Chapter - V

A Facile Spectrophotometric Method for the Determination of Hydrazine with Pyrogallol Red-Iodate System
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6.1: SUMMARY

A new, simple, sensitive and selective spectrophotometric method was developed for the determination of hydrazine at microgram level. The method was based on inhibitory effect of hydrazine on the reaction of Pyrogallol red with potassium iodate in presence of hydrochloric acid in the solution. The absorbance of the Pyrogallol red after the reaction was measured Spectrophotometrically at 475 nm. Actually as a result of the reaction the absorbance decreased, depending on the concentration of hydrazine. Beer's law was obeyed over the concentration range of 1.2–12 µg hydrazine per 25 ml of final solution. The molar absorptivity and Sandell's sensitivity were found to be $6.2 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ and 0.0036 µg cm$^{-2}$, respectively. The variables including reagents concentration, time, temperature and effect of foreign species, were studied in order to optimize the reaction conditions. The developed method was successfully applied for the determination of hydrazine in water and biological samples.

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5.2: INTRODUCTION

Hydrazine and its derivatives are relatively strong reducing agents and react readily with most oxidizing agents. These are highly toxic compounds with a variety of biological effects (1). The first large-scale use of hydrazine was as a rocket fuel by the German during World War II (2). The reducing property of hydrazine is utilized for the prevention of corrosion in boilers by scavenging oxygen from water (3). Other commercial uses are, in metal plating, solder fluxes, antioxidants, as an intermediate in the manufacture of soap additives, pharmaceuticals, photographic development, blowing agents for plastics etc (3-6). A potentially large-scale application of hydrazine is in fuel cells for electric power generation.

Hydrazine is absorbed through skin contact and by inhalation. The exposure to hydrazine occurs during its production and use as herbicides, tuberculostatic agent, given therapeutically and prophylactically (7). Hydrazine is suspected of causing human cancer in the lungs, nervous system, liver, kidney, hematopoietic organs, breast and subcutaneous tissues (8,9). After subcutaneous absorption it causes mucous membrane irritation, convulsions, anorexia, weight loss, weakness, vomiting and can also produce dermatitis (10). Inhalation results in eye and pulmonary tract irritation with lung congestion, bronchitis and pulmonary edema. Liver damage is an important feature of hydrazine toxicity, after chronic exposure (5, 11). A portion of hydrazine is excreted in urine, after its intravenous or subcutaneous administration (12). Due to the extensive use and high toxicity the threshold limit value for hydrazine has been reduced to 0.1 ppm from 1.0 ppm for 8 h time weight average TWA (5,12,13). Thus, because of its toxicological effects and extensive use as industrial chemical and rocket fuel, it is very important to develop sensitive method for the determination of micro quantities of hydrazine in environmental samples.

Several methods are described in the literature for the determination of hydrazine, using different analytical techniques such as: Spectrofluorimetry (14-16), Voltametry (17-19), Coulometry (20), Amperometry (21,22), Titrimetry (23,24), Ion Selective Electrode (25,26), Chemiluminescenceometry (27-29), Gas Chromatography (30,31), Flow Injection Spectrophotometry (32,33) and High Performance Liquid Chromatography (34,35).
However, most of the methods lack sufficient sensitivity, require complicated and expensive instruments, demand carefully controlled conditions, required trained personals and are not applicable for the rapid detection and determination of the hydrazine at the sample source.

