettes: Special bulb pipettes of 1.0 and 2.0 ml were used for withdrawing aliquots from reaction mixture. Graduated corning pipettes of 1 x 0.01 ml and 5 x 0.01 ml were also used.

[D] Measuring cylinders: Graduated measuring cylinders of 10 x 0.1 ml, 50 x 0.1 ml and 25 x 0.1 ml were used.

[E] Volumetric flasks: Volumetric flask of 1 litre, 500 ml, 100ml, 50ml and 25 ml were used.

[F] Beakers: Borosil beakers of 25ml, 50ml, 250ml and 500ml were used in the experiments.

3.2 PREPARATION OF HETEROCYCLIC HYDROXAMIC ACIDS

Five heterocyclic hydroxamic acids 2- Furohydroxamic acid (FHA), N-Methyl-2-furohydroxamic acid (MFHA), N-Phenyl-2-furohydroxamic acid (PFHA), N-p-Tolyl - 2-furohydroxamic acid and N-p-Chlorophenyl-2-furohydroxamic acid were synthesized as per literature procedure [1-7]. Their preparation method described in Chapter - II.
3.3 KINETIC MEASUREMENTS

For each kinetic run, in a glass-stoppered reaction vessel, about 7-8mg of heterocyclic hydroxamic acid dissolved in suitable solvent, was taken. In another glass-stopped vessel catalysing acid solution was taken. Both reaction vessels were thermostated for about 15 minutes and then catalysing acid solution was transferred to the reaction vessel containing hydroxamic acid. After mixing, from this reaction mixture 2ml aliquots were withdrawn at regular intervals of time and added to 10ml volumetric flask containing 2ml of acidic ferric chloride. A double purpose, quenching of the reaction and colour development, was thus served. The volume of the coloured solution was made up to 10ml and its absorbance was measured at 540 nm using a reference solution containing 2ml of the same ferric chloride in 10ml of water. The kinetic runs were studied generally up to two half-lives. For measuring absorbance, a Systronics UV-VIS spectrophotometer model 106 was used.

For a pseudo-first order reaction, a plot of log absorbance vs. t will give a straight line of slope, \(-k_v/2.303\) (where \(k_v\) is the pseudo first order rate constant for the reaction). The rates of hydrolysis were determined spectrophotometrically by following the decrease in the characteristic absorption of the heterocyclic hydroxamic acid-ferric chloride complex. Beer's law is obeyed by the system. The concentration of reacting species is proportional to the absorbance \([\log A \propto \log(a-x)]\). To obtain the rate constant \(k_v\), \(\log(a-x)\) was plotted against time \(t\), from the slope of the plot, \(k_v\), was determined. The fact that a straight line was obtained for all the plots of \(\log(a-x)\) vs. \(t\) measured in this investigation was, in itself, an indication that the reactions were all first order with respect to substrate. The initial concentration of the heterocyclic hydroxamic acid in the reaction mixture was \(2.7 \times 10^{-3}\) M. The experimental errors in the respective runs were
generally less than 1.0% and reproducibility of the rate constants were within ± 1.5%.

3.4 PRODUCT IDENTIFICATION

The acid catalysed hydrolysis of heterocyclic hydroxamic acids give parent carboxylic acids and unsubstituted and N-substituted hydroxylamines according to reaction 3.1.

\[ R - N - OH + H_2O \xrightarrow{[H^+]} RNHOH + COOH \] (3.1)

The nature of hydrolysis products depends on the nature of heterocyclic hydroxamic acid. Products obtained in case of different heterocyclic hydroxamic acids studied are given in Table 3.1. The carboxylic acids were separated and analysed by m.p. The unsubstituted and N-substituted hydroxylamines were unstable and decomposed into a variety of complex products[8].

3.5 EXPERIMENTAL SECTION FOR COMPLEXATION OF HETEROCYCLIC HYDROXAMIC ACID (FHA) WITH VANADIUM (V)

3.51 Chemicals And Reagents

[A] Stock solution of Vanadium : The stock solution of vanadium 500ppm was prepared by dissolving 1.1482 gm ammonium metavandate (BDH, AR) in double distilled water and diluted to 1 litre. The solution was standardized volumetrically using potassium permanganate[9]. The working standard solutions were prepared by appropriate dilution of the stock solution.
Table 3.1 Products Of Hydrolysis Of Heterocyclic Hydroxamic Acids

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Heterocyclic Hydroxamic Acid</th>
<th>Unsubstituted and N-Substituted Hydroxylamine</th>
<th>Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><img src="image" alt="2- Furohydroxamic acid" /></td>
<td><img src="image" alt="NH₂OH" /></td>
<td><img src="image" alt="COOH" /></td>
</tr>
<tr>
<td></td>
<td>2- Furohydroxamic acid</td>
<td>Hydroxylamine</td>
<td>2-Furoic acid</td>
</tr>
<tr>
<td>2.</td>
<td><img src="image" alt="N- Phenyl - 2 - furohydroxamic" /></td>
<td><img src="image" alt="N-Phenyl hydroxylamine" /></td>
<td>2-Furoic acid</td>
</tr>
<tr>
<td>3.</td>
<td><img src="image" alt="N-p-Tolyl-2-furo hydroxamic" /></td>
<td><img src="image" alt="N-p- Tolyl hydroxylamine" /></td>
<td>2-Furoic acid</td>
</tr>
<tr>
<td>4.</td>
<td><img src="image" alt="N-p-Chlorophenyl- 2-furohydroxamic acid" /></td>
<td><img src="image" alt="N-p- Chlorophenyl hydroxylamine" /></td>
<td>2-Furoic acid</td>
</tr>
</tbody>
</table>

(77)
[B] Hydrochloric acid: 10M hydrochloric acid solution was used for acidity adjustment.

