STUDIES ON ANALYSIS OF SOME ORGANIC POLLUTANTS IN WATER

SUMMARY

THESIS
SUBMITTED FOR THE AWARD OF THE DEGREE OF
Doctor of Philosophy
IN
CHEMISTRY

BY
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ALIGARH (INDIA)
1989
SUMMARY

Pesticide is the general term for acaricides, fungicides, herbicides, insecticides, molluscicides, rodenticides and similarly active substances used to kill or control pests. The commonly used pesticides, their LD\textsubscript{50} values, action and applications are summarized in Table 1.

The pesticides mix up in natural water, thereby polluting public water supplies in many ways including accidental fall-out of sprays from agricultural treatments, discharge of urban and domestic wastewater and sewage, fall-out from the atmosphere, release of industrial effluents, road run-offs and surface run-off from agricultural land. The concentration of pesticides is increasing day by day in water due to their indiscriminate and frequent use. The regular consumption of water containing above substances will certainly have devastating consequences such as skin trouble, sterility, lesions of liver, vomiting, loss of weight, jaundice, intestinal swelling, abdominal pain, gradual poisoning and cancer.

The existing concentration of the pesticides in water is low and it is generally below the limit of detection of the analytical methods used. Therefore, any organic pollutant analysis technique for water samples should include a dependable preconcentration method. The following preconcentration methods have been used:

1. Concentration Techniques
   1.1 Freeze concentration
   1.2 Lyophilization
   1.3 Evaporation
   1.4 Distillation
1.5 Reverse-osmosis
1.6 Ultrafiltration

2. Isolation Techniques
2.1 Liquid-liquid extraction
2.2 Solid-phase extraction
2.3 Precipitation process
2.4 Impregnation materials
2.5 Ion-retardation resins and Immobilizing ion-exchange site resins

But no single method has been found to be adequate for concentrating all the organic pollutants in water. The choice of method or combination of methods of preconcentration depends on factors such as volatility and solubility of the organic pollutant, the degree of concentration required and the nature of the test to be used.

Many instrumental and non-instrumental techniques have been used for the analysis of organic pollutants in water. Instrumental methods are highly sensitive and they require a small amount of the test material. Unfortunately they are costly and sophisticated. So they are either not available or have not been installed in many places. Though non-instrumental methods are less sensitive, they are simple and inexpensive and can be used at any spot on field detection. They have been proved to be of utmost importance in the preliminary characterization of the test material. Therefore, there is a growing interest in developing new non-instrumental methods of analysis. In this thesis efforts made to develop simple and inexpensive methods for the detection, separation and determination of some organic pollutants have been described and discussed. The results
obtained are summarized below and they are discussed in detail in Chapters 1-5 of this thesis.

A new indicator and test paper have been developed for the semi-quantitative determination of carbaryl in traces in water. A chromatographic paper impregnated with sodium hydroxide (test paper) and spotted with a carbaryl solution is dipped in 2,6-dichloroquinone-4-chloroimine (Gibb's reagent) in benzene, it turns blue with violet tinge. The colour formation is based on the hydrolysis of carbaryl to give 1-naphthol which reacts with 2,6-dichloroquinone-4-chloroimine to produce indophenol that turns blue with violet tinge in alkaline medium. The results of detection on the test paper of the carbaryl and related compounds are summarized in Table 2. The test paper can be used for the semi-quantitative determination of carbaryl, 2,4-D-ester, sodium salt of 2,4-D (2,4-D-Na), 1-naphthol and phenol. The intensity of the colour increases with the increasing concentration of the test material. The method becomes very sensitive when it is coupled with a suitable preconcentration method (extraction).

