CHAPTER V

EXTRACTIVE SPECTROPHOTOMETRIC DETERMINATION OF HYDROXYLAMINE

SUMMARY

An extractive spectrophotometric method based on the diazotising and coupling reaction is described for the determination of hydroxylamine. Hydroxylamine is oxidised by iodine to nitrite, which then diazotises p-nitroaniline to form a diazonium salt, which is later coupled with diphenylamine in acidic medium. The pink coloured chloroform extract has a maximum absorption at 540 nm. Beer's law is obeyed in the range of 0.02 to 0.16 ppm. The molar absorptivity and Sandell's sensitivity of the method is found to be $3.30 \times 10^4 \text{ l.mol}^{-1}\text{cm}^{-1}$ and 0.001 $\mu\text{g cm}^{-2}$ respectively. The method has been successfully applied for the determination of simple hydroxamic acids.

** Accepted for publication in JICS.**
Amines find their use in the production of dye-stuffs, emulsifiers, rubber products, soaps and pharmaceutical agents (1). Hydroxylamine is a widely used reagent employed for the detection and determination of various compounds and metals (2-6). It has been found to be highly toxic. Hydroxylamine and its derivatives have a common property to form methemoglobin in man and animal (1). It has also been detected in bacterial media and in tissues of a number of organisms (7). Hydroxylamine induces point mutations by reaction with cytosine, but in the presence of trace metals and oxygen it also produces radicals which rapidly inactivate DNA (8). Hence, the determination of hydroxylamine in traces is very important both in studies of industrial and biological purposes.

The general methods for the determination of hydroxylamine cited in the literature are based on its oxidation or reduction. Spectrophotometric methods make use of Griess-Illosvay reaction which involves the determination of nitrite formed by the prior oxidation of hydroxylamine (9-14). Direct determination of hydroxylamine is also done spectrophotometrically (15-21). Other available spectrophotometric methods are less sensitive and suffer from common interferences (22-26). Other instrumental methods are also reported for its determination (27-38).
In this part of investigation a new and sensitive method is described for spectrophotometric determination of hydroxylamine. The method is based on the oxidation of hydroxylamine to nitrite by iodine in acetic acid medium. The nitrite so formed diazotises p-nitroaniline which is subsequently coupled with diphenylamine to give a pink coloured dye extractable in chloroform (39). The chloroform extract of the dye has an absorption maxima at 540 nm. The colour is stable for ~30 hours. The molar absorptivity and Sandell's sensitivity (40) of the system is found to be $3.30 \times 10^4 \text{ l.mol}^{-1}\text{cm}^{-1}$ and 0.001 $\mu\text{g cm}^{-2}$ respectively. The method is sensitive, selective and free from common interferents. The method has been successfully applied for the determination of simple hydroxamic acids.

**EXPERIMENTAL**

**Apparatus:**

All spectral measurements were done at Carl Zeiss spekol with 1 cm matched silica cells.

**Reagents:**

Standard hydroxylamine solution - A stock solution of hydroxylamine was prepared by dissolving AR grade hydroxylamine hydrochloride in demineralised water. A working standard of 4 $\mu\text{g/ml}$ was prepared by appropriate dilution of the stock.
p-Nitroaniline solution - A 0.05% (w/v) solution of p-nitroaniline was prepared in 20% aqueous ethanol.

Iodine reagent - 1.3 gm of iodine was dissolved in 100 ml of glacial acetic acid.

Sodium thiosulphate solution - A 2.5% (w/v) solution of sodium thiosulphate was prepared in demineralised water.

Diphenylamine solution - A 0.2% (w/v) solution of diphenylamine was prepared in 10 M glacial acetic acid.

All chemicals used were of AnalaR grade.

Procedure:

An aliquot of sample (~50 ml) containing 1-8 μg of hydroxylamine was taken in a 100 ml graduated boiling tube. 1 ml of sodium acetate acetic acid solution and 2 ml of iodine reagent were added to the sample solution. To it 3 ml of p-nitroaniline solution was added, shaken and allowed to stand for 2-5 minutes to ensure complete oxidation and diazotisation. Excess of iodine was removed by dropwise addition of sodium thiosulphate solution. 5 ml of diphenylamine solution was added and the acidity was adjusted to 2.5 M. The solution was then thermostated for 60 minutes at 60°C and was transferred to a separatory funnel, and extracted with chloroform in small fractions. The brown coloured chloroform extract was shaken with 0.5 ml hydrochloric acid which turns pink on the addition of the acid. The
pink coloured chloroform layer was transferred to a 10 ml volumetric flask and made up to the mark with glacial acetic acid. The absorbance was measured at 540 nm against chloroform blank. The amount of hydroxylamine was deduced from the calibration curve.

RESULTS AND DISCUSSIONS

Spectral characteristics:

The absorption spectra shows that the pink coloured dye has a maximum absorption at 540 nm (Fig. 1). The absorbance of the reagent blank was negligible in this region hence all the measurements were made against demineralised water.

