# Facile and Sensitive Spectrophotometric Determination of Carbosulfan in Formulations and Environmental Samples

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**Abstract** Facile, selective and sensitive spectrophotometric method has been developed for the determination of carbosulfan in insecticidal formulations, fortified water, food grains, agriculture wastewater and soil samples with newly synthesized reagents. The method was based on acid and alkaline hydrolysis of the carbosulfan pesticide, and the resultant hydrolysis product of carbosulfan was coupled with 2,6-dibromo-4-methylaniline to give a yellow color product with $\lambda_{max}$ of 464 nm or interaction with 2,6-dibromo-4-nitroaniline to produce yellow colored product with $\lambda_{max}$ of 408 nm or coupling with 2,4,6-tribromoaniline to form red colored product has a $\lambda_{max}$ of 471 nm. Under optimal conditions, Beer's law range for 2,6-dibromo-4-methylaniline (DBMA) was found to be 0.2–12.0 μg ml$^{-1}$, 0.6–16.0 μg ml$^{-1}$ for 2,6-dibromo-4-nitroaniline (DBNA) and 0.4–15.0 μg ml$^{-1}$ for 2,4,6-tribromoaniline (TBA). The molar absorptivity of the color systems were found to be $3.112 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ for DBMA, $3.214 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ for DBNA and $3.881 \times 10^4$ l mol$^{-1}$ cm$^{-1}$ for TBA. Sandell's of the color reactions are 0.013 μg cm$^{-2}$ (DBMA), 0.012 μg cm$^{-2}$ (DBNA) and 0.011 μg cm$^{-2}$ (TBA) respectively. The effect of the non-target species on the determination of carbosulfan was studied to enhance the selectivity of the proposed methods. The formation of colored derivatives with the coupling agents is instantaneous and stable for 28, 30, and 26 h. Performance of the proposed methods were compared statistically in terms Student's $F$ and $t$-tests with the reported methods.

**Keywords** Carbosulfan · 2,6-Dibromo-4-methylaniline · 2,6-Dibromo-4-nitroaniline · 2,4,6-Tribromoaniline · Spectrophotometry · Water · Grains · Environmental samples

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## 1 Introduction

Carbosulfan is a broad spectrum carbamate pesticide closely related to its main metabolite carbofuran, a major pesticide in its own right. Carbosulfan is available as emulsifiable concentrates, dusts and granular formulations (wettable powder formulations are under development) for the control of soil and foliar pests of a variety of commodities. It may be applied to soil or foliage and is said to be effective through direct contact or stomach ingestion. Foliar

pests may be controlled by soil applications *via* systemic action, although as discussed under 'Fate of Residues' most of the systemic activities is not due to carbosulfan. It is also registered for seed treatment and may be applied from the ground or air. Treatment may be in furrow, by band, broadcast, side dress, preplant, injection or pre-emergence.

Carbosulfan belongs to the carbamates class of insecticide and sulfonylated derivatives of carbofuran. Carbosulfan has got the property of reversible cholinesterase inhibition capable of causing toxic effects as a result of oral ingestion or inhalation. Carbosulfan influences the toxicity on insects and likely to cause extensive mortality to some species of nematodes at relatively high dosages in soil (Tomlin, 1994). Carbosulfan [2,3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutyldiaminothio) methyl carbamate] is an insecticide widely used throughout India in agriculture and households in particular to Andhra Pradesh. The indiscriminate use of insecticides is increasing for agriculture, commercial pest control and residential consumer use. In scrutiny of literature reveals that several techniques have been reported for the determination of carbosulfan such as spectrophotometry (Harikrishna, Prasad, & Naidu, 2003; Rajeswari & Naidu, 1986; Suresh Kumar, Prabhakara Rao, Lokanath Swaroop, Sivasankara Reddy, & Chiranjeevi, 2003), voltammetry (Augustina et al., 2001), gas chromatography (Quintana, Marti, &Ventura, 2001; Tae Hyo, Won Chul, Jung Hag, Taeowan, & Yong Tae, 2003) and high performance liquid chromatography (Consuelo, Beatriz, & Tadeo, 2004; Morrica, Fidente, & Seccia, 2005; Yu, Wang, & Chu, 1998). These techniques suffer from several disadvantages such as poor sensitivity and selectivity (spectrophotometry), few others are expensive (GC and HPLC) and some of them have been reported to require solvents for the extraction of color derivatives. So, it is desirable to develop facile, sensitive, reliable and rapid method for the determination of carbosulfan in its formulations and environmental samples by spectrophotometry.