The newly developed technique, sequential thin-layer chromatography (S-TLC) has been found to be a fast method for the analysis of complex mixtures. S-TLC behaviour of some carboxylic pollutants has been studied on calcium sulphate coating in simple organic solvents such as acetone, benzene, carbon tetrachloride, chloroform, dioxan, ethyl acetate and propanol. Alkaline bromophenol is used for locating the chromatograms. S-TLC is used to separate herbicides such as 4-chlorophenoxyacetic acid (CPAA), 2,4-dichlorophenoxyacetic acid (2,4-D), phenoxyacetic acid (PAA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and trichloroacetic acid (TCA) and plant growth regulators such as benzoic acid (BOA), cinnamic acid (CIA),
gallic acid (GA), indole-3-acetic acid (IAA), 2-naphthaleneacetic acid (2-NPAA) and 2-naphthoxyacetic acid (2-NXAA) and other carboxylic acids such as citric acid (CA), oxalic acid (OA), salicylic acid (SA) and tartaric acid (TA). By S-TLC many ternary separations have been achieved which are not possible by normal-phase thin-layer chromatography (NP-TLC). Some important separations are recorded in Table 3.

Ion-pair reversed-phase thin-layer chromatography (IP-RP-TLC) on calcium sulphate coating containing an ion-pair reagent cetrimide and an oil (coconut or olive or paraffin or silicone) has been used to separate phenoxyacid herbicides such as 4-chlorophenoxyacetic acid (CPAA), 2,4-dichlorophenoxyacetic acid (2,4-D), phenoxyacetic acid (PAA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and trichloroacetic acid (TCA) and plant growth regulators such as benzoic acid (BOA), cinnamic acid (CIA), gallic acid (GA), indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), 1-naphthaleneacetic acid (1-NPAA), 2-naphthaleneacetic acid (2-NPAA) and 2-naphthoxyacetic acid (2-NXAA). By IP-RP-TLC many ternary and quaternary separations have been achieved which are not possible by NP-TLC or S-TLC. Some important quaternary separations are recorded in Table 4. Detection of the phenoxy acid herbicides on IP-RP-TLC is a sensitive method as the lower limit of the detection is found to be 20 μg.

A selective spot test for the detection of trichloroacetic acid has been developed. It is based on the formation of yellow-greenish fluorescent salicylaldazine under UV light. Trichloroacetic acid gives off chloroform on heating with aqueous sodium hydroxide. Chloroform so formed reacts with phenol to give salicylaldehyde which condenses with hydrazine to produce salicylaldazine. Hydrazine in presence of alkaline phenol is used
as a fluorescence reagent. The lower limit of detection of trichloroacetic acid in environmental samples is shown in Table 5. This method can also be used successfully for the detection of persistence of trichloroacetic acid in different soils.
## TABLE 1 COMMONLY USED PESTICIDES, THEIR ACTION AND APPLICATIONS

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Action</th>
<th>LD$_{50}$ (mg/kg)</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Organochlorines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1 DDT</td>
<td>Insecticide</td>
<td>87</td>
<td>Mosquito vector control for the eradication of malaria.</td>
</tr>
<tr>
<td>1.2 BHC</td>
<td>Insecticide</td>
<td>125</td>
<td>Extensively used for the control of cotton insects.</td>
</tr>
<tr>
<td>1.3 Dieldrin</td>
<td>Insecticide</td>
<td>40</td>
<td>Control of soil insects, public health insects, termites and many other pests.</td>
</tr>
<tr>
<td>1.4 Toxaphene</td>
<td>Insecticide</td>
<td>40</td>
<td>Control of grasshoppers, armyworms, horn flies, lice, ticks etc.</td>
</tr>
<tr>
<td><strong>2. Organophosphorus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1 Malathion</td>
<td>Insecticide</td>
<td>885</td>
<td>Control a wide variety of insects including aphids, spider mites, house flies and mosquitoes.</td>
</tr>
<tr>
<td>2.2 Dichlorvos</td>
<td>Insecticide</td>
<td>25</td>
<td>Control of household and public health pests, stored product insects, horn flies, house flies, gnats and mosquitoes on lactating dairy animals and beef cattle.</td>
</tr>
<tr>
<td>2.3 Demicron</td>
<td>Insecticide</td>
<td>17-30</td>
<td>Mainly used against sucking insects. Also used against stem borers in rice and aphids in crops.</td>
</tr>
<tr>
<td>2.4 Ethyl parathion</td>
<td>Insecticide</td>
<td>3</td>
<td>A wide range of application on many crops against numerous insect species.</td>
</tr>
<tr>
<td>2.5 Methyl parathion</td>
<td>Insecticide</td>
<td>9</td>
<td>Control of many insects, especially effective for boll weevil control.</td>
</tr>
</tbody>
</table>
### TABLE 1 (Continued)

