Chapter - 7
Spectrophotometric Determination of Carbosulfan in Environmental Samples

Summary

Rapid, simple, sensitive and an extractive method for the determination of carbosulfan is described. The method is based on alkaline hydrolysis of carbosulfan into its corresponding phenol and subsequent coupling with diazotized p-amino acetophenone in alkaline medium. The red brown coloured dye has absorption maximum at 465 nm. The dye can be extracted in isoamyl alcohol, which makes the reaction more sensitive with same $\lambda_{\text{max}}$. Beer's law is obeyed in the range of 0.8-5.6 $\mu$g ml$^{-1}$ and 0.1-0.6 $\mu$g ml$^{-1}$ in the aqueous and extractive system respectively. The apparent molar absorptivity and Sandell's sensitivity were found to be $4.46 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ and 0.0085 $\mu$g cm$^{-2}$ respectively. The method has been satisfactorily applied for the determination of carbosulfan in various environmental samples.
Introduction

Carbosulfan, a widely used broad spectrum insecticide, nematicide and miticide used to control insects, mites, ants and nematodes by soil, foliar and seed treatment applications(1). Carbosulfan [2, 3 dihydro-2, 2-dimethyl-7-benzofuranyl [(dibutylamino) thio] methyl carbamate] is the derivative of carbamic acid, which is effective against aphides, mites and their larva with stomach and contact action. Bruce et al have studied its properties in water, soil and plants. It is safe to crops and effective to leaf hopper, whitfly, aphid, shoot and fruit borer pests and their larva by good systemic properties, low residue and long term effect (2-5).

It is used to protect apple, corn, citrus, vegetables, maize, rice, sugarcane, potato sorghum etc. Acute oral LD₅₀ value for male rat is 90-250 mg kg⁻¹. The acute RfD for carbosulfan is 0.02 mg kg⁻¹(6, 7). Carbosulfan is very effective against citrus leprosies mite and the citrus rust mite. It is highly effective, rapid in curative action, and adoptable to most situations and relatively economical (8-10). In the environment, carbosulfan is first metabolized to carbofuran then to 3-hydroxy-carbofuran and 3-keto carbofuran. This is a interesting case in which a less toxic pesticide (LD₅₀-250 mg kg⁻¹ for rats) is transformed into a more toxic one carbofuran (LD₅₀ - 8 mg kg⁻¹ for the same species) after its application (11).

The most sensitive effect of the oral administration of carbosulfan was the inhibition of cholinesterase activity, accompanied at the same or higher doses by clinical signs indicative of cholinesterase inhibition such as salivation, lacrimation, ataxia, tremors, anogenital staining, and diarrhea. It is minimally irritating to the eye, slightly irritating to the skin and is a dermal sensitizer. Long term exposure of carbosulfan is responsible for reductions in body weight, inhibition of brain and erythrocyte cholinesterase activity and pathological changes in the eye i.e. focal irisatrophy, iris coloboma and absence of iris tissue. It also causes malaise, muscle weakness, dizziness and sweating, headache, nausea vomiting, abdominal pain, depression and pulmonary edema (12-17).
Because of the wide usage and toxicity of carbosulfan several analytical techniques like gas chromatography (18, 19), liquid chromatography-mass spectrometry (20, 21), high performance liquid chromatography (22, 23), gas chromatography-mass spectrometry (24-26), and thin layer chromatography (27) have been reported for its determination. A few spectrophotometric methods involving reagents like 2-aminobenzophenzone (28), diazotized p-amino benzoic acid (29), 2, 4-dimethoxyaniline (30), p-chloro aniline (31), 2,4,6-trichloroaniline (32) etc have been reported in the literature.

In the present paper a simple, rapid and inexpensive spectrophotometric method based on the alkaline hydrolysis of carbosulfan and its subsequent coupling is proposed. The proposed method can be easily applied for its determination in water and grain samples.

**Experimental**

**Apparatus**

A Toshaniwal spectrophotometer (model TVSP-25) and Systronics pH meter (model 331) were used for the spectral and pH measurement.