The present paper describes a simple and low cost procedure for the preparation of reagents by one step process and the present method explains certain advantages such as stability of the color products, selectivity, sensitivity and good recoveries of the carbosulfan residues over the reported methods in the literature (Harikrishna et al., 2003; Rajeswari & Naidu, 1986). Here, authors developed facile, rapid, economical and

sensitive spectrophotometric technique for analyzing the carbosulfan in its formulations, food grains and soil samples using 2,6-dibromo-4-methylaniline, 2,6-dibromo-4-nitroaniline and 2,4,6-tribromoaniline as coupling agents.

## 2 Experimental

### 2.1 Preparation of reagents

Two grams of *p*-toluidine was dissolved in 25 ml of 1:1 HCl and 2 ml of bromine (premixed with 5 ml of acetic acid) added with a dropping funnel, while stirring, maintaining the reaction temperature at 38 °C. After stirring for 3 h, the precipitate was filtered, washed with distilled water and dried at 40 °C. 2,6-dibromo-4-methylaniline was obtained (yield 80%, m. p. 75–77 °C). 2,6-dibromo-4-nitroaniline and 2,4,6-tribromoaniline were prepared by employing the same procedure for the preparation of the reagents were shown in Figure 1.
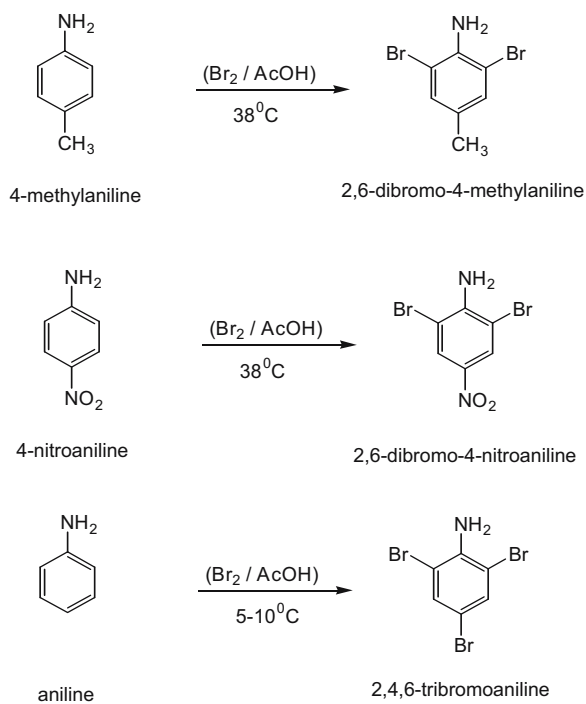


Figure 1 Preparation of coupling reagents.

Figure 2 Interactions of carbosulfan with coupling reagents.
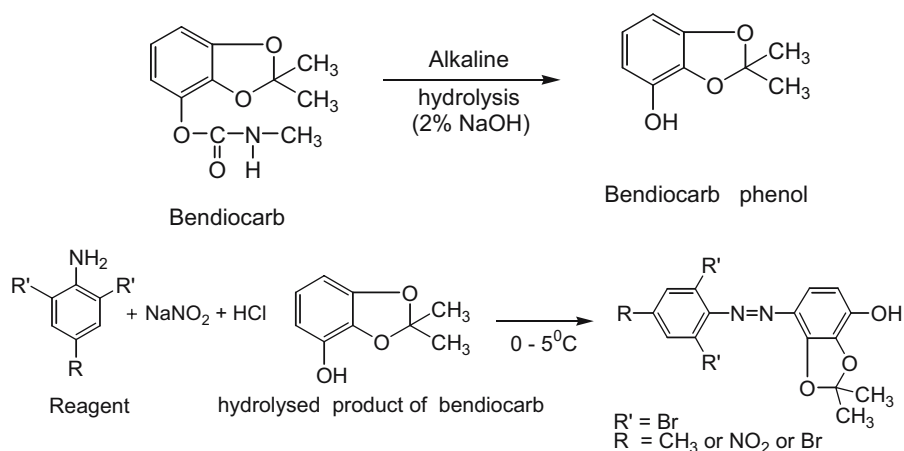


## 2.2 Materials and methods

A HITACHI U 2001 spectrophotometer with 1 cm matched quartz cell was used for the absorbance measurements. All chemicals used were of the best available quality. A stock solution (1 mg ml$^{-1}$) of carbosulfan (Rallis India Limited, Bangalore, India) was dissolved in methanol and working standard solution (10 μg ml$^{-1}$) was prepared by appropriate dilution with distilled water. Two percent of NaOH, 0.3% NaNO$_2$ and 0.2% reagent solutions were used in the experiments.