The colour reaction:

Three steps are involved in the colour reaction. In the first step hydroxylamine is oxidised to nitrite (I) with iodine reagent. In the second step p-nitroaniline reacts with nitrite ion to form p-nitrophenyl diazonium chloride (II). And in the final step the diazonium ion is coupled with diphenylamine in acidic medium to form the dye (III) which is measured spectrophotometrically. Schematic representation of the reaction is as follows –

1. Oxidation of hydroxylamine:

\[
\text{NH}_2\text{OH} + 2\ I_2 + \text{H}_2\text{O} \rightarrow \text{HNO}_2 + 4\ \text{HI}
\]  
(I)
FIG. 1. ABSORPTION SPECTRA OF THE DYE AND REAGENT BLANK.
A. CONCENTRATION OF NH$_2$OH = 5µg/50ml.
B. CONCENTRATION OF NH$_2$OH = 3µg/50ml.
C. REAGENT BLANK
2. Diazotisation:

$$\text{O}_2\text{N} - \bigcirc - \text{NH}_2 + \text{NO}_2^- + 2 \text{H}^+ \rightarrow$$

$$\text{O}_2\text{N} - \bigcirc - \overset{\dagger}{\text{N}} = \text{N} = \text{N} + 2 \text{H}_2\text{O}$$

(II)

3. Coupling:

$$\text{O}_2\text{N} - \bigcirc - \overset{\dagger}{\text{N}} = \text{N} = \text{N} + \overset{\dagger}{\text{N}}$$

$$\bigcirc - \text{NH} - \bigcirc$$

$$\downarrow \text{Acidic medium}$$

$$\text{O}_2\text{N} - \bigcirc - \overset{\dagger}{\text{N}} = \text{N} = \text{N} - \bigcirc - \overset{\dagger}{\text{N}}$$

(III)

**COLOUR REACTION**

**Effect of various reaction conditions**

**Oxidation of hydroxylamine:**

Iodine oxidation of hydroxylamine takes place at pH 2.2 to 3.0 (Fig. 2) which was maintained by the addition of acetic acid sodium acetate solution. The oxidation of hydroxylamine to nitrite was completed within 2 minutes. Excess of iodine must be removed by the dropwise addition of sodium thiosulphate solution within 5 minutes, as low absorbance values are observed otherwise (Fig. 3). Excess of sodium thiosulphate solution caused in low absorbance values.
FIG. 2. EFFECT OF pH ON THE OXIDATION OF HYDROXYLAMINE TO NITRITE
CONCENTRATION OF NH$_2$OH = 5µg/50ml.

FIG. 3. EFFECT OF TIME ON OXIDATION REACTION
CONCENTRATION OF NH$_2$OH = 5µg/50ml.
Diazotisation and Coupling

Acidity

The diazonium chloride was formed at the same pH which was required for the oxidation of hydroxylamine to nitrite, which was then coupled with diphenylamine. For coupling the most suitable acidity range was found to be 1.5 M to 3.5 M hydrochloric acid (Fig. 4).

Effect of time and temperature

The diazotisation reaction was fast at room temperature and it was completed within 1 minute. The temperature required for coupling was also studied by developing colour at different temperatures for 60 minutes. Best results were obtained at 60°C. Below 50°C the colour reaction was found to be incomplete (Fig. 5a). To study the time for complete colour development, coupling reaction was carried out for various lengths of time at 60°C. It was found that from 60 to 80°C almost constant results were obtained (Fig. 5b).

The dye was found to be stable for ~30 hours.

Beer's law, molar absorptivity and Sandell's sensitivity:

The colour system was found to obey Beer's law in the range of 1 to 8 μg/50 ml (0.02 to 0.16 ppm) of the sample (Fig. 6). Their molar absorptivity and Sandell's sensitivity (40) were found to be 3.30x10^4 l.mol^-1 cm^-1 and 0.001 μg cm^-2 respectively.
FIG. 4. EFFECT OF ACIDITY ON COUPLING REACTION
CONCENTRATION OF NH$_2$OH = 5µg/50ml.

FIG. 5. EFFECT OF (a) TEMPERATURE AND (b) TIME ON COUPLING REACTION.
CONCENTRATION OF NH$_2$OH = 5µg/50ml.
FIG. 6. CALIBRATION CURVE FOR THE DETERMINATION OF HYDOXYLAMINE.
Reproducibility

The reproducibility of the method was studied by replicate analyses of a solution containing 4 μg hydroxylamine over a period of 7 days. The standard deviation and relative standard deviation computed from the data in Table I were found to be ± 0.05 and 1.4% respectively.

Effect of diverse species

A specific study was carried out to see the effects of diverse species commonly present with hydroxylamine. 4 μg of hydroxylamine was determined in presence of known amount of foreign species. The tolerance limits of these species are shown in Table II. Nitrite interferes with the method, which however can be removed from the sample by adding 1 ml of 3% sulphamic acid before iodine oxidation.

