



A Sensitive Determination of Carbofuran by Spectrophotometer using 4,4-azo-bis-3,3',5,5'-tetra bromo aniline in various Environmental Samples

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Abstract: A simple and sensitive spectrophotometric technique was developed for the determination of carbofuran in its formulations, water and grain samples. The method was based on the alkaline hydrolyzed product of carbofuran phenol interacted with diazonium salt of 4,4-azo-bis-3,3',5,5'-tetra bromo aniline. The maximum absorbance of the red coloured derivative was measured at 470 nm. The beer's law was obeyed in the concentration range of 0.1-16.0 µg/ ml. The interference of the non target species were studied on the determination of carbofuran which increases the selectivity of the method. The present method was successfully applied for the determination of carbofuran in its formulations, water and grain samples.

Key words: Carbofuran, 4,4-azo-bis-3,3',5,5'-tetra bromo aniline, spectrophotometry, water and grain samples

Introduction

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuran 7-methyl carbamate) belongs to the carbamate class of insecticides. It is a broad spectrum of systemic acaricide, insecticide and nematicide. Its insecticidal properties have been described¹. It is widely used in agriculture

for the control of soil insects and mites, soil-dwelling and foliar-feeding insects. Formulations of carbofuran are now being extensively used to control the corn root worm on field corn, rice, water weevil, land mosquitoes on rice, nematicides and wireworms on sugar cane. It is highly toxic to mammals and wild birds^{2,3}. Indiscriminate use of carbofuran for controlling the insecticides and also leads to serious environmental problems. Spectrophotometry⁴, GLC¹⁸⁻²², GC²³⁻²⁶ and HPLC²⁷⁻²⁹ methods have been reported for the determination of carbofuran. The above reported methods suffers from several disadvantages such as less stability, pH dependent, poor sensitivity and most of them required specific solvents for the extraction of colour derivatives. Here, authors developed sensitive, facile and rapid spectrophotometric method for the determination of carbofuran in its formulations, water and grain samples. In the present method carbofuran undergoes alkaline hydrolysis to produce carbofuran phenol and is coupled with diazotized 4,4-azo-bis-3,3',5,5'-tetra bromo aniline.

Experimental

Synthesis of 4,4-azo-bis-3,3',5,5'-tetra bromo aniline

Acetanilide and 4-amino acetanilide were dissolved in 25 ml of 1:1 HCl / HNO₃ and 2 ml of bromine (pre-mixed with 5 ml of acetic acid) added with a dropping funnel while stirring maintaining 38^oC. After stirring for 3 h the precipitate was filtered, washed with distilled water and dried at 40^oC, 3,5-dibromo acetanilide and 4-amino 3,5-dibromo acetanilide were obtained (yield 80% mp 180-185^oC). 3,5-dibromo acetanilide and 4-amino 3,5-dibromo acetanilide were dissolved in methanol. 4-amino-3,5-dibromo acetanilide is diazotized with 0.5 % of 2.5 ml sodium nitrite and 1.5 ml of concentrated HCl at 0-5^oC to form azo solution (a). The resulting azo solution (a) is added with 3,5-dibromo acetanilide at 0-5^oC of pH 7-9 to form azo solution (b). The resulting azo solution (b) is washed with distilled water and added 5 ml of concentrated H₂SO₄ and water refluxed for 3 h at 70-75^oC.

The precipitate is filtered, washed with distilled water and dried at 40^oC on water bath. 4, 4-azo-bis-3,3',5,5'-tetra bromo aniline is obtained (yield 70-80% mp 120-125^oC). The synthesis of 4, 4-azo-bis-3,3',5,5'-tetra bromo aniline was shown in **Scheme 1**. Elemental analysis was calculated for C₁₂ N₄ Br₄ H₈ (m/z) 527.838 (C-27.30%, N-10.61%, H-1.52%) was found to be C. 27.16%, N-10.35%, H-1.63%. The bond at 1250-1020 cm⁻¹ is due to the C-N, the bond at 650-500 cm⁻¹ is due to the C-Br and the bond at 1575 cm⁻¹ is due to the N=N.