**Reagents**

All chemicals used were of analytical grade or of the best available quality. Demineralised distilled water was used throughout the experiment.

**Carbosulfan (Rallis, India)**

A stock solution of 1 mg ml\(^{-1}\) was prepared in ethanol. Working standard was prepared by the appropriate dilution of the present stock.

**P-Aminoacetophenone (pAAP, Loba Chemie)**

1% (w/v) solution was prepared in 1:4 hydrochloric acid.
Sodium Nitrite (Merck)

0.2% (w/v) aqueous solution was prepared.

Sodium Hydroxide (Loba Chemie)

4 M aqueous solution was prepared in distilled water
1 N sulphuric acid was prepared.

Diazotised p-Aminoacetophenone (DpAAP)

To 25 ml of pAAP, 1.5 ml of 0.2% sodium nitrite was added dropwise with stirring. The diazotized compound was stable for about 4 hrs when kept in a brown bottle in cold.

Procedure

In Aqueous System

To an aliquot of a working standard solution containing 8-56 µg of carbosulfan in graduated flask, 0.3 ml of 1 N sulphuric acid was added, and was hydrolyzed in the corresponding phenol by adding 0.5 ml of sodium hydroxide. To it 1.5 ml of DpAAP was added and the solution was kept for 5 min with occasional shaking to ensure complete coupling. Then 1.0 ml of 4 M sodium hydroxide was added and volume was made up to 10 ml. The absorbance of the red brown dye was measured at 465 nm against a reagent blank.

In Extractive System

An aliquot containing 10-60 µg of carbosulfan was taken in a 100 ml separatory funnel. To it 0.3 ml of sulphuric acid was added and 0.5 ml of 4 M sodium hydroxide was added to hydrolyze it. Solution was allowed to stand for 2 min with occasional shaking. Then 1.5 ml of DpAAP was added and after 5 minutes 1 ml of 4 M sodium hydroxide was added to maintain pH between 12-13. Then red brown coloured complex was extracted with 3 x 5 ml portions of isoamyl alcohol. The extracted dye was dried over anhydrous sodium sulphate and then absorbance was measured at 465 nm against reagent blank.
Results and Discussion

The proposed method involves the formation of carbofuran (a derivative of carbosulfan) by the reaction of carboxulfan with sulfuric acid. After alkaline hydrolysis it is subsequently coupled with diazotised p-aminoacetophenone to give red brown dye in the alkaline medium.

Spectral Characteristics

The absorption spectrum of the dye against a reagent blank was measured at 465 nm in the aqueous as well as in extractive system (Fig1).

Effect of Varying Reagent Concentration

The effects of varying reagent concentration on the colour reaction were studied. It was found that under optimum conditions 0.5 ml of sodium hydroxide was sufficient for alkaline hydrolysis and 1.5 ml of DpAAP was required for maximum colour intensity.

Effects of Time and Temperature

The time required for diazotization and coupling was found to be 10 min and 5 min respectively. After adding DpAAP no noticeable changes in the absorbance values were observed at low or high temperature, so the reaction was carried out at room temperature. The dye was stable for 48 hours in aqueous medium as well as in extractive medium.

Effects of Acidity

Higher absorbance was obtained at pH 12-13, which was maintained by adding 1 ml of 4 M sodium hydroxide. Excess of alkali caused turbidity.

Sensitivity and Precision

Beer's law is obeyed over the concentration range of 8-56 µg of carbofuran per 10 ml (0.8–5.6 µg ml⁻¹) in the aqueous medium and 10-60 µg of carbofuran per
100 ml (0.1-0.6 μg ml⁻¹) in the extractive system. Sandell’s sensitivity and molar absorptivity for the proposed reaction were calculated and mentioned in Table 1.