## 2.3 General procedure

Twenty milliliter of the carbosulfan standard solution was taken in a clean dry 100 ml beaker; 5 ml of 2% NaOH

solution was added and kept for 15 min for complete hydrolysis to yield respective phenol. The diazonium salts of the 2,6-dibromo-4-methylaniline, 2,6-dibromo-4-nitroaniline and 2,4,6-tribromoaniline were prepared by using 3 ml of 0.3% NaNO$_2$ and 3 ml of 1 N HCl for coupling reactions at 0–5 °C. The pH of the solutions was maintained in the range of 9–12. The color derivatives of the carbosulfan with coupling reagents were shown in Figure 2. The absorption spectra of the above color derivatives were recorded and analytical characteristics of the color derivatives were shown in Table I.

## 2.4 Determination of carbosulfan in its formulations

Carbosulfan in 10 and 70% granules formulations were analysed using the aforesaid procedure with the cou-

Table I Optical characteristics, precision, and accuracy of the spectrophotometric determination of carbosulfan with the reagents

| Characteristics | 2,6-Dibromo-4-methylaniline | 2,6-Dibromo-4-nitroaniline | 2,4,6-tribromoaniline |
|---|---|---|---|
| Concentration range (μg ml$^{-1}$) | 0.2 – 12.0 | 0.6 – 16.0 | 0.4 – 15.0 |
| Color | Yellow | Yellow | Red |
| Stability (h) | 28 | 30 | 26 |
| $\lambda_{max}$ (nm) | 464 | 408 | 471 |
| Limit of detection (μg ml$^{-1}$) | 0.181 | 0.524 | 0.326 |
| Limit of quantification (μg ml$^{-1}$) | 1.089 | 1.572 | 1.76 |
| Molar absorptivity (l mol$^{-1}$ cm$^{-1}$) | $3.112 \times 10^4$ | $3.214 \times 10^4$ | $3.881 \times 10^4$ |
| Sandell's sensitivity (μg cm$^{-2}$) | 0.013 | 0.012 | 0.011 |
| Regression[a] slope $b$ | 0.098 | 0.105 | 0.081 |
| Regression intercept $a$ | 0.102 | 0.154 | 0.106 |
| Correlation coefficient ($r$) | 0.9971 | 0.9985 | 1.0002 |
| Relative standard deviation (%)[b] | 1.42 | 1.19 | 1.32 |
| Relative error (%) | 1.15 | 1.34 | 1.08 |

[a] Regression curve: $y = ax + b$, where $x$ is the concentration of carbosulfan (μg ml$^{-1}$) and $y$ is absorbance.

[b] Determination for $n = 5$.

**Table II** Average recovery of carbosulfan from fortified water and grain samples as determined spectrophotometrically with coupling reagents

| Reagents | Fortification ($\mu g \; ml^{-1}$) | Recovery[a] | | | |
|---|---|---|---|---|---|
| | | Tap water | Distilled water | Rice | Wheat |
| 2,6-Dibromo-4-methylaniline | 0.2 – 6.4 | 98.88 ± 1.05 | 97.85 ± 1.12 | 97.14 ± 1.02 | 96.35 ± 1.26 |
| 2,6-Dibromo-4-nitroaniline | 0.6 – 5.4 | 98.57 ± 1.01 | 98.33 ± 0.95 | 96.65 ± 1.21 | 95.70 ± 0.98 |
| 2,4,6-Tribromoaniline | 0.4 – 4.8 | 98.75 ± 0.86 | 98.62 ± 0.91 | 95.60 ± 0.71 | 94.20 ± 1.22 |

[a] Mean ± standard deviation ($n = 5$).

pling reagents. The methods were compared with the reported methods in the literature (Harikrishna et al., 2003) and shown in Table III.

2.5 Determination of carbosulfan in environmental samples

Water (distilled and tap) and grain (rice and wheat) samples were fortified with 0.4–14.0 ppm levels with carbosulfan and left for 24 h to give closer simulation of environmental samples. These fortified samples were extracted independently with chloroform and the combined extracts were washed with 0.1 M $K_2CO_3$ solution to break any emulsion formed during the extraction and dried over anhydrous sodium sulphate. Finally, chloroform was evaporated to dryness on a steam bath and the residue was dissolved in methanol and the amount of the carbosulfan was determined using the procedure described earlier. The percentage recoveries along with the fortification levels were also shown in Tables II and III.