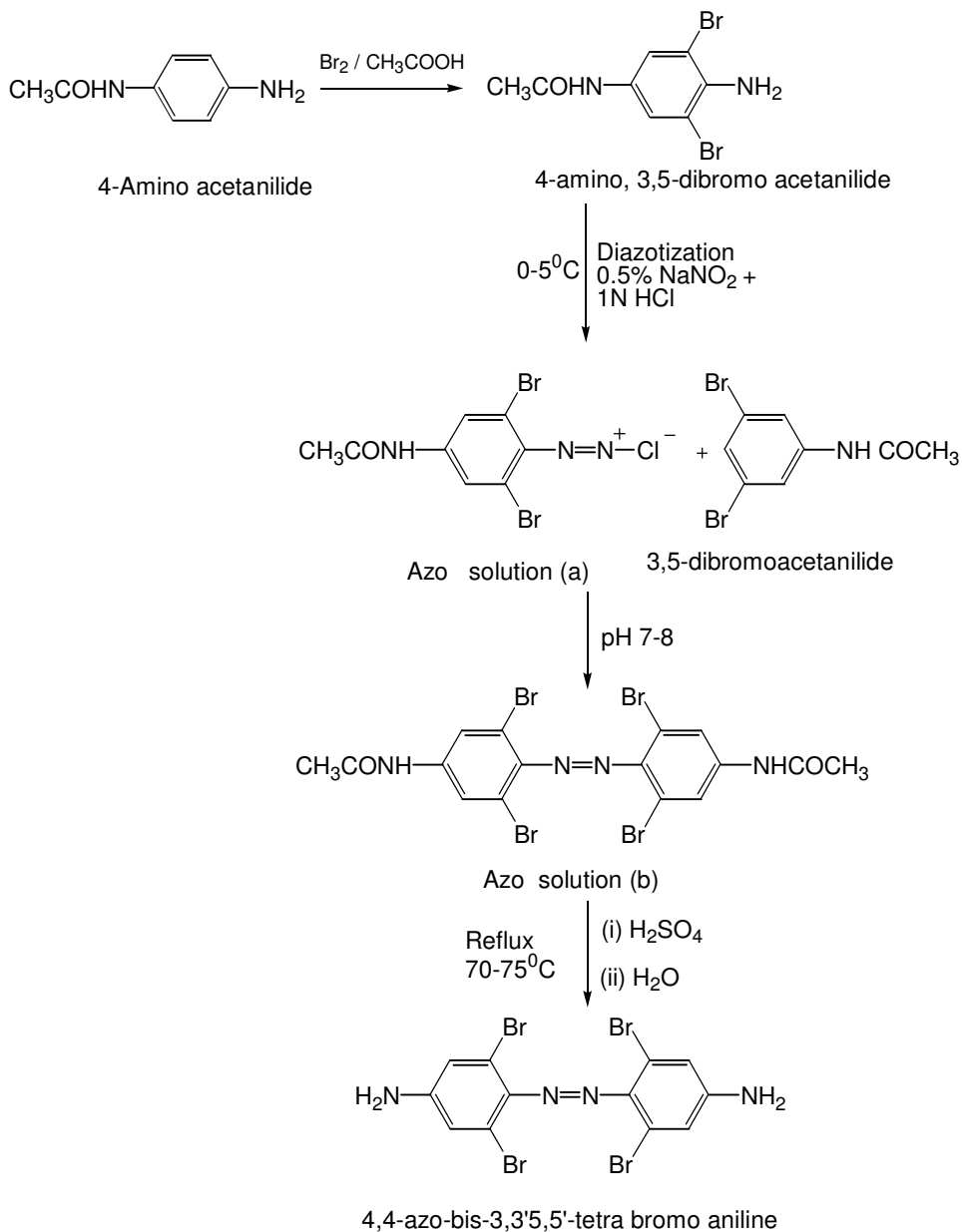
Instrumentation

The spectral recording in the UV-VIS region for the coloured compound was obtained on a HITACHI U 2001 spectrophotometer. Elemental analysis was carried out by Perkin-Elmer 240 C elemental analyzer. An Elico Li-129 model pH meter with combined calomel-glass electrode was used for all pH measurements.