3. **Organosulphurs**

| 3.1 Ovex  | Acaricide | 2000 | Most effective as an ovicide but also toxic to active stages of spider mites. Used on cotton, deciduous fruits, nuts and ornamentals. |

4. **Carbamates**

| 4.1 Carbaryl  | Insecticide | 307 | For the control of insect pests on different crops including citrus, fruit, forage crops, forests, field crops, lawns, nuts, as well as poultry and pets. |

| 4.2 Aldicarb' | Insecticide, Acaricide, Nematicide | 1 | Used to control insects, mites and nematodes on cotton, sugar beets, potatoes, peanuts, oranges, soybeans and ornamentals. |

| 4.3 Baygon  | Insecticide | 95 | Particularly effective against insects affecting man and animals, such as cockroaches, flies and mosquitoes. |

5. **Triazines**

| 5.1 Simazine  | Herbicide | 5000 | For the control of most annual grasses and broadleaf weeds in corn, alfalfa, cherries, peaches, citrus, grapes, nuts, apples, pears and ornamentals. |

6. **Carboxylic compounds**

| 6.1 TCA  | Herbicide | 3200-5000 | An effective soil sterilant, used for perennial weed grass control on noncrop land. |

| 6.2 Dalapon  | Herbicide | 6500 | Effective against quackgrass, bermudagrass, and other perennial and annual grasses as well as cattails and rushes. |
### TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>6.3 Dicamba</th>
<th>Herbicide</th>
<th>1040</th>
<th>For control of brush under utility lines, along highway rail roads and both annual and perennial broadleaf weeds on noncropland areas.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4 2,4-D</td>
<td>Herbicide</td>
<td>370</td>
<td>Used on grasses, wheat, barley, oats, sugar cane and noncrop areas for postemergent control of weeds.</td>
</tr>
<tr>
<td>6.5 2,4,5-T</td>
<td>Herbicide</td>
<td>300-500</td>
<td>Widely used for the control of woody plants. Also used extensively for weed control in rice.</td>
</tr>
<tr>
<td>6.6 MCPA</td>
<td>Herbicide</td>
<td>700</td>
<td>For postemergent control of many annual and perennial broadleaf weeds in grains, rice, peas, grassland and turf.</td>
</tr>
<tr>
<td>6.7 IAA</td>
<td>Plant growth regulator</td>
<td>-</td>
<td>An important natural auxin responsible for many physiological changes in plants.</td>
</tr>
<tr>
<td>6.8 1-Naphthaleneacetic acid</td>
<td>Plant growth regulator</td>
<td>1000</td>
<td>Including root formation on cutting and transplants, including pineapple, flowering, thinning olives and otherwise controlling fruit set.</td>
</tr>
<tr>
<td>6.9 2-Naphthoxyacetic acid</td>
<td>Plant growth regulator</td>
<td>-</td>
<td>Blossom set and growth regulant for pineapples, strawberries, tomatoes etc.</td>
</tr>
</tbody>
</table>
### TABLE 2 DETECTION ON TEST PAPER

