2.1 Materials

The following materials were used in the investigation.

1. Methanol
2. Chloroform
3. Acetone
4. Sodium hydroxide
5. Sodium nitrite
6. p-Nitroaniline
7. p-Aminobenzoic acid
8. p-Chloroaniline
9. 2,4-Dichloroaniline
10. 2,5-Dichloroaniline
11. 3-Aminopyridine
12. Hydrochloric acid
13. Sulphuric acid
14. Potassium permanganate
15. Silica gel (TLC grade)
16. Sodium sulphate (anhydrous)
17. Potassium carbonate
18. Distilled water
19(a) Carbofuran (analytical grade) and the formulations
75% Wettable powder, 50% soluble powder, 10% granules
and 3% granules

(b) Bendiocarb (analytical grade) and 96% technical grade
formulation

(c) Carbosulfan (92%) and 25% emulsion.

All the pesticide samples were supplied by Rallis
India Ltd., Bangalore.

2.2 Purification of solvents

Methanol, chloroform and acetone were purified
by the standard methods described in the literature\textsuperscript{1-4}.

\underbrace{\text{Methanol (BDH)}} was shaken with 500 g per litre
of anhydrous calcium oxide and refluxed for about eight
hours. Finally the sample was distilled using a fractionating
column. The fraction boiling between 64\textdegree C and 65\textdegree C was
collected for use.

\underbrace{\text{Chloroform (BDH)}} was six times with about half
its volume of water, then dried over anhydrous sodium
sulphate for 24 hours and distilled employing a fractionating
column. The fraction boiling between 61\textdegree C and 62\textdegree C was
collected.
Acetone (RDH). Solid potassium permanganate was added to acetone until the colour of the solution turned to permanent pink. Then the solution was dried over fused calcium chloride, filtered and distilled using a fractionating column.

2.3 Preparation of reagents

Unless otherwise specified, all reagents used were of analytical grade.

Sodium hydroxide (2%). 2 g of sodium hydroxide were dissolved in 100 ml of distilled water.

Sodium nitrite (0.3%). 0.3 g of sodium nitrite was dissolved in distilled water and diluted to 100 ml. Everytime it was prepared freshly.

p-Nitroaniline (0.2%). 0.2 g of p-nitroaniline was dissolved in 100 ml of 1N hydrochloric acid. It was prepared freshly for everytime.

p-Aminobenzoic acid (0.1%). 0.1 g of p-aminobenzoic acid was dissolved in 1N hydrochloric acid and diluted to 100 ml (freshly prepared).

p-Chloroaniline (0.1%). This was prepared freshly by dissolving 0.1 g of p-chloroaniline in 100 ml of 1N hydrochloric acid.
2,4-Dichloroaniline (0.1%). This solution was prepared freshly by dissolving 0.1 g of 2,4-dichloroaniline in 1% sulphuric acid and diluted to 100 ml.

2,5-Dichloroaniline (0.1%) (freshly prepared). 0.1 g of 2,5-dichloroaniline was dissolved in 100 ml of 1% sulphuric acid.

3-Aminopyridine (0.1%). It was prepared freshly by dissolving 0.1 g of 3-aminopyridine in 100 ml of 1N hydrochloric acid.

Potassium permanganate solution. 200 mg of potassium permanganate was dissolved in 10 ml of distilled water and mixed with 5 ml of 50% sulphuric acid.

Potassium carbonate solution (0.1M). 1.4 g of potassium carbonate was dissolved in 100 ml of distilled water.

2.4 Apparatus

Elico spectrocolorimeter model CL-23 with 10 cm borosil cells used for colorimetric determinations.

Shimadzu recording UV-240 spectrophotometer with 4 cm quartz cells used for absorbance measurement.
Elico digital pH meter with combined glass electrode was employed for pH measurement.

2.5 Preparation of standard solutions of pesticides

50 mg of analytical grade carbofuran was dissolved in 500 ml of methanol in a volumetric flask. This solution contains 100μg/ml of carbofuran. Similarly standard solutions of bendiocarb and carbosulfan were prepared (100μg/ml).

2.5.1 Preparation of solutions of pesticide formulations

(a) Carbofuran 75% wettable powder

50 mg of 75% carbofuran was accurately weighed into a 50 ml volumetric flask and dissolved in 50 ml of methanol. 5 ml aliquot of the solution was subsequently diluted with 50 ml of methanol. Aliquots of this solution were used for the determination. The sample was cleaned up by using thin layer chromatography prior to determination.

