CHAPTER VII
SPECTROPHOTOMETRIC DETERMINATION OF ENDOSULFAN USING A NEW ABSORBING REAGENT

SUMMARY

The use of succinyl dihydrazide as an absorbing reagent for determination of sulphurdioxide liberated from endosulfan (Thiodon, Benzopin), a widely used sulphur containing organo chloro pesticide, has been investigated. The absorbed sulphurdioxide is then estimated by using p-amino azo benzene and formaldehyde in acidic medium to give a pink coloured dye which has an absorbance maxima at 500 nm. Beer's Law is obeyed in the range of 0.6-4.2 ppm of endosulfan. This method has been applied to river water, soil and vegetable samples to determine endosulfan at low levels. This method is free from the interference of most of the commonly used pesticide and other common species.

Part communicated in Asian Environment
Endosulfan, also called Thiodon or Benzoepin is an organochlorine contact insecticide and miticide containing sulphur and oxygen and is technically called 6,7,8,9,10 hexachloro 1,5,5a,6,9,9a hexahydro 6, 9 methano- 2, 4, 3,- benzodioxathiopin -3- oxide. Two isomers of endosulfan, alpha-endosulfan (MP 108-110°C) and beta-endosulfan (MP 208-210°C) are known (1). The structure of endosulfan is as given below.

Endosulfan is very effective against insects, and mites effecting cauliflower, pigeon pea, rice (stem borer and gall midge), black pepper (Phytophthora palmivora causing foot rot), potato, chickpea, coffee (Hypothenemus lampier, berry borer), maize, spinach, grapes, peach, strawberries etc (2-11).

The toxic symptoms to human beings caused by endosulfan are similar to those caused by BHC such as hyperexcitability, tremor, dypsnea and salivation. It has been proved to be a potential mutagen in mammalian microsome. It reduced protein, carbohydrate and lipid content in liver tissues. Chromosomal fragmentation, chromatid breaks, chromosomal and chromatid gaps, intrachromatid exchanges took place in bone marrow cells of mice after endosulfan intake (12-15).
Fishes thriving on endosulfan poisoned river water suffered from dyscrasia, erythropenia, anemia, lymphocytosis, thrombocytosis, monocystosis and neutropenia which ultimately leads to its death. The neurobehavorial toxicity of endosulfan in rats include learning and memory deficits, deteriorated muscle co-ordination (16-18). Very low concentration of endosulfan caused erosion and disturbance in basement membrane, degenerated gill lamella. Endosulfan has been proved to be tumor promoting agent acting by clonal expansion of initiated cells (19,20).

The insecticide finds its way inside the body by oral ingestion (LD50 for rats is 40-100 mg/kg) and dermal application (LD50 for rats is 35 mg/kg) (21).

In view of its use in the agricultural sector and its toxicity to aquatic being, rats and human beings, a number of methods have been cited in the literature for the determination of endosulfan. Some of these are based on gas chromatography (22,23), thin layer chromatography (24-27), gas liquid chromatography (28-30), high performance liquid chromatography (31,32), liquid chromatography (33), mass spectroscopy (34), spectrophotometry (35-37) etc.

The most commonly used spectrophotometric methods are based on liberation of sulphur dioxide from endosulfan by p-toluene sulphonlic acid and alcoholic potassium hydroxide as reported (38) which is subsequently absorbed in glycerol-alkali solution and later estimated by p-rosaniline (PRA) and
formaldehyde in acidic medium following West and Gaeke's method for sulphur dioxide (39). These methods have many discrepancies such as instability of sulphur dioxide in glycerol alkali solution (35), purity of PRA (40) and formation of unstable complexes (41).

In the proposed method, the sulphur dioxide liberated from endosulfan has been absorbed in a new absorbing reagent, succinyl dihydrazide and then estimated by p-aminoazo benzene and formaldehyde (42) in acidic medium to give a pink orange dye with an absorbance maxima at 500 nm. This method has also been applied to determine endosulfan in polluted water, agricultural field, soil and vegetable samples and the result obtained are found to be satisfactory.

EXPERIMENTAL

APPARATUS: A Carl Zeiss spekol with 1 cm matched glass cell was used for spectral measurements. Fritted midget impingers of 35 ml capacity, a flow rate adjustable calibrated rotameter and a vacuum pump were used for liberation and absorption of sulphur dioxide from endosulfan.