Effect of solvent extraction

Solvent extraction using different solvents were employed to enhance the sensitivity of the reaction. Among the various solvents tried, chloroform was found to be the most suitable solvent. The dye was found to be unstable in iso-amyl alcohol and complete extraction of the dye did not take place in benzene, n-hexanol, n-octanol. Extraction in chloroform enabled to determine submicrograms of hydroxylamine in large volume of sample.
### TABLE - I

**REPRODUCIBILITY OF THE METHOD**

Concentration of hydroxylamine = 0.08 ppm.

<table>
<thead>
<tr>
<th>Days</th>
<th>Absorbance $\lambda_{max} = 540$ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.395</td>
</tr>
<tr>
<td>2</td>
<td>0.405</td>
</tr>
<tr>
<td>3</td>
<td>0.400</td>
</tr>
<tr>
<td>4</td>
<td>0.410</td>
</tr>
<tr>
<td>5</td>
<td>0.395</td>
</tr>
<tr>
<td>6</td>
<td>0.400</td>
</tr>
<tr>
<td>7</td>
<td>0.405</td>
</tr>
</tbody>
</table>

Mean = 0.40

Standard deviation = ±0.57

Relative standard deviation = 1.4%

### TABLE - II

**EFFECT OF DIVERSE SPECIES**

Concentration of hydroxylamine = 0.08 ppm

<table>
<thead>
<tr>
<th>Diverse species (Tolerance limit in ppm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline (100), Hydrazine (1000), Phenyl hydrazine (1000), Formaldehyde (2000), Benzaldehyde (2000), Phenol (2000), Acetaldehyde (2000), Ethanol (2000), 2,4-Dinitrophenyl hydrazine (1500), Toluene (2500), Benzene (2500), Cu$^{2+}$ (800), Ca$^{2+}$ (800), Mg$^{2+}$ (600), NH$_3$ (600), Fe$^{3+}$ (900), PO$_4^{3-}$ (2000), Zn$^{2+}$ (2000), Cd$^{2+}$ (2000), Pb$^{2+}$ (2000).</td>
</tr>
</tbody>
</table>

* Amount causing an error within ±2%. 

* Amount causing an error within ±2%. 

FIG. 7. EFFECT OF pH ON OXIDATION.

FIG. 8. CALIBRATION CURVE FOR THE DETERMINATION OF BENZOHYDROXAMIC ACID.
**Beer's law, molar absorptivity, Sandell's sensitivity and reproducibility**

The colour system was found to obey Beer's law in the range of 10 to 80 µg per 50 ml of the sample solution (Fig. 8). The molar absorptivity and Sandell's sensitivity were found to be 16.3 l.mol$^{-1}$cm$^{-1}$ and 0.0025 µg cm$^{-2}$ respectively. The reproducibility of the method was checked by replicate analyses of the solution containing 40 µg benzohydroxamic acid over a period of seven days. The standard deviation and relative standard deviation were found to be ± 0.007 and 1.7% respectively.

**Comparison with other methods**

The present method can be favourably compared with other methods (Table III) and has been found to be more sensitive than the various reported methods.

**CONCLUSION**

The proposed method can be successfully employed for the estimation of hydroxylamine and simple hydroxamic acids. The method is simple, reasonably sensitive and free from common interferences.
### Table III

**Comparison with Other Colorimetric Reagents**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Reagent for hydroxylamine</th>
<th>Limit of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Triphenyl tetrazolium chloride ( a )</td>
<td>25 ( \mu )g/ml</td>
</tr>
<tr>
<td>2.</td>
<td>1(-Naphthyl)Ethylene diamine dihydrochloride ( b )</td>
<td>1-8 ( \mu )g/25 ml</td>
</tr>
<tr>
<td>3.</td>
<td>Ferrozene ( c )</td>
<td>1-10 ( \mu )g/25 ml</td>
</tr>
<tr>
<td>4.</td>
<td>8-Quinolinol ( d )</td>
<td>0.2 to 100 ( \mu )g</td>
</tr>
<tr>
<td>5.</td>
<td>Tetra cyanophenanthroline ferrate ( e )</td>
<td>3 to 30 ( \mu )g/100 ml</td>
</tr>
<tr>
<td>6.</td>
<td>o-Dianisidine ( f )</td>
<td>0.1 to 4 ( \mu )g/ml</td>
</tr>
<tr>
<td>7.</td>
<td>Diphenyl amine ( g )</td>
<td>1-8 ( \mu )g/50 ml</td>
</tr>
</tbody>
</table>

\( a \) Ref. 12, \( b \) Ref. 14, \( c \) Ref. 15, \( d \) Ref. 19, \( e \) Ref. 23, \( f \) Ref. 24

\( g \) Present study (extractive).