In a similar manner solutions containing 0.1 mg/ml of 96% bendiocarb and 1 mg/ml of 25% carbosulfan were prepared. These were also cleaned up using TLC.
(b) **Carbofuran 50% soluble powder**

100 mg of 50% carbofuran was accurately weighed into a clean and dry centrifuge test tube and dissolved in 100 ml of methanol. It was centrifuged for 5 min at 5000 rpm and the supernate was decanted into a 50 ml volumetric flask. The residue in the centrifuge test tube was extracted with another 10 ml portion of methanol, the extracts were combined and diluted to 50 ml with methanol. This solution has light pink colour due to presence of dye and it was cleaned up using TLC method.

Solutions of 10% and 3% carbofuran were similarly prepared using aforesaid procedure and these samples were also cleaned up to remove dyes employing TLC method.

2.6 **Clean up procedure**

In the present investigation Thin Layer Chromatography (TLC) described by Hanla and Dikshit\(^5\) was used.

A layer of 1 mm thickness of silica gel was spread over 200x200 mm glass plates. The slurry was prepared by dissolving 1 part by weight of silica gel & in 2 parts by weight of water. The plates were allowed
to set for 5 minutes, then placed in an oven and dried at 110°C for an hour. A measured volume say 100μl of the pesticide formulation along with analytical grade was spotted on the thin layer of silica gel at 1.5 cm apart with a micropipette. The spots were dried and the plates were placed in the TLC tank. The TLC tank was presaturated with chloroform-acetone (9:1) and the plates were developed in the tank containing the solvent. The ascending development was stopped before the solvent reached the top of the plate. The plate was removed from the tank and allowed to air dry. The migrated spot was located by spraying potassium permanganate solution. The spot zones were scrapped into a minichromatographic column and parent compound was eluted with 50 ml of methanol and concentrated to 10 ml in Kuderna-Danish evaporator. Suitable aliquots were taken from this solution for further determination.

2.7 Visible spectrophotometry

Visible spectrophotometry or colorimetry makes use of that portion of spectrum, covering the range, 400-800 nm. Visible spectrophotometry is one of the analytical techniques of analysis which is widely used for the quantitative determination of pesticides in formulations and pesticide residues. Visible spectrophotometric method
of analysis includes the treatment of substance in solution with a reagent that develops colour and the absorbance of which bears a simple relation to the concentration of the substance. The relationship is usually linear. Generally, pesticides are colourless and colours are to be developed specifically for each pesticide. Essential conditions here are that the developed colour must be quantitative, adequately intense and should remain stable for a reasonable period of time.

Photoelectric colorimeters and spectrophotometers are the instruments used for colorimetric and spectrophotometric analysis of pesticides. Spectrophotometer permit one to measure transmittance or absorbance over a wide range of wavelength. Hence these are regarded as refined filter photoelectric photometers. Single beam spectrophotometers such as Unicam SP 600 spectrophotometer, Unicam SP 3-100 UV-visible spectrophotometer, Beckman DU ultraviolet and visible spectrophotometer and Bausch and Lomb spectronic 505 are used for pesticide analysis. Even double beam spectrophotometers, viz., Perkin Elmer 402 spectrophotometer and Unicam SP 1700 spectrophotometer are also used for the determination of pesticides.

In the present work the Elico spectrocolorimeter model CL-23, with an interference graded density
filter was employed to record absorbance data of the coloured compounds produced by using diazotized p-nitroaniline and p-aminobenzoic acid as coupling agents. In this instrument the filter covered the entire visible range with continuously variable path wavelength ranging from 400 nm to 700 nm. The instrument had a spectral band width of 20 nm.

Shimadzu recording UV-240 spectrophotometer, with a recorder for recording data, was also employed. It is a double beam spectrophotometer covering the wavelength region 190 nm to 900 nm. The absorbance values of the coloured compounds obtained using diazotized p-chloroaniline, 2,4-dichloroaniline, 2,5-dichloroaniline and 3-aminopyridine as coupling agents were measured with the aid of this instrument. The instrument has a spectral band width variable from 0.003 to 5 nm and it offered a resolution of 0.1 nm.