REAGENTS:

Standard Endosulfan solution: (Northern Minerals Limited, India): A 1% solution of endosulfan was prepared in ethanol. A working standard of 52.5 μg/ml was prepared daily by appropriate dilution of the stock.

Acid Reagent: 15.2 g of p-toluene sulphonic acid dissolved in 50 ml of isopropanol and 10 ml of water.
Succinylidihydrazide (SDH): It was prepared by adding hydrazine hydrate (0.1 mole in alcohol) to the alcoholic solution of diethyl succinate (0.5 mole in alcohol) in 2:1 ratio. The white solid was recovered from water. 0.1 M of SDH was used as an absorbing reagent.

p-Amino azo benzene: 0.02% (W/V) solution of crystallised p-amino azo benzene in 25% ethanol.

Formaldehyde: 0.2% (v/v) solution in water prepared from 40% formaldehyde.

Potassium hydroxide: 2% alcoholic potassium hydroxide.

EDTA: 10% solution of disodium salt of EDTA was used as a masking agent.

Hydrochloric acid: 0.16 M solutions of hydrochloric acid as well as concentrated hydrochloric acid.

All chemicals were of the best quality available and demineralized water was used for the preparation of the solution.

PROCEDURE: Preparation of Calibration Curve, Liberation, Absorption of Sulphur Dioxide.

5 ml of acid reagent and 1 ml of alcoholic potassium hydroxide were added to an aliquot of working standard solution containing 15-105 µg of endosulfan taken in an impinger placed in a hot water bath. This impinger was connected to two other impingers of same capacity containing 10 ml SDH and 2 ml EDTA.
and connected to a suction source through a flow rate adjustable rotameter. Temperature of 90°C was required for complete liberation of sulphur dioxide from the solution of endosulfan.

**ANALYSIS**: An aliquot of absorbing solution was transferred to a volumetric flask of 25 ml capacity. 2 ml of p-amino azobenzene was added and acidity was maintained between 0.02 - 0.16 M by addition of hydrochloric acid. 1 ml of formaldehyde solution was then added and acidity of solution was increased to get a stable coloured complex by addition of 1 ml of concentrated hydrochloric acid. The volume was made up to the mark with demineralised water. After the waiting time of 15 minutes, absorbance of pink dye was measured at 500 nm against demineralised water. Actual absorbance of sample solution was calculated by subtracting the absorbance value of the blank from that of sample.

**RESULT AND DISCUSSION**: 

**SPECTRAL CHARACTERISTICS**: The dye has maximum absorbance at 500 nm. The reagent blank also absorbs at the same wavelength (Fig 1). Therefore actual absorbance of sample was calculated by subtracting reagent blank value from the value obtained for the sample.

**EFFECT OF VARIABLES ON COLOUR DEVELOPMENT**: 

**EFFECT OF ACIDITY**: 1 ml of concentrated hydrochloric acid was sufficient for the development of maximum colour. The acidity of the solution was maintained in two steps, the first step
FIG. 1 - ABSORPTION SPECTRA OF THE DYE AND REAGENT BLANK

A - REAGENT BLANK
B - CONCENTRATION OF ENDOSULFAN = 30 µg/25 ml.
FIG. 2 - EFFECT OF ACIDITY ON COLOUR REACTION (AFTER ADDITION OF P-AMINO BENZOIC ACID).

[ENDOSULFAN] = 30 μg/25 ml

CONCENTRATION OF HCl, M

ABSORBANCE
FIG. 3- EFFECT OF ACIDITY ON COLOUR REACTION (AFTER ADDITION OF FORMALDEHYDE).
FIG. 4 - CALIBRATION CURVE FOR DETERMINATION OF ENDOSULFAN.
after the addition of p-amino azo benzene when the acidity was maintained to 0.02 - 0.16 M with hydrochloric acid (Fig 2). In the second step, acidity was maintained by the addition of 1 ml concentrated hydrochloric acid after the addition of formaldehyde (Fig 3).

**EFFECT OF TIME**: About 20-30 minutes were required for the colour development. The pink dye with maximum absorbance at 500 nm was found to be stable for 12-18 hours at 25-30°C.