Spectrophotometry is a simple and widely used technique for the quick determination of analyte, even at the sample source in the remote areas. Most of the Spectrophotometric methods are based on formation of aldazine by the condensation of aromatic aldehyde with hydrazine. Some of the reagents, like; 2-Hydroxy-1-Naphthaldehyde (36), Cinnamaldehyde (37), Benzaldehyde (38), Salicylaldehyde (39-41), p-Dimethylaminobenzaldehyde (42,43), Pentfluorobenzaldehyde (44), 5-Nitro-2-hydroxybenzaldehyde (45), Vanillin (46,47), Veratraldehyde (48) II-Point Standard Addition Method (49) etc have been reported. Various other reagents, such as; Copper (II) neopropine (2,9-dimethyl-1,10-Phenothrofin) (50), Silver gelatin complex (51), 2, 3, 5-Triphenyltetrazolium chloride (52), Fe (III) in the presence of Ferrozine (53), 2,2'-Dipyridyl (54), Perinaphthalene 2,3,4-Trione hydrate (56), Phosphomolybdic acid (57), Fe (III) 1,10-Phenothrofin (58), 2,3-Dichloro-5,6-dicyano p-benzoquinone (59), Neutral red (60), N-(1-naphthyl) Ethylenediamine, NEDA (61) etc were also used for the Spectrophotometric determination of hydrazine. Although, they were sensitive, but involved poor selectivity, reproducibility, less stability of colour system, interferences of various foreign species. Therefore, a simple, sensitive and selective and reliable method for the determination of hydrazine was felt to be essential.

The flow injection method based on inhibitory effect of hydrazine on the reaction of Pyrogallol red with potassium iodate, in presence of hydrochloric acid had however, been reported earlier (62) but, this method required expensive instrument, demanded carefully controlled conditions and was less sensitive. In the present work a Spectrophotometric method has been developed using the same reaction based on measuring the change in absorbance of Pyrogallol red dye at 475 nm. Beer’s law was obeyed over the concentration range of 1.2 –12 μg hydrazine per 25 ml of final solution, with molar absorptivity and Sandell’s sensitivity of 6.2 X 10^4 and 0.0036 μg cm^-2, respectively. The method was simple, rapid, selective, reliable and more sensitive than FIA method for the determination of hydrazine in water and biological samples.
5.3: EXPERIMENTAL

5.3.a: Apparatus:
Spectral measurements were made with Systronics 104 digital Spectrophotometer with 1cm quartz cuvettes. Calibrated glasswares were used throughout the experiments.

5.3.b: Reagents:
All the chemicals used were of AnalR grade or the best quality available. Double distilled deionised water was used throughout the experimental studies.

5.3.b-I: Hydrazine Solution: 1000 μg/ml solution was prepared daily by dissolving 0.4143 g of hydrazine sulphate (BDH) in water and making the volume to 100 ml. A working solution of lower concentration was prepared by appropriate dilution of this solution with water. All the solutions were kept in amber coloured bottles in dark.

5.3.b-II: Potassium Iodate (Merck) Solution: 0.1% aqueous solution was prepared.

5.3.b-III: Hydrochloric Acid Solution: 0.5 M aqueous solution was used

5.3.b-IV: Pyrogallol Red Solution (PGR): 0.02% solution was prepared by dissolving 0.02g PGR (Merck) in ethanol / water (1:1) in 100 ml volumetric flask.

5.3.c: Procedure:
Aliquots of working standard solutions containing 1.2 - 12 μg hydrazine were transferred into a series of 25 ml calibrated tubes. Then, 1 ml of 0.1% potassium iodate solution was added to each of them, followed by the addition of 1 ml of 0.5 M hydrochloric acid. To them 1 ml of PGR solution was added and shaken for 2 min, and contents of the tubes were diluted up to the mark and allowed to stand for 5 -7 min, at 25° -30°C, for completion of the reaction. The absorbance of the PGR dye was measured at 475 nm, against the reagent blank, which was prepared in the similar manner.

5.4: RESULTS AND DISCUSSION

Pyrogallol red is a dye that can be oxidized by hydrogen peroxide (63), potassium bromate (64) and potassium iodate. The reaction between PGR and trace amounts of iodate in acidic medium was fast and gave product with maximum absorbance at 475 nm (Fig 5.1). On the other hand, when trace amount of hydrazine was added to the reaction mixture, the
reaction rate decreased considerably. This was due to the fast reaction of hydrazine and potassium iodate (65) in acidic medium, which inhibited the reaction of PGR with iodate. Increasing concentration of hydrazine resulted in further decrease of the rate of the reaction of PGR with iodate, which caused decrease in the absorbance at 475 nm. The absorbance change was measured against the hydrazine concentration.