[C] Heterocyclic hydroxamic acid: 2-Furohydroxamic acid (FHA) was synthesized by standard method [1-2] and its 0.12 M (1.6% w/v) solution was employed.

[D] Solution of diverse ions: The solutions of diverse ions were prepared by West's method [10]. Suitable amounts of metal salts were taken to get approximately 5mg of the metal ion per ml of the solution.

3.52 Procedure

To an aliquot of working standard of 0.2-1.6µg ml⁻¹ of Vanadium, 0.05ml of 3 M hydrochloric acid was added followed by addition of 5ml of 1.6% (w/v) of FHA. The mixture was shaken for approximately 1 minute for complete colour development. Then the final volume was made up to 25ml adding distilled water. The absorbance of the purple coloured complex was measured at 475 nm using Systronics 106 spectrophotometer against the similarly prepared reagent blank. pH measurement were made by using Systronics digital pH meter 331. The amount of Vanadium in samples were calculated from calibration curve.

3.6 EXPERIMENTAL SECTION FOR BIOLOGICAL ACTIVITY OF HETEROCYCLIC HYDROXAMIC ACID

3.61 Nutrient Medium

[A] COMPOSITION OF BACTERIAL NUTRIENT MEDIUM

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3gm</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5gm</td>
</tr>
<tr>
<td>Peptone</td>
<td>5gm</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
</tbody>
</table>
Agar - Agar - 15gm
Congo Red dye - 2.5ml
pH - 7.2

[B] COMPOSITION OF FUNGAL NUTRIENT MEDIUM POTATO DEXTROSE AGAR MEDIA (PDA)

Potato - 200 gm
Dextrose - 20 gm
Agar - Agar - 20 gm
Distilled water - 1000 ml
pH - 6.2

[C] Preparation of biodisc: The biodisc were prepared of the five synthesized heterocyclic hydroxamic acids in DMSO (Dimethyl sulphoxide) in the concentration level 1000 μg/ml.

3.62 Apparatus

Inoculation chamber, Inoculating needle, spirit lamp, petriplates.

3.63 Procedure

The antimicrobial activity was tested by paper-disc plate method [11-12]. The five synthesized compounds [FHA, MFHA, PFHA, p-TFHA and p-CIPFHA] were screened for antimicrobial activity against certain bacteria Escherichia coli (Gram-ve), Lactobacillus (Gram +ve), Pseudomonas (Gram-ve), Rhizobium (Gram -ve) and fungal strains like Alternaria, Curvularia and Rhizoctonia. The reference drugs used were norfloxacin and penicillin. Bacterial suspensions were prepared in nutrient medium and fungal suspensions were prepared in PDA media. Each test tube containing 10 ml medium and one loopful bacteria or fungus were inoculated. 1 ml of bacterial suspension and 10ml of nutrient agar medium were inoculated in petriplate.
Similarly 1 ml of fungal suspension and 10 ml of PDA medium were inoculated in petriplate.

After plating and solidification of the medium biodiscs were placed upon the surface of solidified nutrient agar medium and PDA medium. The plates were incubated for 24 hrs at 28±°C and in case of fungus the plates were incubated for 72 hrs at 28±°C. Zone of inhibition was measured in mm[13].

3.7 STATISTICAL ANALYSIS OF EXPERIMENTAL DATA

The experimental data were subjected to statistical analysis[14]. The standard deviation 'S' was calculated from the expression, 3.2 and relative standard deviation 'RSD' from the expression, 3.3,

\[ S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} \]  

(3.2)

where

\[ \bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i \]

\[ RSD = \frac{100 \times S}{\bar{x}} \]  

(3.3)

The method of least square analysis was adopted whenever necessary. For straight line plot, following equation may be treated by the method of least squares,

\[ Y = mx + C \]

The slope 'm' and intercept 'C' parameters for straight line can be calculated by linear regression analysis using computer. The straight line selected by common linear regression analysis is that which minimizes the sum of the squares of the deviations of the variable from the line. The fit of linear relations was judged by calculating the correlation coefficient 'r' from the expression 3.4, using computer.

(80)
\[ r = \sqrt{\frac{\sum (x_i - \bar{x})(y_i - \bar{y})^2}{(x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}} \]  \hspace{2cm} (3.4)

where \( \bar{x} = \frac{1}{n} \sum x_i \) and \( \bar{y} = \frac{1}{n} \sum y_i \)
REFERENCES