Solutions

Carbofuran analytical grade sample (99.5%) supplied by Rallis India Ltd., Bangalore in India. Standard carbofuran (1000 µg/ml) solution was prepared by dissolving 100 mg of analytical grade carbofuran in 100 ml of methanol. Further dilution was made to get working standard solution. The reagents like sodium nitrite 0.5% (w/v) solution, aqueous sodium hydroxide 2% (w/v) solution dipotassium hydrogen phosphate (10.5g) and 7.3g of potassium dihydrogen phosphate were dissolved in distilled water and diluted to 100 ml,

aqueous 4,4-azo-bis-3,3',5,5'-tetra bromo aniline. 0.1% (w/v) solution was prepared in methanol were purified and employed for the present study.



Scheme 1 Synthesis of 4,4-azo-bis-3,3',5,5'-tetra bromo aniline

Procedure

Aliquots of standard carbofuran (0, 0.25, 0.5, 1.0 - 8.0 ml) pesticide solution were taken into a series of 25 ml standard flasks. To each one of these 2.5 ml of 2 % sodium hydroxide, 2.5 ml of 0.5 % sodium nitrite, 1.5 ml of concentrated HCl and 2.0 ml of 4,4-azo-bis-3,3',5,5'-tetra bromo aniline and 5 ml of phosphate buffer (pH 9.0) were added. The solution was made up to the mark with distilled water. The resulting red coloured derivatives shows maximum absorption at 470 nm as shown in **Scheme 2** against a reagent blank and the colour of the derivative stable for more than 36 h. Absorbance values were recorded spectrophotometrically as shown in **Figure 1**.