The influence of the reagents concentration and effect of variables (hydrochloric acid, potassium iodate, Pyrogallol red concentration etc) on the inhibitory effect of hydrazine were studied, to optimize the conditions for the determination.

\[ IO_3^- + N_2H_4 + 2H^+ + Cl^- \rightarrow ICl + N_2 + 3H_2O \]

Pyrogallol red dye
\( \lambda_{max} \) 475 nm

Bleached dye,
5.4.a: Adherence to Beer's Law, Molar Absorptivity and Sandell's Sensitivity:

By using the present method, under optimized conditions, a linear curve between hydrazine concentration and change in absorbance was obtained, over the concentration range of 1.2 - 12 µg hydrazine per 25 ml of final solution (Fig 5.2). The calibration graph had a correlation coefficient of 0.9940. The Molar absorptivity and Sandell's sensitivity were found to be $6.2 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ and $0.0036$ µg cm$^{-2}$, respectively.

5.4.b: Effect of the Reagents Concentration:

To optimize the concentration of potassium iodate, different volumes of 0.1% iodate solution were added to the contents. It was found that 1 ml of iodate was sufficient for constant and maximum difference in absorbance (Fig 5.3).

The influence of PGR concentration was studied by adding different volumes of 0.02% PGR solution to the reaction mixture. It was found that 1 ml PGR solution was sufficient for constant absorbance value (Fig 5.4).

The effect of hydrochloric acid concentration on reaction was studied by using different concentrations of hydrochloric acid. It was found that 1 ml of 0.5 M of HCl was sufficient to ensure constant and maximum difference in absorbance. The sensitivity and reproducibility were markedly affected while using higher concentrations of hydrochloric acid (Fig 5.5).

5.4.c: Effect of Temperature and Time:

The maximum difference in absorbance was found to be independent of temperature. By increasing the temperature the sensitivity increased slightly, whereas temperature higher than 30°C caused the sensitivity to decrease. This was due to the fact that the reaction rate of PGR with iodate increased with temperature to a greater extent, as compared to the inhibitory effect of hydrazine at higher temperature. Therefore, it was more convenient, to carry out the determinations at room temperature. The time required for the final inhibition of the colour reaction was found to be 5 min.

5.4.d: Analytical Data:

The reproducibility of the method was checked by seven replicate measurements for seven days, each containing 6.0 µg hydrazine per 25 ml of final solution. The standard
deviation and relative standard deviation were found to be ± 0.0078 and ± 1.7%, respectively. The lower relative standard deviation value was ± 1.7% and the range of the error at 95% confidence level in terms of absorbance was ± 0.0076, indicating good precision and accuracy of the method.

5.4.c: Effect of Foreign Species:

To assess the validity of the method, the effect of various diverse ions and interfering species on the determination of hydrazine was examined, by adding known amount of these compounds to a solution containing 6.0 μg hydrazine per 25 ml of final solution. The tolerance limits of these compounds (in ppm) are shown in Table 1. It was clear from the results in Table 5.1, that most of the common ions and other major toxicants did not interfere with the determination under optimum conditions.

5.5: APPLICATIONS

The developed method was successfully applied for the determination of hydrazine in water and biological samples. The accuracy of the method was checked by performing recovery test to the various samples by the standard addition method. The recovery values in percentage, obtained were quantitative, close to 100% and in good agreement with the reference method (46), indicating that the method worked satisfactorily.

5.5.a: Determination of Hydrazine in Polluted Water Samples:

Polluted water from the nearby river, which received effluents from steel plants, fertilizer factories etc was filtered through Whatman filter paper No.42 and then tested for hydrazine. This water was found to be free from hydrazine; hence synthetic samples were prepared by adding known volumes of standard hydrazine solution and analyzed by the method described above, Table 5.2.

Tap water and tube well water samples were brought to the laboratory in pre-washed polyethylene bottles from various sites, spiked with known amount of hydrazine and then these samples were analyzed by the general procedure, Table 5.2.

5.5.b: Determination of Hydrazine in Biological Samples:

The urine and blood serum samples were found to be free from hydrazine and so, synthetic samples were prepared by the addition of known amounts of hydrazine. The
samples were deprotenised by adding trichloroacetic acid (66) and were analyzed for hydrazine content by the method described above, Table 5.2.