**REAGENT CONCENTRATION**: For the liberation of sulphurdioxide from endosulfan, 5 ml of p-toluene sulphonie acid and 1 ml of alcoholic potassium hydroxide were sufficient. 2 ml of 0.02% of p-amino azobenzene and 1 ml of 2% formaldehyde were sufficient for maximum colour development.

The temperature required for liberation of sulphurdioxide from endosulfan was found to be 90-100°C.

**BEER'S LAW, MOLAR ABSORPTIVITY, SANDELL'S SENSITIVITY**: Beer's law was obeyed in the range of 15-105 μg of endosulfan in 25 ml of final volume (0.6-4.2 ppm) (Fig.4). The molar absorptivity and Sandell's sensitivity were found to be $33 \times 10^4$ lit/mol/cm ($\pm 100$) and 0.0123 μg/cm² respectively.

**APPLICATION OF THE METHOD TO SOIL, WATER AND VEGETABLE SAMPLES**

Water samples (250 ml) or 100 gm of finely ground soil sample or 100 gm of finely mashed vegetable samples (potato, spinach) were first spiked with known amount of standard endosulfan solution and thoroughly mixed and kept for 30 minutes. These were then extracted with 2 x 50 ml portions of
petroleum ether in a glass bottle. The extract from soil and vegetable samples were decanted and washed thoroughly with 2 x 100 ml portion of water. The extracts were dried over anhydrous sodium sulphate and then made upto the mark by petroleum ether in a 100 ml volumetric flask. Aliquots of the washed extract containing 15-105 µg of endosulfan was evaporated off under suction. The residue was mixed with 5 ml of p-toluene sulphonie acid and sulphur dioxide was liberated, absorbed and measured after colour development as described earlier. Recoveries of samples were found to be satisfactory. The recovery of endosulfan from various samples are given in Table 3.

COMPARISON WITH OTHER SPECTROPHOTOMETRIC METHODS REPORTED:

The proposed method has been compared with other spectrophotometric methods reported for the determination of endosulfan and it has been found to have some definite advantages. Table 4 gives an account of other methods in comparison to the proposed method.

CONCLUSION: By the proposed method, endosulfan can be determined down to 0.6 ppm. The pink dye was also stable.

REACTION MECHANISM: Hydrazine sulphinic acid (II) was formed by the absorption of sulphur dioxide released from endosulfan (I) by the action of p-toluene sulphonie acid and alcoholic potassium hydroxide. The structure is similar to that obtained by the reaction between phenyl hydrazine and sulphur dioxide (42).
Hydrochloric acid added, releases the sulphur dioxide from succinyl dihydrazide which combines in situ with p-amino azo benzene to give a pink coloured dye having amino methane sulphonie acid structure (III)
Table 1

REPRODUCIBILITY OF THE METHOD

<table>
<thead>
<tr>
<th>No. of Days</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.720</td>
</tr>
<tr>
<td>2</td>
<td>0.750</td>
</tr>
<tr>
<td>3</td>
<td>0.730</td>
</tr>
<tr>
<td>4</td>
<td>0.720</td>
</tr>
<tr>
<td>5</td>
<td>0.740</td>
</tr>
<tr>
<td>6</td>
<td>0.725</td>
</tr>
<tr>
<td>7</td>
<td>0.735</td>
</tr>
<tr>
<td>8</td>
<td>0.740</td>
</tr>
</tbody>
</table>

Mean = 0.732

Standard deviation = 0.0123

Relative standard deviation = 1.68%

Table 2

EFFECT OF FOREIGN SPECIES

<table>
<thead>
<tr>
<th>Foreign species</th>
<th>Tolerance Limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td>250</td>
</tr>
<tr>
<td>Malathion, Pb&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>500</td>
</tr>
<tr>
<td>Zn&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>800</td>
</tr>
<tr>
<td>Karathane, Trichloroacetic acid</td>
<td>1,000</td>
</tr>
<tr>
<td>Paraquat, Zineb</td>
<td>2,000</td>
</tr>
<tr>
<td>Pyridine, Formic acid, DHC</td>
<td>4,000</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>7,000</td>
</tr>
<tr>
<td>Cu&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>10,000</td>
</tr>
</tbody>
</table>

