Improved Method For The Estimation Of Carbaryl Residues In Various Environmental Samples

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ABSTRACT

A rapid, sensitive and simple spectrophotometric method has been developed for the determination of alkaline hydrolysis product of carbaryl (1-naphthol) using 4-hydroxy anilinium hydrochloride in the presence of copper (II) as a catalyst. The absorbance is measured at 630 nm. Beer's law is valid over the concentration range of 0.1-2.75μg ml^{-1} and molar absorptivity found to be 4.56 X 10^4 l mol^{-1}cm^{-1}. The Sandell's sensitivity and relative standard deviation were found to be 4.41 ng cm^{-2} and 0.907 %. The method is successfully employed for the determination of carbaryl in various environmental samples. The results compare favorably with those of official and reported methods.
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INTRODUCTION

Carbaryl is one of the most frequently used carbamate insecticide and widely used for the control of a variety of pests on fruits, vegetables, forage, crops, livestock and pets[1]. Like most carbamates, carbaryl acts as an inhibitor to cholinesterase, one of many important enzymes in the nervous systems of humans, vertebrates and insects[2]. Acetylcholinesterase (AChE) works to break down another chemical, acetylcholine, which is essential in transmitting impulses between nerves. Therefore, when carbaryl is used, AChE becomes unable to break down acetylcholine, which consequently accumulates in nerve cells. This abnormal acetylcholine build-up can cause in co-ordination, rapid twitching, paralysis and death[3,4].

Carbaryl has deleterious effects on a variety of wildlife including beneficial arthropods, birds, fish, earthworms, plants and bacteria. Because it is a broad spectrum insecticide with a mode of action common to the majority of living organisms, its target insects (mites) are not the only organism killed by its use, but numerous beneficial insects. This has been

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found in both agricultural and non-agricultural systems[5]. Toxicity to fish is varied but can cause death in concentrations as low as 2-16ppm in water[6]. Sub lethal effects such as damage to gill and liver cells, kidney lesions were visible at concentrations less than 1ppm[7].

Because of the aforementioned toxic effects of carbaryl, it is inevitable to find a rapid, simple and sensitive and reliable method to analyse the residues of carbaryl in various environmental samples. Through the comprehensive literature survey it is found that some of the spectrophotometric methods[7-14] are reported.

The present communication is the improved method of Sastry et al[10]. Sastry employed p-aminophenol for estimation of carbaryl. The colored product obtained by Sastry et al is less stable and less sensitive. In this proposed method, stability of the colored product, sensitivity and recovery are very much improved as compared to Sastry et al. This paper explains simple method developed for the estimation of hydrolyzed carbaryl using 4-hydroxy anilinium hydrochloride in the presence of copper as a catalyst in alkaline medium. The method offers the advantages of simplicity, sensitivity and rapidity without need for extraction.

EXPERIMENTAL

Instrumentation

"Systronics Spectrophotometer-106" model with 10mm matched quartz cells was used throughout the spectral measurement.

Chemicals and glassware

All the chemicals and solvents used were of AR and pesticide grade. Distilled water was used for diluting the other reagents throughout the experiment. Class-A glassware were used for present study.

Standard Carbaryl solution (100 µg ml^{-1})

Carbaryl pesticide (99.9%) was obtained from Bayer India Ltd. A stock solution of carbaryl (100 µg ml^{-1}) was prepared by dissolving 10mg of carbaryl in acetone in 100ml volumetric flask and diluted to 100ml with acetone. The stock solution was

further diluted to 10 µg ml^{-1} using acetone and it can be used as a working solution.

Hydroxy anilinium hydrochloride (0.025%)

Prepared by transferring 0.025 g of 4-hydroxy anilinium hydrochloride obtained from Merk (Germany), in to 100 ml volumetric flask, dissolved and diluted to mark with methanol.

Other reagents

Copper (II) solution (500 µg ml^{-1}) was prepared in 100ml volumetric flask using 0.5ml concentrated sulfuric acid and diluted with water.

5 M NaOH solution was prepared in methanol.

Standard procedure

An aliquot of a sample containing 0.1-2.75µg ml^{-1} of working standard carbaryl solution and 1.0 ml of 5 M of methanolic sodium hydroxide solution was added into a series of 10 ml of calibrated volumetric flask and allowed to stand for 2 min to complete hydrolysis. To this content, 0.5 ml of 0.025% of methanolic solution of 4-hydroxy anilinium hydrochloride and aqueous solution of 0.1 ml of 500 ppm of Cu^{2+} were added. After 5 min, the colored solution was diluted using methanol and absorption of the resulting blue-colored product was measured at 630 nm against a corresponding coreless reagent blank. A calibration graph was constructed.