Agriculture wastewater samples (10 ml) were collected from Sri Venkateswara Agricultural Research Station, Tirupati, India and the water samples were extracted with chloroform (2 × 10 ml). The chloroform extracts was then evaporated to dryness under reduced pressure and the residue was dissolved in 25 ml of 50% methanol and amount of the carbosulfan was determined by the described procedure earlier. Ten grams of the soil samples and plant material (cabbage) samples were extracted according to the reported method (Harikrishna et al., 2003) and the amount of the carbosulfan was analysed by the aforesaid procedure.

# 3 Results and Discussion

The analytical parameters of the coupling reagents were shown in Table II. The present analytical method is based on alkaline hydrolysis of carbosulfan, which gives respective phenol. The latter was coupled with diazotised salts 2,6-dibromo-4-methylaniline, 2,6-dibromo-4-nitroaniline, or 2,4,6-tribromoaniline and the resulting azo derivatives were spectrophotometrically determined in solution.

A linear calibration graph for the spectrophotometric determination of carbosulfan was obtained for concentrations with reagents in the range of 0.2 to 16.0 $\mu g$ $ml^{-1}$. The limit of quantification (LOQ) was determined by taking the ratio of standard deviation ($\sigma$) of the blank with respect to water and slope ($s$) of calibration curve multiplied by a factor of 10. This means that the LOQ was 5.4 times limit of detection (LOD). Naturally, LOQ slightly crosses the limit of the range given by *Lambert–Beer's* law; however, the LOD is well below the corresponding lower-limit. The upper range was determined by a plot of absorbance (at $\lambda_{max}$) against concentration. The calibration graph had correlation coefficients from 0.9971 to 1.0002. The molar absorptivity was observed from $3.112 \times 10^4$ to $3.881 \times 10^4$ $1 \; mol^{-1} \; cm^{-1}$. Ringbom's plot is the established standard adopted to know the optimum range of concentration for a system that obeys Beer's law. The plot is drawn between log C of carbosulfan and (1-$T$; where $T$ is the transmittance). The optimum concentration ranges were evaluated by Ringbom's method and were found to be 0.35–6.5 $\mu g \; ml^{-1}$ (DBMA), 0.45–6.0 $\mu g \; ml^{-1}$ (DBNA) and 0.28–5.8 $\mu g \; ml^{-1}$ (TBA) respectively. The optical characteristics of the colored derivatives were given in Table I.

The effect of NaOH concentration on hydrolysis of carbosulfan was studied by varying the amount of base added (1–7 ml of 2% NaOH solution). It was found that 4–7 ml of 2% NaOH solution were necessary for complete hydrolysis; thus, a volume of 5 ml was selected for all experiments. Absorbance values were obtained within a pH range of 11–12 without using the buffer for stabilization of the color. Other alkaline

**Table III** Comparison of different methods for carbosulfan determination in environmental samples

| Sample | Carbosulfan added (μg) | 2,6-Dibromo-4-methylaniline | | 2,6-Dibromo-4-nitroaniline | | 2,4,6-Tribromoaniline | | Spectrophotometry (Harikrishna et al., 2003) | Gas Chromatography (Tae Hyo et al., 2003) |
|---|---|---|---|---|---|---|---|---|---|
| | | Found | Recovery[a] | Found | Recovery[a] | Found | Recovery[a] | Recovery[a] | Recovery[a] |
| 25% Seed treat $(F, t)$ | – | 24.78 | $99.00 \pm 0.08$ (0.49, 0.91) | 24.80 | $99.20 \pm 0.05$ (0.91, 1.21) | 24.79 | $99.16 \pm 0.05$ (0.64, 0.75) | $94.50 \pm 3.85$ (2.3, 0.89) | $99.80 \pm 0.01$ (1.04, 0.80) |
| 2% Emulsion $(F, t)$ | – | 1.98 | $99.00 \pm 0.02$ (0.34, 1.06) | 1.97 | $98.50 \pm 0.07$ (1.09, 0.45) | 1.96 | $98.00 \pm 0.04$ (1.03, 0.97) | | |
| Distilled Water $(F, t)$ | 7.0 | 6.78 | $96.87 \pm 1.56$ (0.73, 1.15) | 6.71 | $95.85 \pm 0.08$ (0.74, 1.04) | 6.79 | $97.00 \pm 0.07$ (0.65, 0.89) | | |
| Tap water $(F, t)$ | 7.0 | 6.85 | $97.85 \pm 0.09$ (2.18, 0.72) | 6.77 | $96.71 \pm 1.01$ (0.75, 0.66) | 6.70 | $95.74 \pm 1.06$ (2.61, 1.47) | $95.67 \pm 2.21$ (1.67, 0.71) | $98.40 \pm 1.48$ (1.78, 0.64) |
| Rice | 10.0 | 9.54 | $95.40 \pm 1.05$ | 9.58 | $95.80 \pm 0.09$ | 9.52 | $95.20 \pm 0.85$ | – | – |
| Wheat | 10.0 | 9.42 | $94.20 \pm 1.27$ | 9.41 | $94.10 \pm 0.98$ | 9.49 | $94.90 \pm 1.27$ | – | – |
| Agricultural wastewater[b] | 15.0 | 14.12 | $94.13 \pm 1.11$ | 14.39 | $95.93 \pm 1.15$ | 14.36 | $95.73 \pm 1.23$ | – | – |
| Plant material (Cabbage)[b] | 15.0 | 14.58 | $97.20 \pm 1.35$ | 14.48 | $96.53 \pm 1.17$ | 14.38 | $95.86 \pm 1.18$ | – | – |
| Agriculture soil[b] | 20.0 | 18.90 | $94.50 \pm 1.62$ | 18.94 | $94.70 \pm 1.71$ | 19.10 | $95.50 \pm 1.42$ | – | – |

