CHAPTER TWO

Modified Fujiwara Reaction
For the Determination of Trichloroacetic Acid
Modified Fujiwara Reaction For the Determination of Trichloroacetic Acid

SUMMARY

A new sensitive spectrophotometric method for the determination of trichloroacetic acid (TCA) at the ppm level is described. The method involves the modification of the Fujiwara reaction i.e. discharging the colour with acetic acid followed by the addition of sulphanilic-formic acid reagent which results in the formation of an orange-yellow coloured polymethine dye having an absorption maxima at 505 nm. The sensitivity after this modification is found to be about five times more than that of the conventional Fujiwara procedure. "Beers' law is obeyed in the range of 10 to 100 μg (1-10 ppm) of trichloroacetic acid per 10 ml of the solution. The method is found to be free from the interference of various organic compounds including carbon tetrachloride, benzene and chloroform under the conditions employed. The method has been successfully applied for the determination of trichloroacetic acid in air, serum and urine samples.

INTRODUCTION

Trichloroacetic acid (TCA) is used in various syntheses and as a reagent for albumin. It is also widely used as a protein precipitant. It is given off during chlorination of acetic acid in the presence of a catalyst. Trichloroacetic acid has medicinal and herbicidal uses. The sodium salt of TCA is used primarily as a grass killer. It has been proved useful as a nonselective treatment on perennial weedy grasses such as Johnson grass, Bermuda grass and quack grass. TCA is also used as a selective pre emergence treatment in sugar beets to control annual grasses. In sugarcane on pre emergence or early post emergence treatment controls seedling grasses including Johnson grass seedling. When applied to foliage TCA causes rapid necrosis by contact action. It inhibits the growth of both shoots and roots and causes leaf chlorosis and formative effects especially to the shoot apex. Sodium salt of TCA controlled weeds and increased the yield of fodder beets, semisugar beets and sugar beets (1, 2).

Oral intake of TCA causes narcosis and severe damage to human tissue by direct contact and is a strong acid. Its application to the cornea causes severe coagulation immediately. Higher amounts of TCA (20 ppm) in urine indicates possible exposure to trichloroethylene. Human erythrocytes and frog muscle sarcolemma on exposure to TCA causes maximum thermolysis to blood cells and maximum damage to sarcolemma. It is also reported to decrease number of protein released by erythrocytes (1, 3, 4).

The LD_{50} value of TCA in rats is reported to be 3.3 g/kg. TCA is extremely corrosive to skin and its sodium salt is a skin and eye irritant (5). The importance of TCA as a widely used herbicide has led to the development of various instrumental methods like gas-liquid chromatography using sophisticated detectors (6), differential pulse polarography (7), head space gas chromatography (8), anion selective membrane electrode (9), gas chromatography (10, 11), ion chromatography (12) etc.

The most popular spectrophotometric methods used for determination of TCA are based on the Fujiwara reaction (13, 14, 20), which involves the measurement of a red colour developed by the reaction between polyhalogenated compound and pyridine in the presence of alkali. The reaction has several critical parameters such as temperature, concentration and time of heating (15, 16).

In the present method a modified Fujiwara reaction has been developed for the detection of TCA. The method is based on the reaction of TCA with pyridine and alkali which on mild heating forms a red coloured chromophore. The red colour is
discharged by addition of a few drops of acetic acid, and sulphanilic-formic reagent is then added to obtain a yellow-orange coloured chromophore with a maximum absorbance at 505nm. This modification increases the sensitivity of the Fujiwara reaction about five times.

Benzenidine-formic acid reagent reported earlier for such modification uses carcinogenic benzidine (17) and hence its use is not recommended. Sulphanilic acid used here is non-toxic and has earlier been reported for the determination of cyanide (18) and acrylonitrile (19). The method has been successfully applied for the determination of TCA in air and biological samples.

EXPERIMENTAL

APPARATUS:
A Carl Zeiss spectrophotometer with 1cm matched silica cells was used for all spectral measurements.

REAGENTS:

**Trichloro acetic acid:**

Standard solution of Trichloro acetic acid :Dow chemical company.

A 1mg/ml stock solution of pure distilled TCA was prepared in distilled water. The working standard was prepared by appropriate dilution of the stock solution.

**Sulphanilic acid - Formic acid reagent:**

A 1% solution of sulphanilic acid in 50% formic acid was used.