In all of the above cases (Table 5.2), parallel determinations were carried out by making use of reference method (46) and results of various analyses showed no significant difference in accuracy and precision of the present and reference method. The recoveries were close to 100%, which indicated that there was no serious interference during the estimation of hydrazine in the above types of samples.

5.6: CONCLUSION

The method developed for determining hydrazine Spectrophotometrically was simple, rapid, selective, and offered the advantage of sensitivity in performing wide range of determinations, without the need for extraction or heating. The method did not involve any stringent reaction conditions. This method was a good alternative to some of the reported costly instrumental methods and its advantage was mainly due to higher stability of the colour of Pyrogallol red dye. The developed method was successfully applied to the determination of hydrazine in water and biological samples. The results summarized in Table 5.2 and 5.3 clearly showed that the present method worked satisfactorily and was superior to the other reported Spectrophotometric methods.
Fig. 5.1: Absorption Spectra of the Dye

- ▲ Reagent Blank
- ■ Concentration of Hydrazine, 6.0 µg per 25 ml

Fig. 5.2: Calibration Curve for the Determination of Hydrazine
Fig. 5.3: Effect of the Concentration of KIO₃ Solution

Fig. 5.4: Effect of the Concentration of PGR Solution

Fig. 5.5: Effect of the Acidity
Table 5.1: Effect of Foreign Species (6.0 μg of Hydrazine per 25 ml).

<table>
<thead>
<tr>
<th>Foreign Species</th>
<th>Tolerance limit ppm*</th>
<th>Foreign Species</th>
<th>Tolerance limit ppm*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺, K⁺, Br⁻, NO₁⁻, B₄O₇²⁻, PO₄³⁻</td>
<td>1000</td>
<td>Lactose, Sucrose, Glycol</td>
<td>1000</td>
</tr>
<tr>
<td>Mg²⁺, Al³⁺, Ni²⁺, NH₄⁺</td>
<td>850</td>
<td>Glycerol, Fructose, Glucose</td>
<td>800</td>
</tr>
<tr>
<td>Ca²⁺, Sr²⁺</td>
<td>500</td>
<td>Acetate, Citric Acid, Oxalic Acid</td>
<td>500</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>400</td>
<td>Uric Acid</td>
<td>300</td>
</tr>
<tr>
<td>Mn²⁺, Fe²⁺</td>
<td>300</td>
<td>Hydroxylamine, Urea</td>
<td>200</td>
</tr>
<tr>
<td>Pb²⁺, Ba²⁺, Ag⁺</td>
<td>100</td>
<td>Phenylhydrazine, Semicarbazide</td>
<td>100</td>
</tr>
<tr>
<td>Sb³⁺, SCN⁻, NO₂⁻, S₂O₃²⁻</td>
<td>50</td>
<td>Ascorbic acid</td>
<td>50</td>
</tr>
</tbody>
</table>