* Amount causing an error of ±2% in absorbance.
### Table 3

**RECOVERY OF ENDOSULFAN FROM SPIKED POLLUTED RIVER WATER, SOIL AND VEGETABLE SAMPLES**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of endosulfan added (µg)</th>
<th>Amount of endosulfan found (µg)</th>
<th>Recovery %</th>
<th>Amount found by reported method</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil **</td>
<td>26.25</td>
<td>25.15</td>
<td>95.83</td>
<td>24.80</td>
<td>94.50</td>
</tr>
<tr>
<td></td>
<td>52.50</td>
<td>50.24</td>
<td>95.70</td>
<td>49.87</td>
<td>95.00</td>
</tr>
<tr>
<td>Potato **</td>
<td>26.25</td>
<td>25.32</td>
<td>97.22</td>
<td>25.47</td>
<td>97.05</td>
</tr>
<tr>
<td></td>
<td>52.50</td>
<td>51.40</td>
<td>97.91</td>
<td>51.02</td>
<td>97.22</td>
</tr>
<tr>
<td>Spinach **</td>
<td>26.25</td>
<td>25.59</td>
<td>97.50</td>
<td>25.56</td>
<td>97.40</td>
</tr>
<tr>
<td></td>
<td>52.50</td>
<td>51.09</td>
<td>97.30</td>
<td>51.29</td>
<td>97.70</td>
</tr>
<tr>
<td>Water ***</td>
<td>26.25</td>
<td>25.88</td>
<td>98.60</td>
<td>25.85</td>
<td>98.50</td>
</tr>
<tr>
<td></td>
<td>52.50</td>
<td>52.13</td>
<td>99.30</td>
<td>52.22</td>
<td>99.50</td>
</tr>
</tbody>
</table>

* Mean of 3 replicate analysis  
** Amount of sample=100 gm  
*** Amount of sample=250 ml

### Table 4

**COMPARISON WITH OTHER SPECTROPHOTOMETRIC METHOD FOR ENDOSULFAN**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Method and Reagent</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>Beer's Law Range</th>
<th>Remarks</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Use of glycerol as an absorbing reagent and ( p )-rosaniline used for estimation.</td>
<td>570</td>
<td>5-50 µg</td>
<td>Absorbing solution not stable for SO₂, purity of PRA used is not fixed, unstable colour complex.</td>
<td>38</td>
</tr>
<tr>
<td>2.</td>
<td>Use of MDH as an absorbing medium and estimation by ( p )-amino azo benzene</td>
<td>505</td>
<td>1-6 ppm</td>
<td>Less sensitive</td>
<td>36</td>
</tr>
<tr>
<td>3.</td>
<td>4-(4-nitrobenzyl) pyridine, alkaline medium.</td>
<td>520</td>
<td>2-100 µg, detection limit 2 µg</td>
<td>Less sensitive</td>
<td>38</td>
</tr>
<tr>
<td>4.</td>
<td>Use of SDH/EDTA as absorbing medium and ( p )-amino azo benzene for estimation.</td>
<td>500</td>
<td>0.6-4.2 ppm</td>
<td>Low detection limit and applicable to vegetable samples.</td>
<td>proposed method.</td>
</tr>
</tbody>
</table>
REACTION MECHANISM OF ENDOXAN

(i) Cl

(II)

Endosulfan

(ii) \[
\begin{align*}
\text{CH}_2\text{-CONHNH}_2 + \text{SO}_2 & \rightarrow \text{CH}_2\text{-CONHNHSO}_2\text{H} \\
\text{CH}_2\text{-CONHNH}_2 & \rightarrow \text{CH}_2\text{-CONHNHSO}_2\text{H}
\end{align*}
\]

Hydrazino sulphinic acid

(iii) \[
\begin{align*}
\text{CH}_2\text{-CONHNHSO}_2\text{H} + \text{HCl} & \rightarrow \text{SO}_2 \\
\text{CH}_2\text{-CONHNHSO}_2\text{H} & \rightarrow \text{SO}_2
\end{align*}
\]

(iv) \[
\begin{align*}
\text{p-amino azo benzene} + \text{SO}_2 + \text{HCHO} & \rightarrow \\
\text{p-amino azo benzene} & \rightarrow \text{NHCH}_2\text{SO}_3\text{H}
\end{align*}
\]

(III)
REFERENCES