**Sodium hydroxide :** A 5M aqueous solution was used.

**Hydrochloric acid:** A 6M solution was used.

**Pyridine :** (Qualigen make)

**Acetic acid (glacial):**

All chemicals used were of B.D.H. or Glaxo or Qualigen A.R grade. All solutions were prepared in double distilled water.
PROCEDURE:

Aliquots containing 10-100 µg (1-10ppm) of TCA were transferred to a 10ml volumetric flask. To this 1ml of pyridine reagent and 3ml of 5M sodium hydroxide were added and thoroughly shaken. The contents were kept in water at 70°C for 3 minutes and shaken from time to time. The red coloured solution so obtained was cooled in ice cold water and then decolourised with 2ml of glacial acetic acid. To this solution 2ml of sulphanilic-formic acid reagent and 1ml of 6 M hydrochloric acid were added and the contents were allowed to stand for 10 minutes. The volume was brought upto 10ml with distilled water and absorbance of the orange-yellow coloured polymethine dye having an absorbance maxima at 505 nm was measured against a colourless reagent blank.

RESULT AND DISCUSSION:

All spectral studies were made at 505 nm with 10ml of final volume containing various amounts of trichloro acetic acid. All spectral measurements were carried out against demineralised water since the reagent blank showed negligible absorbance at this wave length (Fig. 2.1)

Colour reaction:

The reaction takes place in three steps. In the first step TCA reacts with pyridine in alkaline medium to form Schiff's base of glutaric aldehyde I. In the second step by the addition of glacial acetic acid, the red color of Schiff base of glutaric aldehyde I is converted to yellow coloured glutaric aldehyde II, which forms an orange-yellow coloured polymethine dye III with Sulphanilic-formic acid reagents at this wave length (Fig. 2.1)

Effects of varying reaction conditions:

For studying the reaction of trichloro acetic acid with pyridine to form the red coloured dye, the effect of concentration of pyridine and sodium hydroxide have been checked. It was found that 1ml of pyridine and 3ml of 5 M sodium hydroxide were sufficient for full colour development. Excess of sodium hydroxide made the solution slightly cloudy due to undissolved sodium hydroxide (Fig.2.2 & 2.3).

Effect of heating time on the development of red coloured dye was also checked. It was observed that heating the reaction mixture for about 3 minutes at 70°C gave
SCHEME - 1
COLOR REACTION

Trichloroacetic acid + Pyridine

$\text{Cl}-\text{C-C-Cl}$

Pyridine

$\text{Cl}^-$

$\text{Cl}^-$

$\text{NaOH}$

$\text{CH}_2\text{Cl}$

$I$

Glutaric Aldehyde

$\text{CH}_2\text{COOH}$

$\text{HOH}$

Sulphanic acid

$\text{HO}_3\text{S}$

Polymethine dye

$\text{HO}_3\text{S}$

$\text{NH}_2$
optimal absorbance values. Lower absorbance values were obtained on prolonged heating. The orange-yellow coloured dye was found to be stable for 10 minutes and there after showed gradual decrease in intensity with time [Fig 2.4 and 2.5].

For decolourisation of the red coloured dye 2 ml of glacial acetic acid were sufficient. An excess amount however does not affect the reaction conditions [Fig 2.6].

Effect of sulphanilic - formic acid reagent concentration was checked. A minimum of two millilitres of sulphanilic acid in 50% formic acid was required for maximum colour intensity. An excess amount of sulphanilic formic acid reagent decreased the colour intensity of the dye. [Fig 2.7].

**Beer’s Law:**

Molar absorptivity and Sandell’s Sensitivity: The colour system was found to obey Beer’s law in the range of 1-10 ppm (10-100 mg of TCA per 10ml) of solution [Fig 2.8]. Molar absorptivity and Sandell’s sensitivity were found to be $1.63 \times 10^3$ litre/mol/cm ($\pm 100$) and 0.01 mg/cm$^2$ respectively.

**Reproducibility of the method:**

The reproducibility of the method was checked by seven replicate analyses of a solution containing 50µg of TCA per 10ml of solution for a period of seven days. The standard deviation and relative standard deviation were found to be ± 0.017 and 3.36% respectively. [Table-1].

**Effect of foreign species:**

Effect of various copollutants, pesticides and polyhalogenated compounds on the reaction was studied by adding known amounts of these compounds to the solution containing 50µg of trichloro acetic acid. Then TCA was analysed as described above. The method was found to be free from the interference of various common organic solvents such as benzene, alcohol, ether, chloroform and carbon tetrachloride [Table-2].