*Tolerance limit is the amount of foreign species, which causes an error of ± 2% in absorbance value.
### Table 5.2: Determination of Hydrazine in Water and Biological Samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Hydrazine Added (µg)</th>
<th>Present Method µg</th>
<th>% RSD</th>
<th>% Recovery</th>
<th>Reference Method (47) µg</th>
<th>% RSD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polluted a water</td>
<td>4.0</td>
<td>4.03 ± 0.030</td>
<td>0.74</td>
<td>100.75</td>
<td>3.98 ± 0.040</td>
<td>1.00</td>
<td>99.50</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.99 ± 0.040</td>
<td>0.50</td>
<td>99.86</td>
<td>7.93 ± 0.060</td>
<td>0.75</td>
<td>99.12</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.09 ± 0.050</td>
<td>0.49</td>
<td>100.90</td>
<td>10.01 ± 0.060</td>
<td>0.60</td>
<td>100.10</td>
</tr>
<tr>
<td>Tap a water</td>
<td>4.0</td>
<td>3.96 ± 0.060</td>
<td>1.50</td>
<td>99.00</td>
<td>3.94 ± 0.050</td>
<td>1.26</td>
<td>98.50</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.90 ± 0.090</td>
<td>1.13</td>
<td>98.75</td>
<td>7.89 ± 0.080</td>
<td>1.00</td>
<td>98.62</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.82 ± 0.080</td>
<td>0.81</td>
<td>98.20</td>
<td>9.80 ± 0.100</td>
<td>1.00</td>
<td>98.10</td>
</tr>
<tr>
<td>Tube a well water</td>
<td>5.0</td>
<td>4.96 ± 0.050</td>
<td>1.00</td>
<td>99.20</td>
<td>4.92 ± 0.030</td>
<td>0.60</td>
<td>98.40</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>6.92 ± 0.049</td>
<td>0.70</td>
<td>98.85</td>
<td>6.89 ± 0.051</td>
<td>0.74</td>
<td>98.42</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>8.87 ± 0.060</td>
<td>0.67</td>
<td>98.55</td>
<td>8.83 ± 0.090</td>
<td>1.00</td>
<td>98.11</td>
</tr>
<tr>
<td>Blood b serum</td>
<td>4.0</td>
<td>3.93 ± 0.020</td>
<td>0.5</td>
<td>98.25</td>
<td>3.90 ± 0.070</td>
<td>1.79</td>
<td>97.50</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>5.89 ± 0.060</td>
<td>1.0</td>
<td>98.16</td>
<td>5.86 ± 0.058</td>
<td>1.40</td>
<td>97.66</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.86 ± 0.100</td>
<td>1.2</td>
<td>98.25</td>
<td>7.85 ± 0.049</td>
<td>0.62</td>
<td>98.12</td>
</tr>
<tr>
<td>Urine b</td>
<td>5.0</td>
<td>4.95 ± 0.068</td>
<td>0.97</td>
<td>99.00</td>
<td>4.91 ± 0.052</td>
<td>1.00</td>
<td>98.20</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>6.90 ± 0.080</td>
<td>1.15</td>
<td>98.57</td>
<td>6.89 ± 0.090</td>
<td>1.32</td>
<td>98.42</td>
</tr>
<tr>
<td></td>
<td>9.0</td>
<td>8.83 ± 0.098</td>
<td>1.00</td>
<td>98.11</td>
<td>8.81 ± 0.110</td>
<td>1.24</td>
<td>97.88</td>
</tr>
</tbody>
</table>

*Mean ± Standard deviation for five replicate analyses.

RSD, Relative standard deviation.

a, 5 ml, b, 2 ml, after treatment as described in procedure section.
### Table 5.3: Comparison of the Present Method with Other Reported Spectrophotometric Methods.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Beer's law Range ppm</th>
<th>$\lambda_{\text{max}}$ nm</th>
<th>Sensitivity $\epsilon$ 1 mol$^{-1}$ cm$^{-1}$</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylaldehyde (39)</td>
<td>0.290-1.25</td>
<td>360</td>
<td>2.38 X 10$^4$</td>
<td>Less sensitive and selective, absorption in UV region.</td>
</tr>
<tr>
<td>2-Hydroxy-1-Naphthaldehyde(36)</td>
<td>0.035-0.70</td>
<td>412</td>
<td>2.70 X 10$^4$</td>
<td>Reagent unstable, reaction at 100°C and extractive.</td>
</tr>
<tr>
<td>Vanillin (46)</td>
<td>0.065-0.50</td>
<td>400</td>
<td>5.25 X 10$^4$</td>
<td>Less sensitive, many hydrazine derivatives interfere.</td>
</tr>
<tr>
<td>N-(1-naphthyl)Ethylene diamine(61)</td>
<td>0.020-0.40</td>
<td>540</td>
<td></td>
<td>Sensitive but method is indirect and complicated.</td>
</tr>
<tr>
<td>Pyrogallol red + Iodate (Present method)</td>
<td>0.048-0.48</td>
<td>475</td>
<td>6.20 X 10$^4$</td>
<td>Simple, rapid sensitive and selective.</td>
</tr>
</tbody>
</table>
5.7: REFERENCES


13. ACGIH, "Threshold Limit Values for Chemical Substances in Work Room Air" Adopted by ACGIH, Cincinnati, Ohio, USA, 1977.


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