CHAPTER - V

A SIMPLE SPECTROPHOTOMETRIC DETERMINATION OF CARBARYL IN GRAINS AND INSECTICIDE FORMULATIONS USING DIAZOTIZED p-AMINOACETOPHENONE

SUMMARY

The method described for the determination of carbaryl in grains and insecticide formulations is based on the coupling of carbaryl with diazotized p-aminoacetophenone in a fairly alkaline medium to produce a purple coloured dye having absorbance maxima at 545 nm. The dye thus formed is extractable in n-butanol. The extract has a maximum absorbance at 555 nm. Beer's law is obeyed in the range of 0.05 - 0.4 ppm of carbaryl. The method is found to be free from interferences of large amounts of phenol, 1-naphthol and other commonly used carbamate insecticide, propoxur. Other optimum reaction conditions and analytical parameters have been studied.

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Carbaryl (1-naphthyl-N-methyl carbamate), a representative of a group of carbamates having insecticidal properties, appears to show promise in the control of a wide range of pests. It is usually sold under the trade name of Sevin or 7744. Dust and wettable formulations of carbaryl have given good control of all major apple and pear insects, boll weevil, boll worm, and pink leaf hopper on grapes. Considerable promise is being shown against a large variety of insect pests attacking forage crops, forests, cotton, livestock, vegetables, fruits and other economic crops. It is commonly used in soil and also used for mite control in woodlands and orchards (1-3).

The wide usage of carbaryl in agriculture for protection of crops is continually increasing and this is reflecting in the increasing number of criminal cases referred to forensic science laboratories concerning the misuse of this compound (4). The acute LD$_{50}$ in rat is 500-700 mg/kg and the maximum allowable concentration is 5 mg/m$^3$ (0.5 ppm) (3,5). Recently a tolerance of 2.0 ppm has been established for residues of carbaryl in or on
pineapples, under the Federal Food Drug & Cosmetic Act by U.S. Environmental Protection Agency (6).

Carbaryl is a dangerous chemical, being an anticholinesterase compound, a suspected carcinogen, teratogen and a skin irritant, and it is also dangerous to fish and wildlife. It may pose special problems for people eating a low protein diet (7,8). Although carbaryl is acknowledged to be moderately hazardous, even by its proponents, one of the intermediary products, methyl isocyanate, is known to be instantly toxic and regarded as very dangerous. However, because intermediate substances do not require the same safety clearances as formulated pesticides, neither Union Carbide, nor United Nations agencies monitoring toxic substances, knew very much about its toxicity before the accident at Union Carbide, Bhopal (chemical plant mainly producing carbaryl) (9).

The toxicity of carbaryl arises from the combination of atoms known as the carbamate group,

\[ \text{H} \quad \text{N} - \text{C} \equiv \text{O} \quad \text{Compounds containing the amino (\(-\text{NH}_2\)) and carboxy (\(-\text{COOH}\)) groups are termed amino acids. Carbamic acid is an example of an amino acid and carbaryl is the derivative of carbamic acid. At least 26 different amino acids are known to be essential to the structure of protein. An upset of the balance of these structures in the body by carbamic acid derivatives cause sickness and death (10).} \]
Carbaryl, a contact insecticide is also feebly systemic in fruit trees by diffusion through the bark (8). The major symptoms of poisoning by carbaryl are related to the accumulation of acetylcholine and include bradycardia and decreased stroke volume accompanied by diarrhea and vomiting due to gastrointestinal hypermotility of muscles and increased secretion of bronchial, lacrimal, pancreatic, salivary and sweat glands. Atropine is the preferred antagonist, because it blocks the postsynaptic depolarization (11,12).