2.8 Preparation of coloured compounds of the carbamates

In the present investigation azo-coupling reaction was used as the basis for the preparation of coloured compounds of the pesticides. The carbamates, carbofuran, beniocarb and carbosulfan were hydrolysed using two per cent sodium hydroxide solution to generate
corresponding phenols. The hydrolysis step in respect of carbofuran is given below for illustration.

**Hydrolysis of carbofuran**

Here carbofuran and bendiocarb will give their corresponding phenols in alkaline conditions but carbosulfan which is a derivative of carbofuran was stable in alkaline medium. Hence 0.1 ml of 2 N sulphuric acid was added to it to convert it into carbofuran first and then sodium hydroxide was added to get the corresponding phenol.

2.9 **Preparation of diazotized reagents**

Diazonium salt of p-chloroaniline was prepared by addition 15 ml of 0.3% sodium nitrite solution to 30 ml of 0.1% p-chloroaniline dissolved in 1N hydrochloric acid and likewise diazotized 3-aminopyridine reagent was prepared by adding 15 ml of 0.3% sodium nitrite solution to 30 ml of 0.1% 3-aminopyridine dissolved in 1N hydrochloric acid.
The temperature of the diazotized mixtures was maintained below 10°C by using chilled water.

Similarly diazonium salts of 2,4-dichloroaniline and 2,5-dichloroaniline were prepared using 1% sulphuric acid in place of 1N hydrochloric acid. This became necessary as the diazonium salt in the form of sulphate in these cases was more stable. The diazotization reactions are illustrated (cf page 61).

Finally, the azocompounds were produced by adding diazotization mixture to the phenol of the carbonate liberated by alkaline hydrolysis described earlier. However in respect of p-nitroaniline and p-aminobenzoic acids, the two reagents were dissolved in 1N hydrochloric acid and directly added along with sodium nitrite to the hydrolysis product to produce coloured compounds. In all the cases, the pH was maintained between 11 and 12. The schemes of azo coupling of carbofuran phenol with different reagents are illustrated (cf page 62.).

2.10 **Effect of pH**

Coupling of diazotized aromatic amines with phenols will proceed in an alkaline medium.\(^\text{10}\) In order to determine the optimum pH value needed for the production
Diazotization of p-Chloroaniline and 3-Aminopyridine

\[ \text{p-Chloroaniline} + \text{NaNO}_2 + \text{HCl} \xrightarrow{\text{Cold aq.}} \text{Diazonium salt} \]

Diazotization of 2,4-Dichloroaniline and 2,5-Dichloroaniline

\[ \text{2,4-Dichloroaniline} + \text{NaNO}_2 + \text{H}_2\text{SO}_4 \xrightarrow{\text{Cold aq.}} \text{Diazonium salt} \]

\[ \text{2,5-Dichloroaniline} + \text{NaNO}_2 + \text{H}_2\text{SO}_4 \xrightarrow{\text{Cold aq.}} \text{Diazonium salt} \]
(a) \[
\text{HO} \quad \text{CH}_3 \quad \text{CH}_3
\]
+ \[
\text{Cl} \quad \text{Cl}
\]
\[\rightarrow\]
\[
\text{HO} \quad \text{CH}_3 \quad \text{CH}_3
\]
+ \[
\text{Cl}
\]

(b) \[
\text{HO} \quad \text{CH}_3 \quad \text{CH}_3
\]
+ \[
\text{N}_2\text{Cl}
\]
\[\rightarrow\]
\[
\text{Cl} \quad \text{Z}
\]

(c) \[
\text{HO} \quad \text{CH}_3 \quad \text{CH}_3
\]
+ \[
\text{N}_2\text{SO}_4
\]
\[\rightarrow\]
\[
\text{Cl}
\]

(d) \[
\text{HO} \quad \text{CH}_3 \quad \text{CH}_3
\]
+ \[
\text{N}_2\text{HS}_4
\]
\[\rightarrow\]
\[
\text{Cl}
\]

(e) \[
\text{HO} \quad \text{CH}_3 \quad \text{CH}_3
\]
+ \[
\text{N}_2\text{Cl}
\]
\[\rightarrow\]
\[
\text{NO}_2
\]

(f) \[
\text{HO} \quad \text{CH}_3 \quad \text{CH}_3
\]
+ \[
\text{COOH}
\]
\[\rightarrow\]
\[
\text{OH}
\]
of coloured compounds with maximum intensity, the coupling reaction was carried out between pH 7 and 12. Results presented in the Fig. 2.1 show that maximum colour development occurred in the pH range 11-12 regardless the nature of phenols.