RESULTS AND DISCUSSION

Spectral characteristics

The proposed method involves coupling reaction between 4- hydroxy anilinium hydrochloride and hydrolyzed carbaryl in presence of copper (II) as catalyst in alkaline medium to produce the bluish green colored product with λ_{max} of 630 nm. The reagent blank had negligible absorption at this wavelength. The absorption spectrum of the reaction product is shown in figure 1.

Optimization and reagent concentration

Various concentration and volume ranges for all the reagents were studied in detail. It was found that methanolic Sodium hydroxide in the range of 0.5-
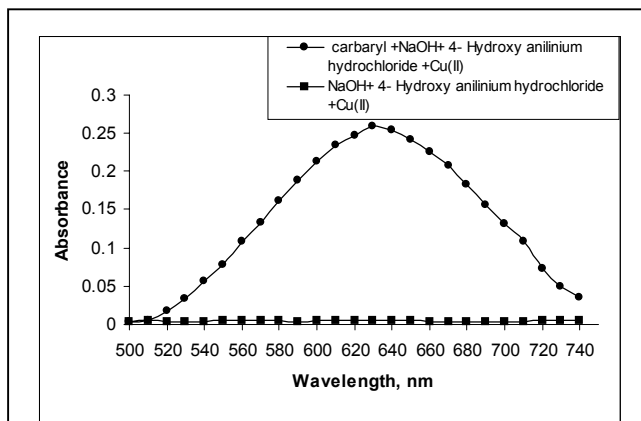
Figure 1: Absorption spectrum of the reaction product of alkaline hydrolyzed carbaryl and 4-hydroxy anilinium hydrochloride. Final concentration of carbaryl 1.2 μg ml^{-1}.

TABLE: 1 Optical characteristics and precision data for the product

Optical parameters	carbaryl with 4–hydroxyl anilinium hydrochloride
Colour	Blue
λ_{max} (nm)	630
Stability (h)	5
Beer's law range μg ml^{-1}	0.1-2.75
Molar absorptivity l mol^{-1}cm^{-1}	4.53 x 10^4
Sandell's Sensitivity ng cm^{-2}	4.40
Detection limit μg ml^{-1}	0.03
Quantitation limit μg ml^{-1}	0.09
Regression equation, Y*	Y= ax + b
Slope (a)	0.248
Intercept (b)	-0.0187
Correlation Coefficient (r)[b]	0.9990

*Y= ax+b, Where x is the Concentration of carbaryl (Sevin) in μg ml^{-1}
[b]n = 5

1.5 ml, 4-hydroxy anilinium hydrochloride 0.5-1.25 ml, and Cu^{2+} in the range of 0.1-0.6 ml were necessary to obtain a stable blue colored product with maximum absorption. Required volumes of the reagents were hence used as noted in the standard procedure. Further dilution of the reaction mixture was tried with different solvents like ethanol, water, and acetone. Methanol gave the best result with maximum color intensity and stability.

Quantification

Adherence to Beer's law by the colored product of alkaline hydrolyzed carbaryl with 4-hydroxy anilinium hydrochloride was determined by measuring the absorbance at 630 nm. Constructed a calibrated graph using values obtained by the series of solutions containing varying amounts of analyte and specified amounts of reagents, against colorless reagent blank. Beer's law obeyed over the carbaryl concentration 0.1-2.75 μg ml^{-1}. Limit of quantification (LOQ) is determined by taking the ratio of standard deviation (σ) of the blank with respect to water and the slope of the calibration curve (s) multiplied by a

factor 10. Limit of detection (LOD) is determined by taking the ratio of standard deviation (σ) of the blank with respect to water and the slope of the calibration curve (s) multiplied by a factor 3.3. The upper limit of the Beer-Lambert range is determined by series of concentrated solutions and lower limit of Beer-Lambert range determined by series of diluted solutions at the value of λ_{max}. Beyond this limit, the correlation results were really affected. Hence, the measurements were excluded above and below these limits to keep the relationship linear. The analytical parameters are given in TABLE 1.