Looking into its importance as a health hazard many spectrophotometric methods have been reported in the literature (13-32). The general instrumental methods used for the determination of carbaryl involve the use of gas chromatography (33,34), liquid chromatography (35), high performance liquid chromatography (36,37), thin layer chromatography (38), fluorimetry (39,40), enzymatic inhibition radiometry (41), mass spectrophotometry (42) and phosphorimetry (43). Spectrophotometric methods for determining carbaryl residues are generally based on alkaline hydrolysis of carbaryl to 1-naphthol, with subsequent coupling of this with various chromogenic reagents (13-24). These methods suffer from interference by 1-naphthol present in residue. The method described by Yuen (27) using diazotized sulphanilic acid is attractive, because preliminary hydrolysis is not required, but it suffers from the disadvantages of poor
dye stability and low sensitivity. The commonly used chromogenic reagents for the spectrophotometric determination of carbaryl are: p-aminophenol (13), p-N,N-dimethyl phenylene diamine (13), 1-amino-2-naphthol-4, sulphonic acid (13), 3-methyl benzothiazoline 2-one hydrazone hydrochloride (15), 4-dimethyl amino benzaldehyde (16), 4-dimethyl amino cinnamaldehyde (16), bis (4-aminophenyl) sulphone (17), 4-aminoantipyrine + K₃Fe(CN)₆ (18,19), 4-nitrobenzene diazonium tetrafluoroborate in acetone methanol (1:1) (20), sulphanilamide (21,29), vanillin (22), p-nitrobenzene diazonium fluoroborate in acid or alkaline medium (23,24), o-toluidine (25), 2,5-dichloroaniline (26) and sulphanilic acid (27), etc.

In the present investigation, a new, simple, sensitive and rapid spectrophotometric method is described, where carbaryl is determined by coupling with a new chromogenic reagent, diazotized p-aminacetophenone in fairly alkaline medium, and the blank value is negligible. The dye formed is extractable in n-butanol. The purple coloured extract has absorbance maximum at 555 nm. The dye is stable for ~24 hours. The method has been found to be satisfactory for the determination of carbaryl in grains, pulses, and insecticide formulations.
EXPERIMENTAL

Apparatus:

A Carl Zeiss spekol and a Varian DMS 100S UV-visible spectrophotometer with 1 cm matched silica cells were used for all spectral measurements. A separatory funnel of 250 ml and calibrated flasks were used.

Reagents:

Standard carbaryl solution: A 0.1% stock solution of carbaryl was prepared in ethanol using reference standard material.

A working standard of 10 μg/ml of carbaryl was prepared by appropriate dilution of the stock.

p-Aminoacetophenone (PAAP)(44): A 1% w/v solution of PAAP was prepared in 1:4 hydrochloric acid.

Sodium nitrite solution: A 0.3% w/v solution of sodium nitrite was prepared daily in demineralized water.

Sodium hydroxide: An 8 M solution of sodium hydroxide solution was prepared in demineralized water.

n-Butanol was used for extraction.

Technical grade insecticide formulations (Union Carbide Co., Bhopal, M.P.): Wettable and dust forms were used for analysis.

All reagents unless mentioned otherwise used were of AnalaR grade.
**Procedure:**

**Preparation of calibration graph:**

A 100 ml aqueous solution containing 5-40 μg of standard carbaryl was taken in a 250 ml separatory funnel. To this solution, 2 ml of PAAP reagent was added and the acidity was adjusted to ~0.5 M with hydrochloric acid. After 2 minutes, 5 ml of sodium nitrite solution was added and the color was developed with 10 ml of 8 M sodium hydroxide solution which was added by a fast running pipette. The solution was kept for 5 minutes for full color development. The purple colored dye was then extracted with 10 ml of n-butanol. The extract was dried over anhydrous sodium sulfate and absorbance was measured at 555 nm, against a reagent blank. The calibration graph was obtained by plotting the absorbance versus concentration of carbaryl.

**Determination of recovery in grains:**

100 g of grain samples were blended for 5 minutes with 200 ml of chloroform in a blender. The samples were then spiked with different concentrations of carbaryl (5-40 μg) in 5 ml of ethanol and the spiked samples were blended further for 2 minutes. The chloroform solution was then decanted into a 250 ml calibrated flask through Whatman No. 1 filter paper retaining the residue in the blender. Blending and decanting was repeated twice with 10 ml portions of chloroform. Chloroform extracts were
combined and diluted to the mark. The chloroform extract was evaporated off under reduced pressure using moderate suction on a water bath at about 50°C. The residue was dissolved in 10 ml of ethanol, and the colour was developed as described in the preparation of the calibration graph.

**Determination of carbaryl in insecticide formulations:**

The well mixed insecticide formulations (labelled amount in %) equivalent to 0.25 - 2.0 mg carbaryl were dissolved in 25 ml of ethanol and centrifuged for 5 minutes. The supernatant liquid was decanted into a clean, dry, 50 ml volumetric flask. The residue was washed again with another 10 ml portion of ethanol. The extracts were combined and diluted to the mark with ethanol. The known aliquots of this solution were used for the colour development as described in the preparation of the calibration graph. The results have been compared with the reference method (25)(Table I).

**RESULTS AND DISCUSSION**

Spectral characteristics:

The absorption spectra shows that the purple coloured dye has a maximum absorption at 555 nm in butanol. The absorbance of the reagent blank was found negligible at this wavelength (Fig. 1).
G.1. ABSORPTION SPECTRA OF THE DYE.

A. CONCENTRATION OF CARBARYL = 20 µg/100 ml.
B. CONCENTRATION OF CARBARYL = 30 µg/100 ml.
C. REAGENT BLANK.
Effect of Variables:

Effect of varying reagent concentrations were examined. It was observed that at least 1 ml of FAAP solution and 5 ml of sodium nitrite solution were required for full colour development. Excess amounts of these reagents did not cause any adverse effect (Figs. 2 & 3).

The effect of acidity on the diazotization was studied. Results show that, at least 0.4 M hydrochloric acid was necessary for complete diazotization and constant absorbance values were obtained over the acidity range of 0.4 - 1.0 M hydrochloric acid (Fig. 4).

It was observed that at least 0.6 M and above sodium hydroxide in final volume was needed for the colour development (Fig. 5). 2 minutes were sufficient for diazotization and coupling reaction.

No significant changes were observed in the absorbance values within the temperature range of 15-30°C (Fig. 6). The dye was found to be stable for ~ 24 hours at this temperature range.

Beer's law, Molar absorptivity and Sandell's sensitivity:

The colour system was found to obey Beer's law in the range of 5-40 µg of carbaryl per 100 ml solution (0.05 - 0.4 ppm) (Fig. 7). The molar absorptivity and Sandell's sensitivity were found to be $4.0 \times 10^5$ lit mol$^{-1}$cm$^{-1}$ (± 100) and 0.005 µg cm$^{-2}$ (± 100), respectively.
FIG. 2. EFFECT OF AMOUNT OF P-AMINOACETOPHENONE ON COLOUR DEVELOPMENT

CONCENTRATION OF CARBARYL = 15 µg/100 ml.

FIG. 3. EFFECT OF AMOUNT OF NITRITE ON COLOUR DEVELOPMENT.

CONCENTRATION OF CARBARYL = 15 µg/100 ml.
4. EFFECT OF ACIDITY ON COLOUR DEVELOPMENT.
CONCENTRATION OF CARBARYL = 15 μg/100 ml.

5. EFFECT OF SODIUM HYDROXIDE ON COLOUR DEVELOPMENT
CONCENTRATION OF CARBARYL = 15 μg/100 ml.
FIG. 6. EFFECT OF TEMPERATURE ON THE FINAL ABSORBANCE

CONCENTRATION OF CARBARYL = 15 μg/100 ml.

FIG. 7. CALIBRATION FOR THE DETERMINATION OF CARBARYL.
Reproducibility of the method:

To check the reproducibility of the method a 100 ml solution containing 10 μg (0.1 ppm) of carbaryl was studied for a period of 7 days. The standard and relative standard deviations were found to be ± 0.0056 and ± 2.7% respectively (Table II).

Effect of foreign species:

To assess the validity of the method the effects of various foreign species associated with 0.2 ppm of carbaryl have been studied. The tolerance limits of various foreign species are given in Table - III.

In carbaryl formulation normally 1-naphthol is present as a contaminant or formed by decomposition during storage, which gives an orange colour when the solution is rendered alkaline. Under normal conditions separation of 1-naphthol or phenol from the compound is not required because up to a tenfold excess of these have no appreciable effect on the determination. But if substantial amounts of naphthol or phenols are suspected, the extract should be washed twice with 0.2 - 0.3 M sodium hydroxide solution and then with water to remove these contaminants before the chloroform is evaporated off and then the colour is developed as reported (28). Studies conducted to determine the optimum concentration of sodium hydroxide required to hydrolyze carbaryl revealed that least 0.6 M and above sodium hydroxide
**TABLE - I**

**DETERMINATION OF CARBARYL IN INSECTICIDE FORMULATIONS**

<table>
<thead>
<tr>
<th>Insecticide formulations of carbaryl amount of Technical Grade</th>
<th>Labelled</th>
<th>Carbaryl found* by present method</th>
<th>Carbaryl found by reference method (27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>1. Dust form</td>
<td>2</td>
<td>1.86</td>
<td>1.87</td>
</tr>
<tr>
<td>2. Dust form</td>
<td>5</td>
<td>4.37</td>
<td>4.25</td>
</tr>
<tr>
<td>3. Wettable form</td>
<td>50</td>
<td>49.15</td>
<td>48.62</td>
</tr>
<tr>
<td>4. Wettable form</td>
<td>100</td>
<td>98.92</td>
<td>97.88</td>
</tr>
<tr>
<td>5. Wettable form</td>
<td>25</td>
<td>24.81</td>
<td>24.61</td>
</tr>
</tbody>
</table>

* Mean of three replicate analyses.

**TABLE - II**

**REPRODUCIBILITY OF THE METHOD**

Concentration of carbaryl - 10 μg/100 ml (0.1 ppm)

<table>
<thead>
<tr>
<th>No. of days</th>
<th>Absorbance, 555 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.205</td>
</tr>
<tr>
<td>2</td>
<td>0.200</td>
</tr>
<tr>
<td>3</td>
<td>0.210</td>
</tr>
<tr>
<td>4</td>
<td>0.215</td>
</tr>
<tr>
<td>5</td>
<td>0.210</td>
</tr>
<tr>
<td>6</td>
<td>0.205</td>
</tr>
<tr>
<td>7</td>
<td>0.200</td>
</tr>
</tbody>
</table>

Mean = 0.206

Standard deviation = ± 0.0056

Relative standard deviation = ± 2.7%
### TABLE - III

**EFFECT OF FOREIGN SPECIES**

Concentration of carbaryl = 0.2 ppm

<table>
<thead>
<tr>
<th>Foreign species</th>
<th>Tolerance limit (ppm)* of individual compound</th>
<th>Foreign species</th>
<th>Tolerance limit (ppm)* of individual compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathion, Malathion</td>
<td>400 Ca(^{2+}), Cd(^{2+}), Pb(^{2+}), CO(^{2-})</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Quinolphos, Monocrotophos</td>
<td>450 Mg(^{2+}), Cu(^{2+})</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>DDT, BHC</td>
<td>600 Cd(^{2+})</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>2** SO(_4^{2-}), Sb(^{3+})</td>
<td>750</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>2** Al(^{3+})</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Propoxur</td>
<td>2**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Amount may vary ± 2%; ** Tolerance limit without its removal from the sample.
in final solution was needed for the hydrolysis. Since hydrolysis of carbaryl was negligible at 0.3 M sodium hydroxide, it was not lost during extraction of 1-naphthol or phenol. The most commonly found carbamate insecticide, which is usually associated with carbaryl, is propoxur (o-iso propoxy phenyl methyl carbamate). The effect of propoxur on carbaryl was studied and it was found that upto a ten fold excess propoxur does not interfere with the method. Studies on high concentration of propoxur have been made and it was found that the dye formed by propoxur shows a maximum absorbance at 484 nm. Other similar N-methyl carbamates, if present, are likely to interfere; hence, they will have to be separated by initial chromatographic clean up methods by using silica gel and sodium sulphate (19,45).

The recovery of carbaryl from grain and pulse samples were found to be ~99%, which is in agreement with the values from the earlier reported methods(16,19,25) (Table IV). A 100 ml synthetic solution containing 20 µg of carbaryl along with various pesticides and phenols such as parathion (200 µg), malathion (150 µg), DDT (200 µg), BHC (200 µg), phenol (100 µg), 1-naphthol (50 µg) and propoxur (100 µg) taken together was analyzed to determine the selectivity of the method and to check recovery of carbaryl. The recovery of carbaryl was found to be ~99% from this synthetic sample, which proves the selectivity of the method.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of carbaryl originally found (µg)**</th>
<th>Amount of carbaryl added (µg)**</th>
<th>Total Amount of carbaryl found (µg)**</th>
<th>Difference (µg)**</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>1.64</td>
<td>10</td>
<td>11.50</td>
<td>0.14</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>2.45</td>
<td>20</td>
<td>22.11</td>
<td>0.34</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>1.32</td>
<td>30</td>
<td>31.05</td>
<td>0.27</td>
<td>99.1</td>
</tr>
<tr>
<td>Rice</td>
<td>6.31</td>
<td>10</td>
<td>16.24</td>
<td>0.07</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td>2.11</td>
<td>20</td>
<td>21.81</td>
<td>0.30</td>
<td>98.6</td>
</tr>
<tr>
<td></td>
<td>2.32</td>
<td>30</td>
<td>31.55</td>
<td>0.82</td>
<td>97.6</td>
</tr>
<tr>
<td>Maize</td>
<td>1.05</td>
<td>10</td>
<td>10.80</td>
<td>0.25</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td>2.15</td>
<td>20</td>
<td>21.39</td>
<td>0.76</td>
<td>96.6</td>
</tr>
<tr>
<td></td>
<td>1.55</td>
<td>30</td>
<td>31.20</td>
<td>0.35</td>
<td>98.9</td>
</tr>
<tr>
<td>Green Peas</td>
<td>1.02</td>
<td>10</td>
<td>9.81</td>
<td>0.19</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>20.52</td>
<td>0.50</td>
<td>97.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>29.34</td>
<td>0.66</td>
<td>97.8</td>
</tr>
<tr>
<td>Black gram</td>
<td>5.08</td>
<td>10</td>
<td>14.98</td>
<td>0.10</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>3.33</td>
<td>20</td>
<td>23.17</td>
<td>0.16</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>4.15</td>
<td>30</td>
<td>33.79</td>
<td>0.36</td>
<td>98.4</td>
</tr>
</tbody>
</table>

* Amount of sample = 100 g;  ** Mean of three replicate analyses.
The proposed method, compared with the other spectrophotometric methods, has been found to be superior in simplicity, sensitivity and selectivity (Table V). The p-aminoacetophenone has been used earlier in the diazotization and coupling reactions for the determination of nitrite and N-(1-naphthyl)ethylene diamine dihydrogen chloride (44,46), and now it has been successfully used as a sensitive chromogenic reagent for the determination of carbaryl.

**CONCLUSION**

The proposed method is rapid, reproducible, sensitive, and free from interference of large amounts of foreign species. Extraction of dye in n-butanol enables to determine very low amount of carbaryl in large volume of samples and the method does not require elaborate clean up procedures as well as preliminary hydrolysis of carbaryl.
<table>
<thead>
<tr>
<th>No.</th>
<th>Method/Reagents</th>
<th>$\lambda_{max}$</th>
<th>Determination range (ppm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 a</td>
<td>4-Dimethyl amino benzaldehyde (16)</td>
<td>560</td>
<td>1.2 - 10</td>
<td>Requires heating at 60°C for color development</td>
</tr>
<tr>
<td>1 b</td>
<td>4-Dimethyl amino cinnamaldehyde (16)</td>
<td>560</td>
<td>0.65 - 6</td>
<td></td>
</tr>
<tr>
<td>2 a</td>
<td>p-Aminophenol (13)</td>
<td>600</td>
<td>0.8 - 10</td>
<td>Dye stability 7 minutes</td>
</tr>
<tr>
<td>2 b</td>
<td>Dimethyl phenylene diamine dihydrochloride (13)</td>
<td>600</td>
<td>0.7 - 8.0</td>
<td>Reagent toxic</td>
</tr>
<tr>
<td>2 c</td>
<td>1-Amino-2-naphthol 4-sulphonic acid(13)</td>
<td>700</td>
<td>3.0 - 35</td>
<td>Reagent unstable</td>
</tr>
<tr>
<td>3 **</td>
<td>Diazotized sulphamid acid or sulphanilamide(27)</td>
<td>520</td>
<td>2 - 10</td>
<td>Dye stability 10 minutes</td>
</tr>
<tr>
<td>4 *</td>
<td>Vanillin (22)</td>
<td>575</td>
<td>2 - 12</td>
<td>Requires heating of 60°C for color development</td>
</tr>
<tr>
<td>5 *</td>
<td>4-Aminophenazone(19)</td>
<td>475</td>
<td>0.5 - 20</td>
<td>Reagent unstable</td>
</tr>
<tr>
<td>6 **</td>
<td>Present method p-Aminoacetophenone</td>
<td>555</td>
<td>0.05 - 0.4</td>
<td>Dye stability 24 hours; More sensitive and rapid</td>
</tr>
</tbody>
</table>

* Methods that require prior hydrolysis of carbaryl to 1 naphthol for diazotization-coupling reaction.

** Methods that do not require prior hydrolysis for diazotization and coupling reactions.
REFERENCES