The precision of the method was checked by the seven replicate analysis of 30 μg of carbosulfan per 10 ml in aqueous system. The detection limit (D_L = 3.3 σ/S) and quantification limit (Q_L = 10 σ/S) [where ‘σ’ is the standard deviation of the reagent blank (n= 6) and ‘S’ is the slope of the calibration curve] of the carbosulfan determined along with other statistical parameters are given in Table 1.

**Effect of Foreign Ions**

Effect of various copollutants pesticide and foreign ions were studied. To a sample containing 30 μg of carbosulfan known amount of foreign species were added. The method was free from the interference of various cations and anions. The tolerance limit of these compounds is given in Table 2.

**Colour Reaction**

The proposed reaction involves following steps:

1. Hydrolysis of carbosulfan into carbofuran in the presence of sulfuric acid.
2. Formation of corresponding phenol from carbofuran by the alkaline hydrolysis.
3. Coupling of diazotized p-aminoacetophenone with phenol derivative of carbosulfan formed in step 2 and formation of yellow brown coloured dye.

[Scheme 1]

**Application**

The proposed method can be applied for the determination of carbosulfan in the soil, water and grain samples. The validation of the method was confirmed by the fortification and percentage recovery.

**Determination of Carbosulfan in Water Samples**

Water samples were collected from agricultural fields where carbosulfan was sprayed. These samples were first extracted twice with 5 ml portion of chloroform in a separating funnel. Extract were then evaporated to dryness under reduced pressure.
The residue obtained was dissolved in 5 ml ethanol and diluted upto 50 ml with double distilled water. Aliquots were then fortified with known amount of carbosulfan and recovery was checked as given under preparation of calibration curve. The recovery is given in Table 3.

**Determination of Recovery in Grain Samples**

50 g of the sample of citrus fruit and rice free from pesticides were taken. The samples were weighed and spiked with known amounts of carbosulfan and kept for 3-4 hours. The samples were washed with 100 ml of double distilled water. It was extracted with 2 x 5 ml portions of chloroform. The chloroform extract was evaporated to dryness on a water bath kept at about 50°C. The residue was dissolved in 5 ml ethanol and volume was made up to 25 ml with distilled water. Aliquots were then analyzed as recommended above and by the reported method (32). The recovery was calculated directly from the correspondent calibration curves for carbosulfan and values are given in Table 3.

**Determination of Carbosulfan in Soil Samples**

Various soil samples were collected from agricultural fields where carbosulfan was sprayed as insecticide. Samples were weighed and finally ground. These samples were washed with 2x10 ml portion of chloroform. The washings were collected and then evaporated to dryness under reduced pressure. The residue was dissolved in 5 ml ethanol and volume was made up to 25 ml with distilled water. Aliquots were then spiked with known amounts of carbosulfan and recovery was determined by the proposed and the reported method (32). The recovery of carbosulfan from various samples was about 97-99% and is shown in Table 3.

**Conclusions**

The present method is simple, sensitive and extractive and can be used satisfactorily for the determination of carbosulfan in soil, water and vegetable samples. The validation of the proposed method was based on the fortification studies and was considered satisfactory due to 95-99.5% recoveries.
Scheme 1 - Colour Reaction of Carbosulfan

Step 1 - Hydrolysis

I

\[
\text{Carbosulfan} \xrightarrow{\text{H}_2\text{SO}_4} \text{Carbofuran}
\]

II

\[
\text{Carbofuran} \xrightarrow{\text{Alkaline Hydrolysis}} \text{Phenol Derivative of Carbofuran}
\]

Step 2 - Diazotization

III

\[
\text{p-Aminoacetophenone} + \text{NO}_2^- / \text{H}^+ \xrightarrow{} \text{Diazotized p-Aminoacetophenone}
\]

Step 3 - Coupling

IV

\[
\text{Phenol} + \text{Diazotized p-Aminoacetophenone} \xrightarrow{\text{Alkaline Hydrolysis}} \text{Red Brown Dye (Polymethine Dye)}\]

\[\lambda_{\text{max}} = 465 \text{ nm} \]
Fig 1: Absorption spectrum of dye
A. Concentration of the carbosulfan, 55 µg / 10 ml
B. Reagent blank.