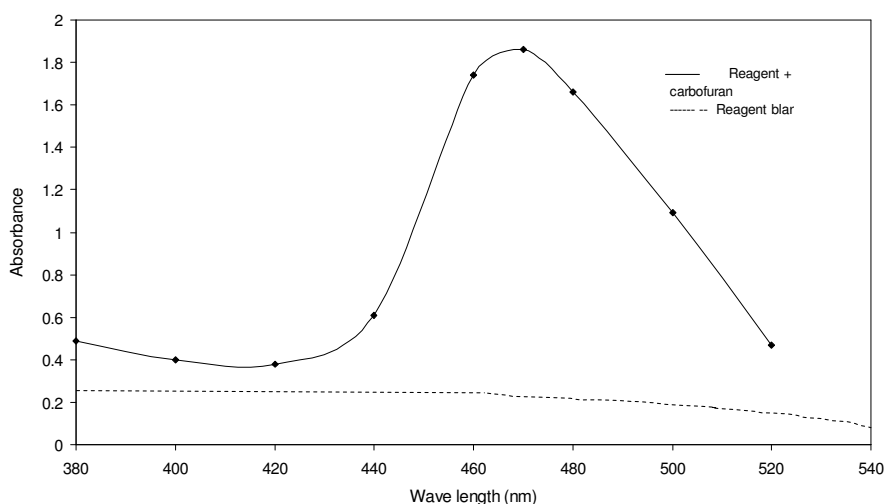


Fig 1 Absorption spectrum of carbofuran derivative

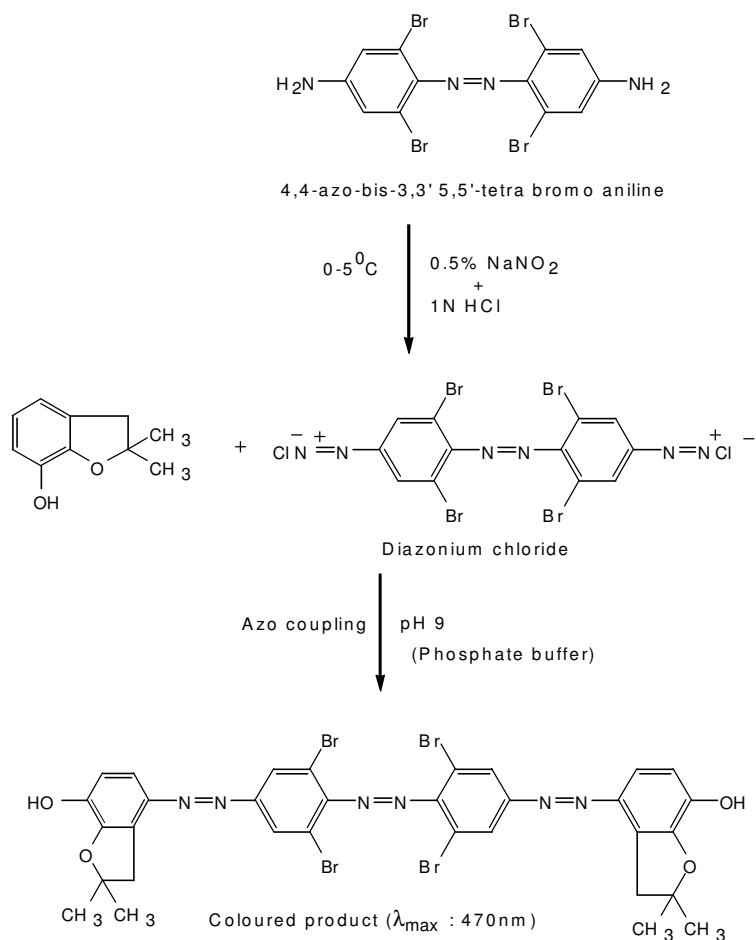
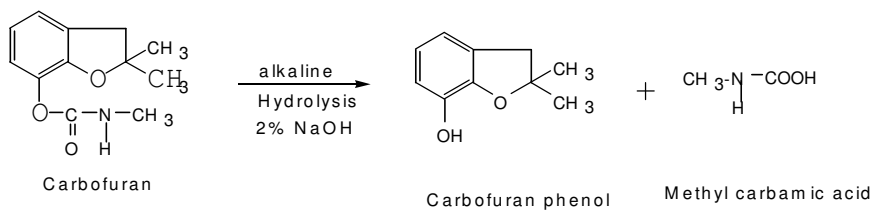
Determination of Carbofuran in insecticidal formulation

Carbofuran in 3% granules, 10% formulations, 50% soluble powder and 75% wetted powder were analysed using aforesaid procedure by coupling diazotized compound. 4,4-azo-bis-3,3',5,5'-tetra bromo aniline which is present in **Table 1**.

Table 1 Determination of carbofuran in insecticidal formulations

Sample	Found ($\mu\text{g ml}^{-1}$)	Recovery (%) ^a
3% Granules	2.97	99.0 \pm 0.016
10% Formulations	9.79	97.90 \pm 0.010
50% Soluble powder	49.74	99.48 \pm 0.36
75% Wetttable powder	74.52	99.37 \pm 0.34

^aMean \pm Standard deviation(n=4).



Scheme 2 Reaction mechanism of 4,4'-azobis-3,3',5,5'-tetrabromo aniline with carbofuran

Determination of carbofuran in water samples

Distilled and tap water samples were fortified with the concentrations ranging from 0.6- 3.6 ppm in methanol. The fortified water samples were extracted with chloroform. The extracts were washed with 0.1 M potassium carbonate solution to breaks 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 was determined by using the procedure described earlier. The results were shown in **Table 2**.

Table 2 Recovery of Carbofuran from fortified water samples 4.4-azo-bis-3,3',5,5'-tetra bromo aniline