**APPLICATION OF THE METHOD:**

The method has been employed for the determination of TCA in air, urine and blood serum.
Determination of TCA

1. In Air:
   A modification of Wilson’s procedure [21,22] was used for determining amount of trichloro acetic acid in air. Since standard air samples of TCA were not available, known concentration of TCA vapours were obtained by dropping a dilute solution of TCA from a microburette in a preheated closed chamber [Fig 1.] connected to an air sampling train.

   Air containing TCA vapour were drawn at the rate of 0.250 litre/minute for about 15 minutes through two midget impingers connected in series and containing 10ml of pyridine forming a part of the sampling train. Pyridine was used as absorbing solution for TCA vapours. After sampling, aliquots of this solution were analysed by the recommended procedure. The recoveries were found to be 98% [Table-3,4].

2. In Blood Serum:
   To check the recovery of TCA from blood serum a known amount of TCA solution was added to TCA free samples of blood serum. The mixture was allowed to stand for sometime, filtered and then analysed by the recommended procedure. The recovery was found to be 96% [Table-5].

3. In Urine Samples:
   To determine the amount of TCA in urine samples, known amounts of TCA solution was added to TCA free urine sample. The solution was allowed to stand for sometime, filtered and then the filtrate was analysed by the above recommended procedure. The results are tabulated in Table-5. The recovery was found to be 96%.

CONCLUSION:

   The method is found to be about five times more sensitive as compared to the method employed for normal Fujiwara reaction. The method is also found to be free from interference of various other organic co-pollutants like carbon tetrachloride and chloroform under the employed conditions and can be applied for industrial hygienic work.
FIG. 1 ASSEMBLY TRAIN FOR SAMPLING OF AIR POLLUTANTS.
FIG. 2: ABOSPTION SPECTRA OF THE DYE.
A. CONCENTRATION OF TRICHLOROACETIC ACID = 70μg/10ml.
B. CONCENTRATION OF TRICHLOROACETIC ACID = 40μg/10ml.
C. REAGENT BLANK.
FIG. 2. EFFECT OF AMOUNT OF PYRIDINE ON COLOUR DEVELOPMENT
CONCENTRATION OF TRICHLOROACETIC ACID = 25µg/10 ml.

FIG. 3. EFFECT OF AMOUNT OF NaOH ON COLOUR DEVELOPMENT
CONCENTRATION OF TRICHLOROACETIC ACID = 40µg/10 ml.
FIG. 4. EFFECT OF HEATING TIME ON FINAL ABSORBANCE

CONCENTRATION OF TRICHLOROACETIC ACID = 40µg/100ml.

FIG. 5. EFFECT OF TIME ON ABSORBANCE VALUES.

CONCENTRATION OF TCA = 40µg/10ml.
AMOUNT OF GLACIAL ACETIC ACID

FIG. 6 EFFECT OF AMOUNT OF GLACIAL ACETIC ACID ON
FINAL ABSORBANCE OF THE DYE.
CONCENTRATION OF TCA = 40μg/10ml.

AMOUNT OF SULPHANILIC FORMIC ACID REAGENT, ml.

FIG. 7 EFFECT OF AMOUNT OF SULPHANILIC-FORMIC ACID
REAGENT ON FINAL ABSORBANCE.
CONCENTRATION OF TRICHLOROACETIC ACID = 40μg/100ml.
FIG. 8 CALIBRATION FOR THE DETERMINATION OF TRICHLOROACETIC ACID.
Table -1
Reproducibility of the method
Concentration of trichloroacetic acid = 50 \( \mu \text{g} \)/10ml (5 ppm)

<table>
<thead>
<tr>
<th>No. of days</th>
<th>Absorbance max * 505 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.530</td>
</tr>
<tr>
<td>2.</td>
<td>0.500</td>
</tr>
<tr>
<td>3.</td>
<td>0.485</td>
</tr>
<tr>
<td>4.</td>
<td>0.495</td>
</tr>
<tr>
<td>5.</td>
<td>0.525</td>
</tr>
<tr>
<td>6.</td>
<td>0.510</td>
</tr>
<tr>
<td>7.</td>
<td>0.490</td>
</tr>
</tbody>
</table>

Mean = 0.505  
Standard deviation = \((\pm) 0.017\)  
Relative standard deviation = 3.36%

*Mean of three repetitive analyses.