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Colour Developed</th>
<th>Lower Limit of Detection (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Bavistin</td>
<td>B</td>
<td>20.00</td>
</tr>
<tr>
<td>BHC</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>BVT</td>
<td>1.20</td>
</tr>
<tr>
<td>Catechol</td>
<td>BVT</td>
<td>0.50</td>
</tr>
<tr>
<td>Cresol-red</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>2,4-D-ester</td>
<td>GB</td>
<td>2.00</td>
</tr>
<tr>
<td>2,4-D-Na</td>
<td>LB</td>
<td>20.00</td>
</tr>
<tr>
<td>Detergent</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Lemon juice</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Malathion</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Milk</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>BVT</td>
<td>0.06</td>
</tr>
<tr>
<td>Phenol</td>
<td>B</td>
<td>1.00</td>
</tr>
<tr>
<td>Phosphamidon</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>RV</td>
<td>0.50</td>
</tr>
<tr>
<td>Soap</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Soil extract</td>
<td>NC</td>
<td>-</td>
</tr>
<tr>
<td>Thymol</td>
<td>B</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Abbreviations used are: NC = no colour, B = blue, BVT = blue with violet tinge, GB = greenish blue, LB = light blue, RV = reddish violet.
<table>
<thead>
<tr>
<th>Carboxylic Pollutants</th>
<th>Separated From</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA (0.0)</td>
<td>MEA (0.5) and 2-NPAA (1.0)</td>
<td>Ethyl acetate - chloroform</td>
</tr>
<tr>
<td>OA (0.0)</td>
<td>MEA (4-5 cm) and 2-NXAA (9-10 cm)</td>
<td>Propanol - chloroform</td>
</tr>
<tr>
<td>OA (0.0)</td>
<td>MA (0.5) and CIA (9-10 cm)</td>
<td>Ethyl acetate - carbon tetrachloride</td>
</tr>
<tr>
<td>OA (0.0)</td>
<td>MEA (0.5) and PAA (1.0)</td>
<td>Acetone - benzene</td>
</tr>
<tr>
<td>OA (0.0)</td>
<td>MEA (0.5) and CIA (9-10 cm)</td>
<td>Acetone - benzene</td>
</tr>
<tr>
<td>OA (0.0)</td>
<td>IAA (0.5) and PAA (1.0)</td>
<td>Ethyl acetate - carbon tetrachloride</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>2-NXAA (0.5) and CIA (8-10 cm)</td>
<td>Ethyl acetate - benzene</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>IAA (3-5 cm) and SA (9-10 cm)</td>
<td>Ethyl acetate - benzene</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>MA (0.5) and SA (9-10 cm)</td>
<td>Ethyl acetate - carbon tetrachloride</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>MA (0.5) and CIA (1.0)</td>
<td>Chloroform - ethyl acetate</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>IAA (4-5 cm) and CIA (1.0)</td>
<td>Ethyl acetate - carbon tetrachloride</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>IAA (4-5 cm) and PAA (9-10 cm)</td>
<td>Ethyl acetate - carbon tetrachloride</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>MEA (0.5) and CIA (1.0)</td>
<td>Chloroform - ethyl acetate</td>
</tr>
</tbody>
</table>

R<sub>f</sub> values are given in parentheses.
### TABLE 4 QUATERNARY SEPARATIONS OF SOME CARBOXYLIC POLLUTANTS BY IP-RP-TLC

<table>
<thead>
<tr>
<th>Carboxylic Pollutants</th>
<th>Separated From</th>
<th>Coatings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIA (0.0)</td>
<td>BOA (2-3 cm) - IAA (0.8) and 2-NXAA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>1-NPAA (0.0)</td>
<td>2,4-D (0.3) - BOA (0.6) and GA (1.0) or PAA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>1-NPAA (0.0)</td>
<td>BOA (0.2) - IPA (0.6) and PAA (1.0)</td>
<td>A</td>
</tr>
<tr>
<td>2-NPAA (0.0)</td>
<td>2,4-D (0.2) - CPAA (5-6 cm) and GA (1.0) or PAA (9-10 cm)</td>
<td>B</td>
</tr>
<tr>
<td>2-NPAA (0.0)</td>
<td>2,4-D (2-3 cm) - IAA (5-7 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2-NXAA (0.0)</td>
<td>2,4-D (0.2) - CPAA (4-5 cm) and PAA (9-10 cm)</td>
<td>B</td>
</tr>
<tr>
<td>2-NXAA (0.0)</td>
<td>2,4-D (2-3 cm) - IAA (5-6 cm) and GA (1.0) or PAA (9-10 cm)</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-T (0.0)</td>
<td>BOA (2-3 cm) - IPA (5-6 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>A</td>
</tr>
<tr>
<td>2,4,5-T (0.0)</td>
<td>2,4-D (2-3 cm) - CPAA (7-8 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B</td>
</tr>
</tbody>
</table>

R<sub>f</sub> values are given in parentheses.