[a] Avarage values for $n = 5$.

[b] Agricultural samples collected from Sri Venkateswara Agricultural Research Institute, Tirupati, A. P., India.

[c] Agricultural samples collected from Perur, Chandragiri Mandal, Chittur District, A.P., India.

solutions (KOH, Ba[OH]$_2$ and NH$_4$OH) were also tested, but NaOH gave the best results.

All diazotizations were carried out at 0–5 °C in the presence of 0.5–1.5 M HCl. In this acidity range, constant absorbances were recorded in the wide acidic range, above this range, a decrease in the absorbance was observed. Thus, all reactions were performed in 1 M HCl, which was optimal for both synthetic and spectral points of view and also studied the effect of reagent concentration (1–6 ml of a 0.2% reagent solution) in 1 M HCl. It was found that 4 ml of 0.2% reagent solution was sufficient for complete color development. Higher concentrations did not enhance the absorbance any further, and lower concentrations gave the poor results.

Possible effects of non-target species on the determination of carbosulfan were studied. Known amounts of foreign species and pesticides were added to standard solution containing 10 µg of carbosulfan in 25 ml of final solution, prior to hydrolysis. Carbaryl, bendiocarb and other non-target pesticides if present, their interfere with the color reactions was removed by washing the chloroform extracts twice with 0.5%–1.0% NaOH solution, followed by 10 ml of water to remove these contaminants. At least 2.0% NaOH solution was needed for the hydrolysis of carbosulfan. The carbosulfan residue was not lost during the removal of carbaryl, bendiocarb and other non-target species. Tolerance limits (µg/25 ml) of some pesticides as follows: carbaryl (850), bendiocarb (650), dimethioate and phorate (580).

Similar high levels of recovery were obtained for carbosulfan in both fortified water and grain samples, as summarized in Tables II and III. Here, coupling reagents turned out to provide the best results, with average recoveries of 94.49%–98.34% from tap water, distilled water, rice and wheat samples. Generally, the recoveries decreased in the order: tap water > distilled water > rice > wheat.

## 4 Conclusion

The performance of the new method was compared statistically in terms of *Student's* test (*t*-values) and the variance ratio of *F*-test. These tests showed no significant difference in accuracy and precision between the proposed methods and reference methods (Harikrishna et al., 2003; Tae Hyo et al., 2003). The low values of the relative standard deviation further reflect the high precision of the method. Theoretical *t*-

values at the 95% confidence level were found to be 2.75. A comparison of the results of our experiments with similar, known procedures is given in Table III. The present methods gave satisfactory results for the determination of carbosulfan in water, grains, waste-water, agricultural soil and environmental samples.

Thus, the present method for the determination of insecticide carbosulfan in a variety of samples (water, grains, and soil) was found to be facile, rapid, inexpensive, and accurate. The coupling reagents 2,6-dibromo-4-methylaniline, 2,6-dibromo-4-nitroaniline, or 2,4,6-tribromoaniline are prepared one step. The proposed methods do not require any special solvent for the extraction of the chromophores, and the color derivatives are quite stable relative to other species reported in the literature (Harikrishna et al., 2003; Tae Hyo et al., 2003).

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