Sample	Added ($\mu\text{g ml}^{-1}$)	Proposed method				Reported method ^{5,6}		
		Found ($\mu\text{g ml}^{-1}$)	Recovery (%) ^a	<i>f</i> -test	<i>t</i> -test	Added ($\mu\text{g ml}^{-1}$)	Found ($\mu\text{g ml}^{-1}$)	Recovery (%) ^a
Tap Water	0.6	0.58	96.66 ± 0.14	0.50	3.82	0.4	0.37	92.50 ± 0.09
	1.2	1.17	97.50 ± 0.16	0.83	0.49	0.6	0.58	96.66 ± 0.10
	1.8	1.78	98.88 ± 0.11	0.20	1.14	0.8	0.78	97.50 ± 0.05
	2.4	2.29	95.41 ± 0.18	0.62	3.75	1.0	0.91	91.00 ± 0.25
	0.6	0.59	98.33 ± 0.03	0.25	3.23	0.4	0.38	95.00 ± 0.07
Distilled water	1.2	1.19	99.16 ± 0.08	0.85	5.67	0.6	0.56	93.33 ± 0.07
	1.8	1.76	97.77 ± 0.05	0.54	1.41	0.8	0.77	96.25 ± 0.03
	2.4	2.39	99.58 ± 0.04	0.90	6.45	1.0	0.93	93.00 ± 0.04

^a Mean \pm standard deviation (n=4)

Determination of carbofuran in grain samples (Rice and wheat)

The grain samples (rice and wheat) of 50 gm each were taken in warming blender and blended for 5 min with 100 ml of chloroform. The samples were fortified with different concentrations of insecticides in methanol and blended for 3 min. The chloroform was filtered and the residue as retained. The residue was washed twice with 10 ml of chloroform and blended for 2 min. The chloroform extracts were evaporated on a steam bath and the residue was dissolved in methanol and the amount was determined by the procedure described earlier. The results were shown in **Table 3**.

Table 3 Recovery of carbofuran from fortified grain samples using 4,4-azo-bis-3,3',5,5'-tetra bromo aniline

Sample	Proposed method					Reported method ^{5,6}		
	Added ($\mu\text{g ml}^{-1}$)	Found ($\mu\text{g ml}^{-1}$)	Recovery (%) ^a	<i>f</i> -test	<i>t</i> -test	Added ($\mu\text{g ml}^{-1}$)	Found ($\mu\text{g ml}^{-1}$)	Recovery (%) ^a
Rice	0.5	0.498	99.6±0.21	0.013	1.16	0.5	0.492	98.3±0.03
	1.0	0.991	99.1±0.21	0.099	0.11	1.0	0.981	98.1±0.07
	1.5	1.481	98.7±0.63	0.0006	0.48	1.5	1.437	95.8±0.03
	2.0	1.975	98.7±0.46	0.008	1.33	2.0	1.916	95.8±0.06
Wheat	0.5	0.496	99.2±0.25	0.003	0.77	0.5	0.495	99.0±0.02
	1.0	0.989	98.9±0.36	0.013	0.23	1.0	0.983	98.3±0.05
	1.5	1.477	98.4±0.39	0.044	0.52	1.5	1.496	97.7±0.09
	2.0	1.955	97.7±0.49	0.001	0.46	2.0	1.917	95.8±0.03

^a Mean \pm standard deviation (n=4)

Results and Discussion

The optical characteristics and precision data was presented in **Table 4**. The molar absorptivity and Sandell's sensitivity were found as $4.126 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and $0.0182 \mu\text{g cm}^{-2}$. These values show that the method is highly sensitive. The correlation coefficient values obtained for this complex is very close to unity, suggesting that the absorbance depends on the concentration of the carbofuran. The values obtained for the standard deviation and percentage error suggest that, this procedure offers a good precision and accuracy.

Ringboom's plot is the established standard adopted to know the optimum range of concentration for a system that obey's Beer's law. The plot is drawn between $\log C$ of Carbofuran with 4,4-azo-bis-3,3',5,5'-tetra bromo aniline (1-*T*) (where *T* is the transmittance). The plot has a sigmoid shape with linear segment at intermediate absorbance values 0.2-1.8 and concentration values – 2.0 – 16.0 $\mu\text{g ml}^{-1}$. The slope of the Ringboom's plot from **Figure 2** is 0.42. Hence, the ratio between the relative error in concentration and photometric error is 6.0 for a concentration of 0.60 for 0.1% photometric error.