Method validation

Accuracy and precision

The accuracy of the methods was established by recovery studies of carbaryl at three levels (within the calibrated graph). The precision was ascertained by calculating the relative standard deviation (RSD)

TABLE 2: Evaluation of accuracy and precision of the proposed method

Carbaryl taken (μg)	Carbaryl found (μg)*	Range (μg)	Standard deviation	Relative standard deviation (%)	Relative error (%)
1.0	1.032	0.047	0.017	1.638	1.636
2.0	1.991	0.032	0.012	0.597	0.596
2.75	2.776	0.074	0.028	1.024	1.023

* Mean value of seven determinations

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of seven replicate determinations at three levels. The relative error (%) and RSD (%) values which were less than 2% are indicative of good accuracy and precision of the method (TABLE -2).

Stability

The colored product of hydrolyzed carbaryl formed by the proposed method was found to be stable for 5 h at room temperature. Reproducible results were obtained in the temperature range of 20-40°C. An increase in temperature of above 40°C decreased the absorbance readings, indicating the decomposition of the product. However, a temperature of 30°C is recommended for the absorbance measurements of the colored product.

## Effect of diverse ions and pesticides

In order to evaluate the suitability of the proposed method, the effect of various ions and pesticides which are likely to interfere in the determination of carbaryl was studied by adding a known amount of diverse ions and pesticides to 1 μg ml^{-1} solution of carbaryl. The tolerance limits of interfering species were established at the concentrations that do not cause more than ± 2 % error in absorbance values. The interference of Ca^{2+} and Mg^{2+} could be masked by adding 1 ml of 2 % EDTA. The tolerance limits of foreign ions are listed in TABLE 3.

TABLE 3: Effect of interfering ions on the determination of carbaryl (1 μg ml^{-1})

Interferents	Tolerance limit (μg ml^{-1})
Parathion, methyl parathion, fenitrothion, EDTA	< 5000
4-nitrophenol, phenol, aldrin	< 1000
Malathion,	900
$Ca^{2+}, Mg^{2+}, Al^{3+}, Pb^{2+}, Cd^{2+}$	650
$SO_4^{2-}, NO_2^-, CO_3^{2-}, PO_4^{3-}$	300

Proposed reaction scheme

Carbaryl on treatment with sodium hydroxide gives 1–Napthol. This hydrolyzed product gives bluish indo dye when reacted with 4-hydroxy anilinium hydrochloride in the presence of Cu^{2+}. The proposed reaction path way is given in SCHEME 1.

SCHEME 1: Proposed reaction path way
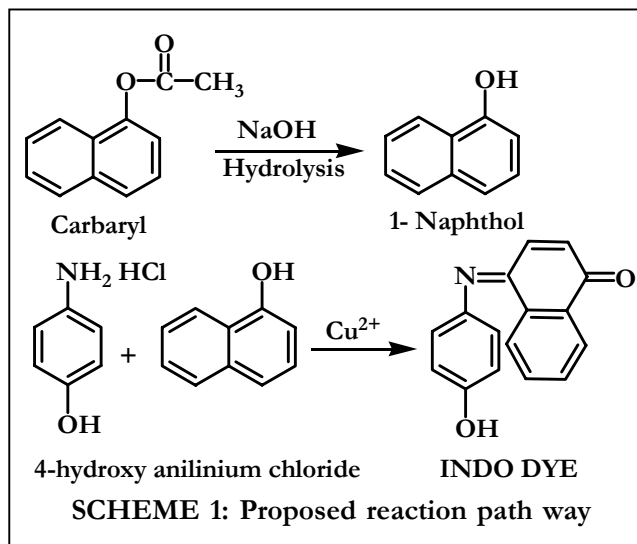
Applications of the method

Determination of carbaryl in vegetables

Vegetables such as beans and cabbage samples of about 100g were meshed and spiked with known amount of carbaryl (μg ml^{-1}); carbaryl was extracted with chloroform and dried to evaporate the solvent. This residue was dissolved in methanol and analyzed as mentioned in standard procedure. Obtained results are shown in TABLE 4.