Table -2
Effect of Foreign Species:
Concentration of Trichloroacetic acid = 50 \( \mu \text{g} \)/10ml (5 ppm)

<table>
<thead>
<tr>
<th>Foreign Species</th>
<th>Tolerance limit * (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl alcohol, Methyl alcohol</td>
<td>700</td>
</tr>
<tr>
<td>Benzene, ether</td>
<td>500</td>
</tr>
<tr>
<td>Carbon tetra chloride</td>
<td>150</td>
</tr>
<tr>
<td>Chloroform</td>
<td>120</td>
</tr>
<tr>
<td>DDT</td>
<td>1200</td>
</tr>
<tr>
<td>Carbaryl, Propoxur</td>
<td>500</td>
</tr>
<tr>
<td>2, 4-D and 2,4,5-T</td>
<td>400</td>
</tr>
<tr>
<td>Paraquat, BHC</td>
<td>200</td>
</tr>
<tr>
<td>Malathion</td>
<td>150</td>
</tr>
<tr>
<td>Parathion</td>
<td>100</td>
</tr>
<tr>
<td>Sn(^{2+}), Ca(^{2+}), Ni(^{2+})</td>
<td>500</td>
</tr>
<tr>
<td>Cu(^{2+}), Cd(^{2+})</td>
<td>1000</td>
</tr>
<tr>
<td>Hg(^{2+})</td>
<td>400</td>
</tr>
<tr>
<td>Pb(^{2+})</td>
<td>600</td>
</tr>
<tr>
<td>NO(_2^–)</td>
<td>500</td>
</tr>
<tr>
<td>PO(_4^{3–})</td>
<td>150</td>
</tr>
</tbody>
</table>

* The amount of foreign species causing an error of \( \pm 2\% \) in absorbance values
ANALYSIS OF TRICHLORO ACETIC ACID IN LABORATORY AIR

Table - 3.
Effect of flow rate on absorption efficiency.
Pyridine is taken as the absorbing solution
sampling time = 15 minutes.
Flow rate = (litre/min)

<table>
<thead>
<tr>
<th>Amount of TCA (µg)</th>
<th>Flow rate (litre/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>10</td>
<td>09.3</td>
</tr>
<tr>
<td>20</td>
<td>18.5</td>
</tr>
<tr>
<td>30</td>
<td>28.1</td>
</tr>
</tbody>
</table>

a = amount of TCA found (µg)
b = % absorbed.

Table - 4.
Effect of time on absorption efficiency.
Flow rate = 0.250 litre/min.
absorbing solution taken is pyridine.

<table>
<thead>
<tr>
<th>Amount of TCA (µg)</th>
<th>sampling time (in minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>20</td>
<td>18.8</td>
</tr>
<tr>
<td>30</td>
<td>28.38</td>
</tr>
<tr>
<td>50</td>
<td>47.9</td>
</tr>
</tbody>
</table>

a = amount of TCA found (µg)
b = % absorbed.
Table - 5.
Determination of TCA in Blood serum and urine samples:

<table>
<thead>
<tr>
<th>Sample **</th>
<th>TCA added (µg)</th>
<th>*</th>
<th>Total TCA found (µg)*</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proposed method</td>
<td>Reported method (2o)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Proposed method</td>
</tr>
<tr>
<td><strong>Blood serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>10</td>
<td>9.42</td>
<td>9.5</td>
<td>94.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>18.8</td>
<td>18.5</td>
<td>84.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>27.85</td>
<td>27.0</td>
<td>92.8</td>
</tr>
<tr>
<td>S2</td>
<td>10</td>
<td>9.85</td>
<td>9.82</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>14.25</td>
<td>14.30</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>18.28</td>
<td>18.20</td>
<td>91.4</td>
</tr>
<tr>
<td>S3</td>
<td>10</td>
<td>9.60</td>
<td>9.66</td>
<td>96.0</td>
</tr>
<tr>
<td></td>
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<td>14.5</td>
<td>14.35</td>
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<td>25</td>
<td>23.8</td>
<td>23.0</td>
<td>95.2</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>10</td>
<td>9.55</td>
<td>9.60</td>
<td>95.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>18.48</td>
<td>18.45</td>
<td>92.4</td>
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<tr>
<td></td>
<td>30</td>
<td>28.65</td>
<td>28.50</td>
<td>95.5</td>
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<tr>
<td>S5</td>
<td>15</td>
<td>14.35</td>
<td>14.30</td>
<td>95.66</td>
</tr>
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<td></td>
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<td></td>
<td>30</td>
<td>28.95</td>
<td>28.80</td>
<td>96.5</td>
</tr>
</tbody>
</table>

* Mean of three repetitive analyses.

** Volume of sample taken = 2ml.
REFERENCES: