CHAPTER - 4
PART - II
SECTION - A

A NEW THIN-LAYER CHROMATOGRAPHIC SPRAY REAGENT FOR THE SCREENING OF BIOLOGICAL MATERIALS FOR THE PRESENCE OF CARBARYL
A NEW THIN-LAYER CHROMATOGRAPHIC SPRAY REAGENT FOR THE SCREENING OF BIOLOGICAL MATERIALS FOR THE PRESENCE OF CARBARYL

INTRODUCTION

Carbamate insecticides are increasingly being used domestically and in agriculture on a daily basis. Easy availability of these insecticides reflected in the increasing number of criminal cases referred to forensic science laboratories concerning the misuse of carbamates.

A number of chromogenic spray reagents for the identification of carbamate insecticides by thin-layer chromatography (TLC) have been described in the literature. The most common are diazophenol (after alkaline hydrolysis)\textsuperscript{36}, Tollen’s reagent\textsuperscript{35}, and alkaline Fast Blue B\textsuperscript{37}. However, these reagents are normally used for the detection of phenolic compounds or cannabinoids and also susceptible to biological impurities such as amino acids, proteins and peptides and hence are not specific. A spectrophotometric method reported by Appaiah et al.\textsuperscript{43} for the detection of carbaryl in grains and formulations is not generally useful for biological sample owing to interference of constituents of viscera, co-extracted with the insecticides. Hence it was felt necessary to develop a new specific and sensitive chromogenic spray reagent for the detection of carbaryl in biological materials by TLC.
This part describes a new sensitive chromogenic reagent viz. 1% ceric ammonium nitrate in 20% v/v hydrochloric acid, for the detection of carbamate insecticide carbaryl and its breakdown product 1-naphthol. The hydrolysis product of carbaryl, 1-naphthol, reacts with ceric ammonium nitrate to produce a violet colour. If 1% aqueous sodium nitrite solution is sprayed on to the TLC plate, the intensity of the violet colour is enhanced.

EXPERIMENTAL

Reagents

All reagents were of analytical reagent grade. Distilled water was used throughout.

1. **Ceric ammonium nitrate reagent 1%**: A 1 g amount of ceric ammonium nitrate was dissolved in 100 ml of 20% v/v hydrochloric acid.

2. **Sodium nitrite solution 1%**: A 1 g amount of sodium nitrite was dissolved in 100 ml of distilled water.

3. **Sodium hydroxide solution 10%**: A 10 g amount of sodium hydroxide was dissolved in 100 ml of distilled water.

Procedure

A standard glass TLC plate was coated with a slurry of silica gel G (ACME) in water (1+2) by weight to a thickness
of 0.25 mm and the plate was activated at 110°C for about 1 h. An amount of 1 μg of carbaryl in ethanol (1 mg/ml) was spotted on the plate which was then developed in a previously saturated TLC chamber using n-hexane-acetone (4+1) as solvent up to a height of 10 cm.

The plate was removed from the chamber, dried in air and sprayed with 10% sodium hydroxide solution (Warning: Spraying sodium hydroxide solution is hazardous, advice to wear appropriate eye protection) followed by freshly prepared 1% ceric ammonium nitrate reagent. A violet spot was observed immediately on TLC plate at $R_F$ value of 0.45. On spraying 10% sodium nitrite solution on the same plate the intensity of the colour increases. A breakdown product of carbaryl, 1-naphthol, gives a similar colour reaction with the reagent at $R_F$ 0.54, without previous hydrolysis with sodium hydroxide. It was observed that commercial carbaryl in formulation give two spots with $R_F$ values of 0.45 and 0.54, demonstrating that the commercial formulations sometimes contain the hydrolysis product, 1-naphthol.

**Extraction of Carbaryl Insecticide from Biological Material**

Portion of ca 100 g each of various types of visceral tissue (stomach, intestine, liver, spleen and kidney) containing carbaryl insecticide was individually minced in aqueous solution. Extract each sample in a separating funnel
with 150 ml of diethyl ether, shaking the funnel for 2-3 min. Transfer the ether extract into evaporating dish. Re-extract the aqueous phase with 50 ml of diethyl ether.

A known volume (10 μl) of the solution was spotted on an activated TLC plate together with the standard solution of carbaryl insecticide. The plate was then developed as described under procedure and sprayed with 10 % sodium hydroxide followed by 1 % ceric ammonium nitrate reagent and sodium nitrite solution.

RESULTS AND DISCUSSION

Recovery Experiment:

A 1 mg amount of carbaryl was added to 100 g of minced visceral tissue, mixed well and kept for 1 d. The insecticide was then extracted with diethyl ether, solvent evaporated at room temperature and the residue dissolved in 1 ml of ethanol. A 10 μl volume of this solution was spotted on preactivated TLC plate together with 10 μl each of standard technical carbaryl solutions containing known concentrations of 80, 90, 100 and 110 mg of carbaryl per 100 ml of ethanol. The plate was then developed as described under procedure and sprayed with 10 % sodium hydroxide followed by 1 % ceric ammonium nitrate reagent and 10 % sodium nitrite solution. The intensity of the coloured spot for the visceral extract was compared with those obtained for the known standards and was found to correspond to the spot
representing a concentration of 100 mg per 100 ml (average of three experiments). Hence the recoveries appeared to be better than 90%.

This reagent does not react with propoxur and carbofuran but does react with their hydrolysis product giving reddish-orange spots (sensitivity ca 10 μg). Moreover organophosphorus insecticides such as malathion, parathion, dimethoate, fenethion, fenitrothion, monocrotophos methyl demeton, quinalphos, phosalone, ekatin, phorate, phosphamidon, dichlorvos and trichlorfon; organochlorine insecticides such as endrin, DDT, gamma-HCH, and endosulfan and pyrethroid insecticides such as fenvalerate, cypermethrin and deltamethrin gave no reaction with this reagent and hence did not interfere. The sensitivity of the reagent is ca 0.1 μg per spot observed after development.

Carbaryl on alkaline hydrolysis, gives 1-naphthol which then reacts with ceric ammonium nitrate in acidic media to give the violet complex III as shown in the following proposed reaction scheme (Fig. II-A). This reagent is very sensitive and selective for carbaryl and hence can be used for its detection and semiquantitative determination in biological material in forensic toxicological work.
1

**Carbaryl**

\[ \text{OCONHCH}_3 \]

\[ \rightarrow \text{NaOH} \]

\[ \text{Alkaline hydrolysis} \]

\[ \rightarrow \text{2} \]

**1-Naphthol**

\[ \text{OH} \]

\[ \text{2} \]

\[ \text{1-Naphthol} \]

\[ \text{OH} \]

\[ \text{Ammonium cerium(IV) nitrate} \]

\[ \rightarrow \text{3} \]

**Violet complex**

\[ \text{3} \]

\[ \text{Fig.-II-A1} \]
SECTION - B

THIN-LAYER CHROMATOGRAPHIC DETECTION OF CARBARYL USING PHENYLHYDADIZIN HYDROCHLORIDE
THIN-LAYER CHROMATOGRAPHIC DETECTION OF CARBARYL BY USE OF

PHENYLHYDRAZINE HYDROCHLORIDE

INTRODUCTION

Carbaryl, 1-naphthyl N-methyl carbamate, is a good contact insecticide with occasional systemic activity. It is used in pest control in India and many tropical countries. Its use is continually increasing and this is reflected in the increasing number of criminal cases referred to forensic science laboratories concerning the misuse of carbamates. Hence its selective characterisation is therefore necessary. A number of reagents have been used for its detection by thin-layer chromatography (TLC), viz. diazophenol (after alkaline hydrolysis)\textsuperscript{36}, alkaline Fast Blue B\textsuperscript{37} and Tollen's reagent\textsuperscript{35}. However, these reagents are normally used for phenolic compounds or cannabinoids, and are susceptible to biological impurities such as amino acids, proteins and peptides and are not specific. Although a copper(II) chloride followed by ammonium metavanadate reagent\textsuperscript{40} is reported for carbaryl specifically has low sensitivity of detection.

In this part we report the use of 1% phenylhydrazine hydrochloride in an alkaline medium for the detection of carbaryl by TLC yielding intense red colour.
EXPERIMENTAL

Reagents

All reagents were of analytical reagent grade. Distilled water was used throughout.

Alkaline Phenylhydrazine hydrochloride reagent: Equal volumes of 1% (w/v) aqueous phenylhydrazine hydrochloride solution and 10% (w/v) aqueous sodium hydroxide solution are mixed together just before use.

Extraction of Carbaryl from Biological Materials:

Portions of ca. 50 g each of various types of visceral tissue (stomach, intestine, liver, spleen, and kidney) containing the above insecticide were individually minced in 50 ml aqueous solution. The insecticide was then extracted with 200 ml diethyl ether and the solvent was evaporated at room temperature. The residue was dissolved in 1-2 ml ethanol.

A known volume (10 μl) of the solution was spotted on an activated TLC plate together with the standard solution of insecticide. The plate was then developed as described under procedure and sprayed with alkaline phenylhydrazine hydrochloride reagent.

Procedure

A standard glass TLC plate was coated with a slurry of silica gel G in water (1:2) to a thickness of 0.25 mm. The
plate was activated at 110°C for about 1 h. A 10 μl volume of a standard solution of carbaryl in ethanol (1 mg/ml) was spotted on the plate, which was then developed in a previously saturated TLC chamber using n-hexane-acetone (4:1) as the solvent up to a height of 10 cm. The plate was removed, dried in air and sprayed with alkaline phenyl-hydrazine hydrochloride reagent. Intense red spot was observed immediately on the TLC plate at R_F value 0.45.

RESULTS AND DISCUSSION

Recovery experiment:

A 1 mg amount of carbaryl was added to 50 g of minced visceral tissue, mixed well and kept for a day. The insecticide was then extracted with diethyl ether, the solvent was evaporated at room temperature and the residue was dissolved in 1 ml of ethanol. A 10 μl volume of this solution was spotted on an activated thin-layer plate together with 10 μl each of standard technical carbaryl solutions containing known concentrations of 9, 9.5 and 10 mg per 10 ml in ethanol. The plate was then developed as described under procedure and sprayed with alkaline phenyl-hydrazine hydrochloride reagent. The intensity of the red spots developed for the visceral extract were compared with those of the known standards and found to agree with the spot of concentration of 10 mg per 10 ml (average of three experiment). Hence the recovery was ca. 100 %.
This reagent is selective for carbaryl. Other carbamate insecticides such as baygon, carbofuran, zineb; organophosphorus insecticides such as malathion, parathion, dimethoate, quinalphos, phorate, fenithion, fenitrothion and monocrotophos; organochlorine insecticides such as endrin, aldrin, dieldrin, endosulfan, DDT and BHC and pyrethroid insecticides such as fenvalerate, cypermethrin and deltamethrin do not give coloured spot. Moreover, constituents of viscera (amino acids, peptides, proteins etc.) which are generally co-extracted with the insecticides do not interfere. The sensitivity of the reagent is ca 0.1 μg per spot (i.e. ca 350 ng/cm²) observed after development.

On alkaline hydrolysis carbaryl yields 1-naphthol\textsuperscript{112,113}, which then reacts with phenylhydrazine hydrochloride to give red complex III as shown in the following scheme (Fig. II-B\textsubscript{1}). Technical grade carbaryl and 1-naphthol gives one spot each at $R_F$ values 0.45 and 0.54 respectively, whereas carbaryl in formulathrin and extract of biological materials in carbaryl poisoning cases give two spots with $R_F$ values of 0.45 and 0.54, demonstrating that they contain the hydrolysis product, 1-naphthol. The colour of the spots is stable for a couple of days.

The reagent described here is very sensitive and specific for carbaryl and hence can be used routinely for the detection and determination of carbaryl and its breakdown product, 1-naphthol, in biological and non-biological materials in forensic toxicology.
\[ \text{(I)} \quad \text{Carbaryl} \quad \xrightarrow{\text{NaOH}} \quad \text{OCONHCH}_3 \]

\[ \text{Alkaline hydrolysis} \]

\[ \text{(II)} \quad \text{1-Naphthol} \quad \xrightarrow{\text{NaOH}} \quad \text{OH} \]

\[ \text{(III)} \quad \text{Naphthoxide} \]

\[ \text{(IV)} \quad \text{Phenylhydrazine hydrochloride} \quad \xrightarrow{\text{NaOH}} \quad \text{NHNNH}_2 \]

\[ \text{Alkaline hydrolysis} \]

\[ \text{(V)} \quad \text{Phenylhydrazine} \]

\[ \text{Naphthoxide} \quad + \quad \text{HNNH}_2 \]

\[ \text{Phenylhydrazine} \]

\[ \text{(VI)} \quad \text{Red species} \]

\[ \text{Fig-II-B}_1 \]
SECTION - C

SPECTROPHOTOMETRIC DETERMINATION OF CARBARYL USING PHENYLHYDRAZIN HYDROCHLORIDE
SPECTROPHOTOMETRIC DETERMINATION OF CARBARYL USING PHENYL-
HYDRAZINE HYDROCHLORIDE

Carbaryl (1-naphthyl N-methyl carbamate), a carbamate insecticide, is widely used against a broad spectrum of insects in field crops, fruits and vegetables in India and many tropical countries and thus its determination in insecticidal formulations and its residues in water and grain samples has become imperative. Vonesch and de Riveros\textsuperscript{116} reported the colorimetric method based on the reaction of the insecticide with diazotized 2,5-dichloroaniline. This reaction is sensitive to 0.1 µg with a detection limit 0.2 ppm. Miskus et al.\textsuperscript{112} and Johnson\textsuperscript{113} reported that alkaline hydrolysis of carbaryl yields 1-naphthol which when coupled with p-nitrobenzene diazonium fluoborate, produces a color with an absorption maximum at 590 nm in alkaline medium. The reaction is sensitive to 5 µg. Another spectrophotometric method based on the reaction of carbaryl with diazotized o-toluidine has also been reported\textsuperscript{117}. The resultant red product is measured at 520 nm with a sensitivity of 0.1 µg/20 g sample. Klisenko\textsuperscript{118} developed a colorimetric method based on coupling carbaryl with diazotized sulfanilic acid and measuring the dye at 500 nm. This reaction is sensitive to 1 µg carbaryl/2.5 ml. Appaiah et al.\textsuperscript{43} reported a spectrophotometric method based on reaction of 1-naphthol with 4-aminophenazone in presence

\textsuperscript{117} Klisenko, \textit{Analytical Chemistry} \textbf{60}, 2367 (1988).
of an alkaline oxidizing agent. This reaction is sensitive to 0.5 µg carbaryl/ml. Sastry et al.\textsuperscript{44} have described three spectrophotometric methods for determination of carbaryl based on the formation of coloured species with p-amino-phenol, o-dimethyl-phenylenediamine dihydrochloride and 1-amino-2-naphthol-4-sulphonic acid, respectively. These reagents are sensitive to 0.8 µg/ml, 0.7 µg/ml and 3 µg/ml respectively.

In this part the previous work of TLC detection of carbaryl by use of phenylhydrazine hydrochloride\textsuperscript{118} by the authors has been extended to its spectrophotometric determination. The method is based on the reaction that carbaryl on alkaline hydrolysis yields 1-naphthol which in turn reacts with phenylhydrazine hydrochloride to give red complex.

\textbf{EXPERIMENTAL}

\textbf{APPARATUS}:

\textbf{Spectrophotometer}: Spectral measurements were carried out on a Shimadzu-160 A UV-visible double-beam recording Spectrophotometer and absorbance reading were made on a single-beam digital spectrophotometer of Electronic Corporation of India Ltd. Model GS 5700 A, using 1 cm silica matched cells.
SAMPLES:

Carbaryl: Reference standard material supplied by Kamdhenu Pesticides, Pune, India has been used as a working standard.

Preparation of stock solution of carbaryl, 1 mg/ml^-1:
Dissolve 100 mg of carbaryl in ethanol and adjust the volume to 100 ml in a calibrated flask with the same solvent, store in a refrigerator.

Working solution of carbaryl, 100 mg/ml^-1: Dilute 10 ml of the stock solution of carbaryl to 100 ml with ethanol in a calibrated flask. Store this solution in a refrigerator when not in use.

REAGENTS

All chemicals used were of analytical reagent grade. Distilled water was used throughout.

Phenylhydrazine hydrochloride solution 1%: Dissolve 1 g of phenylhydrazine hydrochloride in 100 ml distilled water and filter the solution.

Sodium hydroxide solution 1 M: Prepare 1 M solution of sodium hydroxide by dissolving 4 g of sodium hydroxide in 100 ml of distilled water.

Potassium carbonate: Analytical reagent grade (Qualigen, Bombay, India) Prepare 0.1 M solution in distilled water.
PROCEDURE:

Pipe 0.0, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ml aliquots of carbaryl working standard solution (10-350 \( \mu \)g) into a series of 10 ml graduated clean, dry test tubes, and 1 ml of 1 M sodium hydroxide solution to each test tube. Shake and leave for 5 min for complete hydrolysis. Add 1 ml of 1 % freshly prepared phenylhydrazine hydrochloride solution and dilute to the mark of 10 ml with distilled water. Mix well and allow to stand for 5 min to attain full colour formation. Measure the absorbance of the red species formed against a reagent blank prepared in a similar manner, at 500 nm using 1 cm silica matched cells. The plot of absorbance versus concentration shows a straight line calibration graph indicating that Beer's law is obeyed over a concentration range of 1 \( \mu \)g - 35 \( \mu \)g/ml.

Determination of Recovery in Grains

Place 50 g of grain samples (wheat, rice) in a waring blender and blend for 5 min with 200 ml of chloroform. Spike samples in blender with 80, 100, 120, 150 and 180 \( \mu \)g carbaryl in 5 ml of ethanol and blend the spiked samples for 2 min. Filter the chloroform solution through a Whatman No. 1 filter paper and retain the residue in the blender. Repeat the blending and filtering twice with 50 ml of chloroform. Rinse blender with 20 ml chloroform and add
rinse to filter paper. Wash the residue on the filter paper with 20 ml of chloroform. Combine the chloroform extracts. Evaporate the chloroform in a vacuo in a fume cupboard and residues dissolved in ethanol to 10 ml in calibrated flask. Use known aliquote of the diluted solution for colour development as described under procedure.

**Determination of Recovery in water samples:**

After the collection of water samples (1 l), adjust each sample to a pH 5 with 20% v/v sulphuric acid. Dissolve 10 g of ammonium sulphate in each sample. Then fortify each sample with 80, 100, 120, 150 and 180 µg carbaryl in 5 ml ethanol. The carbaryl was then extracted in separating funnel with 150 ml of chloroform, combine the extract in a funnel and wash the combined extract with 0.1 M potassium carbonate solution. Evaporate the chloroform in a vacuo in a fume cupboard and the residues dissolved in ethanol to 10 ml in calibrated flask. Use known aliquote of the diluted solution for colour development as described under procedure.
Table 1. Recovery of carbaryl added to water and grains

<table>
<thead>
<tr>
<th>Carbaryl added/μg</th>
<th>Water sample</th>
<th>Wheat sample</th>
<th>Rice sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbaryl</td>
<td>Recovery*</td>
<td>Carbaryl</td>
</tr>
<tr>
<td></td>
<td>found*/μg</td>
<td>%</td>
<td>found*/μg</td>
</tr>
<tr>
<td>80</td>
<td>76.66</td>
<td>95.83</td>
<td>74.66</td>
</tr>
<tr>
<td>100</td>
<td>95.33</td>
<td>95.33</td>
<td>96</td>
</tr>
<tr>
<td>120</td>
<td>116</td>
<td>96.66</td>
<td>114</td>
</tr>
<tr>
<td>150</td>
<td>145.33</td>
<td>98.88</td>
<td>145</td>
</tr>
<tr>
<td>180</td>
<td>174</td>
<td>96.66</td>
<td>171.33</td>
</tr>
</tbody>
</table>

* Each value is an average of three determinations.
Determination in Insecticidal formulation

Weigh 2-16 mg of 50% carbaryl and dissolve in 25 ml of ethanol and centrifuge for 5 min at 5000 rpm. Decant the supernate liquid into a clean, dry 50 ml volumetric flask. Re-extract residue with another 10 ml portion of ethanol as before and combine extracts. Dilute to 50 ml with ethanol and use known aliquots of solution for colour development as outlined in preparation of calibration graph.

Table 2. Analysis of 50% wettable powder formulation of carbaryl

<table>
<thead>
<tr>
<th>Batch</th>
<th>Wt of sample/mg</th>
<th>Carbaryl found/mg</th>
<th>Carbaryl %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2</td>
<td>0.95</td>
<td>47.50</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.45</td>
<td>48.33</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.40</td>
<td>48.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>AV.</strong></td>
</tr>
<tr>
<td>2.</td>
<td>6</td>
<td>2.95</td>
<td>49.16</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.95</td>
<td>49.37</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4.35</td>
<td>48.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>AV.</strong></td>
</tr>
<tr>
<td>3.</td>
<td>12</td>
<td>5.85</td>
<td>48.75</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>6.90</td>
<td>49.28</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>7.85</td>
<td>49.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>AV.</strong></td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Absorption spectra

When very dilute alcoholic solution of carbaryl and aqueous solution of phenylhydrazine hydrochloride are mixed in the presence of sodium hydroxide, an intense red species forms immediately. The intense red species formed shows a maximum absorption at 500 nm, in contrast to the reagent blank, which shows almost zero absorption in the visible region (400-800 nm). Fig. 1 shows the spectra of the red species and of reagent blank. A wavelength of 500 nm, characteristic of red species, was therefore used in all subsequent determination.

Effect of alkali concentration

The optimum overall concentration of sodium hydroxide for colour development is 1 M.

Effect of reagent concentration

Volume of 1 ml of 1% freshly prepared phenylhydrazine hydrochloride is necessary for maximum colour development.

Effect of time on colour development

A study of the effect of time on colour development showed that the reaction mixture should stand for 5 min in order to achieve an absorbance that is stable for more than 12 h.
**Fig. 1.** Absorption spectra of:

A. Carbaryl treated as described under preparation of calibration graph and measured against a reagent blank.

B. Reagent blank measured against distilled water.
Accuracy and precision

In order to check the accuracy and precision of the method, carbaryl was determined at five different concentrations (50, 80, 100, 120 and 150 µg). The results are shown in Table 3 and indicate that the method is satisfactory.

Table 3. Relative error and precision of the proposed method.

<table>
<thead>
<tr>
<th>Amount of carbaryl taken / µg</th>
<th>Carbaryl found by proposed method / µg</th>
<th>Relative error* %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>48.6</td>
<td>-0.8</td>
</tr>
<tr>
<td>80</td>
<td>79.2</td>
<td>-1.0</td>
</tr>
<tr>
<td>100</td>
<td>99.6</td>
<td>-0.4</td>
</tr>
<tr>
<td>120</td>
<td>119.4</td>
<td>-0.5</td>
</tr>
<tr>
<td>150</td>
<td>148.2</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

* Five determinations

Recovery Experiment

Recovery experiments were carried out by adding a known amount of carbaryl to different samples of water and grains and analysed. Recoveries of carbaryl from water, and grain samples varied from 92.25 to 98.88 %. The data are given in Table 1.
Determination of carbaryl in 50 % wettable formulation is given in Table 2. Average carbaryl contents in 3 batches of the formulation varied from 47.94 to 49.03 % against the declared value of 50 %.

Interference

The reported method is selective for the determination of carbaryl. Other carbamate insecticides such as propoxur (baygon), carbofuran, organophosphorus insecticides, such as malathion, parathion-ethyl, parathion-methyl, dimethoate, quinalphos, phorate, fenthion, fenitrothion and monocrotophos, organochlorine insecticides such as endrin, endosulfan, DDT and BHC, and pyrethroid insecticides such as fenvalerate, cypermethrin and deltamethrin do not give red coloured species and hence not interfere.

The data incorporated in Table 1 and 2 suggest that the ingredients present in the formulations in addition to carbaryl and other constituents present in water, and grains not interfere in the proposed method.

The proposed method is simple, rapid and sensitive and not involves the elaborate clean-up procedure and hence can be used for the routine determination of carbaryl in formulations, water samples and grains.
**Reaction Mechanism**

The course of reaction in the formation of the coloured species may be postulated as on alkaline hydrolysis carbaryl yields 1-naphthol\(^{112,113}\) which further reacts with phenylhydrazine hydrochloride to give a complex of red species III, as a substitution product shown in the scheme (Fig. II-A\(_1\)).

**Conclusion**

The proposed spectrophotometric method is more sensitive than the method used by Miskus et al.\(^{112}\) that specifies fluoborate as a chromogenic salt. Carbaryl can be estimated at levels as low as 1 \(\mu\)g by this method as opposed to 5 \(\mu\)g by the latter method (i.e. by Miskus et al.). Although the present method is not as sensitive as other spectrophotometric methods \(^{43,17}\) with reported sensitivity of 0.1–0.4 \(\mu\)g, the method is very useful for the determination of carbaryl from grains and water samples.

This method is simple, selective for carbaryl and accurate to within \(\pm\) 1.0 % and hence can be routinely applied for the determination of carbaryl in formulations.