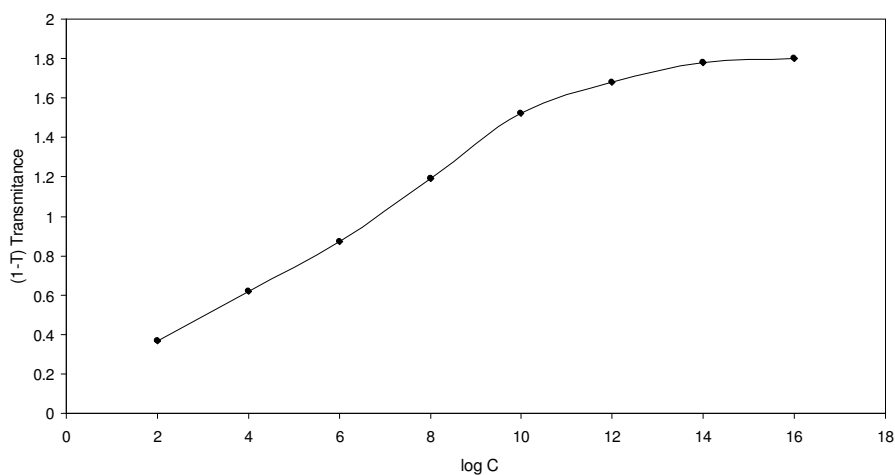
Table 1 show that the active ingredient present in the formulations of carbofuran can be successfully determined spectrophotometrically by using proposed new reagent. The results of these recoveries reveals that the amounts are very close to the manufacturer's specifications and these are favorably compared those reported method in the literature^{5,6}.

The data presented in **Table 2** and **3** suggested that the percentage of carbofuran recovery from fortified water and grain samples range from 95.41 to 99.6% which shows the present method was more reliable, facile and sensitive than the reported method^{5,6}.

Table 4. Optical characteristics and precision data of 4,4-azo-bis-3,3',5,5'-tetra bromo aniline

Optical characteristics and Precision data	Value
Colour	Red
λ_{\max} (nm)	470
Stability (h)	36
Beer's law range ($\mu\text{g ml}^{-1}$)	0.1-16.0
Limit of detection ($\mu\text{g ml}^{-1}$)	0.0621
Limit of quantification ($\mu\text{g ml}^{-1}$)	0.2172
Molar absorptivity ($\text{l mol}^{-1} \text{cm}^{-1}$)	4.126×10^4
Sandwell's sensitivity ($(\mu\text{g cm}^{-2})$)	0.0182
Standard deviation (S.D %) ^b	0.010
Correlation coefficient (\bar{Q})	0.9991
Relative error (%) ^c	1 ± 1.2
Slope (a)	0.0421
Intercept (b)	0.2115

^a $Y=bx+a$; where x is the concentration in $\mu\text{g ml}^{-1}$, ^b $n=4$, ^cFour replicates.

**Figure 2.** Ringboom's plot of carbofuran+4,4-azo bis-3,3',5,5'-tetra bromoaniline complex

These observations suggested that the other ingredients present in these formulations do not interfere in the analysis of carbofuran in various environmental matrices. It is evident from the **Table 2** and **Table 3** that the proposed method is simple, rapid and sensitive. Moreover this method does not involve elaborate cleanup procedures as required by the

other methods^{5,6}. Hence, the proposed method can be adopted as an additional technique for the determination of the carbofuran in its formulations, water and grain samples.

Application

The proposed method was satisfactorily applied to the determination of carbofuran in its formulations, water and grain samples. The results obtained were in good agreement with the reported methods.

Conclusion

The present method describes simple analytical procedure for the determination of carbofuran in its formulations, water and grain samples. The preparation of the coupling reagent 4.4 -azo-bis-3,3',5,5'-tetra bromo aniline was simple and in an classical laboratory. The colour derivatives of the carbofuran is more stable than the other reported method^{5,6}.

The proposed method has the following distinct advantages

- 1) The results of the carbofuran recoveries from fortified water and grain samples with the reagent were superior than the reported methods in the literature^{5,6}.
- 2) The proposed method can be applied as routine analysis for the determination of carbofuran in its commercial formulations and environmental samples.
- 3) All the non-target species do not interfere with the present method.

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