Fig 2: Calibration curve for determination of carbosulfan
Fig 3: Effect of reagent concentration
Concentration of the carbosulfan, 30 μg / 10 ml

Fig 4: Effect of time on colour reaction
Concentration of the carbosulfan, 30 μg / 10 ml
Fig 5: Effect of pH on colour reaction
Concentration of the carbosulfan, 30 μg / 10 ml
Table 1: Photometric and Statistical Parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer's law range (μg ml⁻¹)</td>
<td></td>
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<tr>
<td>In aqueous system</td>
<td>0.8</td>
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<tr>
<td>In extractive system</td>
<td>0.1-0.6</td>
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<tr>
<td>Molar absorptivity (1 mol⁻¹cm⁻¹)</td>
<td>4.46x10⁴</td>
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<td>Sandell's sensitivity (μg cm⁻²)</td>
<td>0.0085</td>
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<tr>
<td>Detection limit (μg ml⁻¹)</td>
<td>0.0225</td>
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<td>Quantification limit (μg ml⁻¹)</td>
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<tr>
<td>Standard deviation</td>
<td>±0.008</td>
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<td>Relative standard deviation (%)</td>
<td>2.12</td>
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<td>Correlation coefficient</td>
<td>0.998</td>
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</table>

Table 2: Effect of Foreign Species

Concentration of carbosulfan = 30 μg per 10 ml

<table>
<thead>
<tr>
<th>Foreign Ions</th>
<th>Tolerance Limit * (μg ml⁻¹)</th>
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<tbody>
<tr>
<td>Carbaryl, propoxur</td>
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<tr>
<td>Phenol</td>
<td>250</td>
</tr>
<tr>
<td>BHC, DDT</td>
<td>700</td>
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<tr>
<td>Monocrotophos, quinolphos</td>
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</tr>
<tr>
<td>Malathion, parathion, phorate</td>
<td>600</td>
</tr>
<tr>
<td>Pb²⁺, Cd²⁺</td>
<td>500</td>
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<tr>
<td>Mg²⁺, Cu²⁺</td>
<td>800</td>
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*Tolerance limit is the amount of interferent that causes an error of ±2%
<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbosulfan Added (µg)</th>
<th>Proposed Method</th>
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<th>Reported Method(32)</th>
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<tbody>
<tr>
<td></td>
<td>Carbosulfan</td>
<td>SD</td>
<td>Recovery</td>
<td>Carbosulfan</td>
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<td></td>
<td>Found (µg)*</td>
<td></td>
<td>%</td>
<td>Found (µg)*</td>
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<td>Water</td>
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<tr>
<td>W₁</td>
<td>30</td>
<td>29.50 ±0.025</td>
<td>98.3</td>
<td>29.35 ±0.032</td>
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<tr>
<td>W₂</td>
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<td>34.65 ±0.017</td>
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<td>34.30 ±0.02</td>
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<td>W₃</td>
<td>40</td>
<td>39.80 ±0.015</td>
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<td>Soil</td>
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<tr>
<td>S₁</td>
<td>20</td>
<td>19.10 ±0.05</td>
<td>95.5</td>
<td>19.05 ±0.04</td>
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<td>S₂</td>
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<tr>
<td>R₁</td>
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<td>19.10 ±0.042</td>
<td>95.5</td>
<td>19.0 ±0.055</td>
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<tr>
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<td>C₁</td>
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<td>29.65 ±0.028</td>
<td>98.8</td>
<td>29.30 ±0.05</td>
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<td>34.15 ±0.035</td>
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<tr>
<td>C₃</td>
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<td>39.40 ±0.03</td>
<td>98.5</td>
<td>39.50 ±0.04</td>
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</table>

*Average of six replicate analysis
a Amount of water sample = 5 ml
b Amount of soil sample = 25 g
c and d, Amount of plant sample = 50 g

130
References