2.11 Determination of the carbamates in formulations

Diazotized p-chloroaniline as coupling reagent

Aliquots of standard carbofuran solution (0, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ml) were taken into 50 ml standard flasks. To each one of these, 2 ml of sodium hydroxide and 1 ml of diazotization mixture (30 ml of p-chloroaniline solution was mixed with 15 ml of sodium nitrite and chilled in an ice bath). The solutions were made up to the mark with distilled water. The resulting yellow-coloured solution had a maximum absorption at 460 ± 5 nm and remained stable for more than 40 hours. Absorbance values were recorded at the wavelength against the reagent blank. Calibration plot of concentration vs absorbance was linear over the composition range, 0.4 ppm to 7 ppm. Calibration plot of carbofuran with p-chloroaniline as coupling agent is illustrated in the fig. 2.2.
Similarly carbofuran in 75%, 50%, 10% and 3% formulations were determined using the aforesaid procedure. Bendiocarb and carbosulfan in formulations were determined in a similar manner. Bendiocarb formed a yellow coloured solution with absorption maximum at 440 nm whereas carbosulfan formed a yellow coloured solution with $\lambda_{\text{max}}$ 465 nm. The colour of these two solutions was stable for more than 12 hours after which there is gradual deterioration. The plot of concentration vs absorbance of bendiocarb was a straight line over the range 0.5 ppm to 7 ppm whereas for carbosulfan, it is linear over the range 1 ppm to 7 ppm. Concentrations of carbamates were read from the standard curves. These concentrations were used to compute the amounts of the carbamates in the formulations employing the formula used by Hanau and Dikshit.\(^5\)

Carbamate by weight = $\frac{A}{A'} \times \frac{g \text{ standard}}{g \text{ sample}} \times P$

where $A$ = absorbance of carbamate in sample

$A'$ = absorbance of carbamate in standard

$P$ = purity of formulation of carbamate (% by weight)

In a similar manner the three carbamates in formulations were also determined using diazotized 3-aminopyridine, 2,4-dichloroaniline, 2,5-dichloroaniline, p-nitroanilinc and p-aminobenzoic acid as coupling reagents. The
absorption maxima, stability period and concentration range that could be covered are given in the table I. The absorption maxima curves of carbofuran, benoicarb and carbosulfan are illustrated (cf. Figs. 2.3-2.6).

2.12 Determination of the carbamates in water samples

One litre of distilled water samples were taken and spiked at concentrations of 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 ppm of analytical grade carbofuran, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 ppm of benoicarb and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ppm of carbosulfan. The pH of these samples was adjusted to 3-4 with 50 per cent sulphuric acid, 10 g of anhydrous sodium sulphate was added to each sample. The carbamates in the samples were extracted with 100 ml of chloroform by shaking for 2-4 minutes. Each sample was again reextracted with 50 ml of chloroform. The combined extracts were dried over 10 g of anhydrous sodium sulphate. Finally chloroform was evaporated to dryness on a waterbath. Residue was dissolved in methanol. To this 2 ml of sodium hydroxide, 1 ml of sodium nitrite and 0.5 ml of p-aminobenzoic acid were added to develop colour and absorbance was recorded. In the similar manner the carbamates in water samples were determined using the coupling agents, p-chloroaniline, p-nitroaniline, 3-aminopyridine, 2,4-dichloroaniline and 2,5-dichloroaniline.
Fig. 2.1 Effect of pH on Colour development

1. Carbofuran with p-Chloroaniline
2. Bendiocarb with 2,5-Dichloroaniline
3. Carbosulfan with 2,5-Dichloroaniline
Fig. 2.2 Carbofuran with p-Chloroaniline
Absorption Curves

Fig. 2.3 Carbofuran with p-Chloroaniline ($\lambda_{\text{max}} = 465 \text{ nm}$)

Fig. 2.4 Carbofuran with 3-Aminopyridine
Absorption Curves

Fig. 2.5 Bendiocarb with 3-Aminopyridine

Fig. 2.6 Carbosulfan with 2,5-Dichloroaniline
Table I

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Reagent</th>
<th>max (nm)</th>
<th>Stability period (hours)</th>
<th>Concentration range (ppm)</th>
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<td>48</td>
<td>0.2-12</td>
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<td>3-aminopyridine</td>
<td>465</td>
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REFERENCES