TABLE 4: Recovery of carbaryl in vegetables

Samples	Amount of carbaryl added μg ml^{-1}	Amount of carbaryl found μg ml^{-1}	Mean recovery In % and ± SD[b]
Cabbage	1.0	0.97	96.5 ± 0.58
	2.0	1.91	95.3 ± 0.56
	2.75	2.65	96.2 ± 0.63
Beans	0.75	0.71	95.0 ± 0.45
	1.5	1.42	94.6 ± 0.57
	2.5	2.41	96.5 ± 0.65

[b] n = 5

Determination of carbaryl in grains

The grain samples such as rice and wheat of 100 g 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 5 min chloroform layer was filtered. The residue was washed twice with 10 ml of chloroform each and blended for 2min and filtered. The chloroform extracts were evaporated to dryness and obtained residue was dis-

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TABLE 5: Recovery of carbaryl residues from grain samples

I Wheat	Amount of carbaryl added $\mu g\ ml^{-1}$	Amount of carbaryl found $\mu g\ ml^{-1}$	Mean recovery In % and $\pm SD^b$
1)	0.5	0.49	97.0 ± 0.45
2)	1.75	1.73	99.0. ± 0.52
3)	1.0	0.97	96.8 ± 0.35
II Rice			
1)	1.5	1.46	97.5 ± 0.35
2)	2.0	1.98	99.0 ± 0.50
3)	2.75	2.70	98.0 ± 0.42

[b]n=5

solved in methanol and analyzed as mentioned in standard procedure. Obtained results are shown in TABLE 5.

Recovery of the carbaryl residues from spiked water samples

Water samples (100ml) were spiked with known amount of carbaryl ($\mu g\ ml^{-1}$), the pH of these samples adjusted to 3-4 with 2M sulphuric acid. The mixture was transferred into a separating funnel and insecticide was extracted using approximately 15 ml of chloroform in each occasion till complete extraction. The combined extracts were washed with 2ml of 0.1M potassium carbonate to break any emulsion formed during the extraction. The extracted pesticide was dried over anhydrous sodium sulphate.

TABLE 6: Recovery of carbaryl residue from spiked water sample

Water sample	Carbaryl added in $\mu g\ ml^{-1}$	Carbaryl found in $\mu g\ ml^{-1}$	Mean Recovery in % and +/- SD^b
Well water (1)	1.0	0.98	98.00 ± 0.49
Well water (2)	0.75	0.74	98.50 ± 0.50
River water(1)	0.5	0.51	101.0 ± 0.44
River water(2	1.5	1.46	97.33 ± 0.45
Tap water (1)	2.0	1.98	99.00 ± 0.50
Tap water (2)	2.5	2.54	101.5 ±0.43

[b]n = 5

Chloroform was evaporated and residue was dissolved in methanol. Determination was carried out by above procedure and data are shown in TABLE- 6.

CONCLUSION

The proposed spectrophotometric method is simple to perform, cheaper, specific and sensitive when compared to many other existing methods (TABLE 7). The sensitivity, simplicity, temperature independence and stability of the colored product are the advantages of this method. This method does not involve extraction step and the use of carcinogenic solvents. This method can be used to detect

TABLE 7: Comparison of other spectrophotometric methods with proposed method

Methods	λ_{max} nm	Beer's law range μgml^{-1} and Molar absorptivity $l\ mol^{-1}\ cm^{-1}$	Remarks
p-Aminobenzoic acid[a]	470	0.1- 0.8 and 2.00 X 10^4	Extraction is necessary Dye stability up to 24 h
p-Dimethylphenylene diamine dihydrochloride[b]	600	0.7-8.0 and 1.41 X 10^4	Reagent itself toxic
p-Aminophenol [b]	600	0.8-10 and 1.33 X 10^4	Not much sensitive Dye stable up to 8 min
2-Amino, 4-nitrophenol [c]	520	0.4-10 and 3.018 X10^4	Moderately sensitive Dye stable up to 24h
2,4-Dimethoxy aniline[c]	505	0.4-10 and 3.42 X10^4	Moderately sensitive Dye stable up to 10h
Hydrolyzed carbaryl with 4-hydroxy anilinium hydrochloride	630	0.1-2.75 4.56 X 10^4	Highly sensitive Dye stable up to 5 h

[a]Ref 8, [b]Ref 10 and [c]Ref 14

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0.03 μg ml^{-1} and quantitatively detect 0.09 μg ml^{-1} of carbaryl. Proposed method does not entail any stringent experimental variables which affect the reliability of the results. From the above aspects it revels that this method can serves as an alternative method for determining carbaryl in various environmental samples.

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