A = Calcium sulphate + cetrimide + coconut oil.

B = Calcium sulphate + cetrimide + olive oil.

C = Calcium sulphate + cetrimide + paraffin oil.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Environmental Samples</th>
<th>Lower Limit of Detection (μg) and Fluorescence Observed by Different Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.3.1</td>
</tr>
<tr>
<td>1.</td>
<td>Soils:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soil (fertile)</td>
<td>(40)-YG</td>
</tr>
<tr>
<td></td>
<td>Soil (pond)</td>
<td>(30)-YG</td>
</tr>
<tr>
<td></td>
<td>Soil (red)</td>
<td>(80)-YG</td>
</tr>
<tr>
<td>2.</td>
<td>Waters:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water (distilled)</td>
<td>(40)-YG</td>
</tr>
<tr>
<td></td>
<td>Water (river)</td>
<td>(50)-YG</td>
</tr>
<tr>
<td></td>
<td>Water (tap)</td>
<td>(40)-YG</td>
</tr>
</tbody>
</table>

Abbreviation used is: YG = Yellowish-green.
CERTIFICATE

This is to certify that the thesis entitled "Studies on Analysis of Some Organic Pollutants in Water" submitted for the award of the degree of Doctor of Philosophy in Chemistry, is a faithful record of the bonafide research work carried out at the Department of Applied Chemistry, Z.H. College of Engineering and Technology, Aligarh Muslim University, Aligarh, by Mr. Sushil Kumar Saxena under my guidance and supervision and it is suitable for submission for the award of Ph.D. degree in Chemistry.

(Dr. H.S. Rathore)
ACKNOWLEDGEMENTS

In the preparation of this thesis, I owe the greatest debt of my gratitude to my supervisor, Dr. H.S. Rathore, Reader, Department of Applied Chemistry, Z.H.College of Engineering and Technology, Aligarh Muslim University, Aligarh, without whose expert guidance, compassionate and inspiring attitude, my toils to complete an investigation of this nature would have been never materialized.

My sincere thanks are due to Prof. A.U. Malik, former Chairman and Prof. K.T. Nasim, present Chairman of the Department of Applied Chemistry, who made laboratory and other facilities freely available to me during the course of my investigation. I also wish to thank Dr. Ishtiaq Ali, Reader, Department of Applied Chemistry, for his active co-operation and help from time to time.

My colleagues, Dr. (Miss) S.R. Sharma, Dr. (Mrs.) Sudha Gupta, Mr. H.A. Khan, Miss Tahira Begum and Miss Rachna Sharma rendered timely assistance and help whenever required for which I am obliged to all of them. Financial support provided by the Council of Scientific and Industrial Research, New Delhi, is gratefully acknowledged.

Last but not least, I wish to extend my sincere thanks to my elder brothers Dr. Sunil Kumar Saxena and Er. Sudhir Kumar Saxena and my friend Mr. Anoop Kumar, whose words of endearment and praise kept all frustrations and defeatism at a distance.

(SUSHIIL KUMAR SAXENA)
DEDICATED
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5.6 CONCLUSION
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BOA</td>
<td>Benzoic acid</td>
</tr>
<tr>
<td>b.p.</td>
<td>Boiling point</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
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<tr>
<td>CPAA</td>
<td>4-Chlorophenoxyacetic acid</td>
</tr>
<tr>
<td>CIA</td>
<td>Cinnamic acid</td>
</tr>
<tr>
<td>CA</td>
<td>Citric acid</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>2,4-D</td>
<td>2,4-Dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>p-DAB</td>
<td>p-Dimethylaminobenzaldehyde</td>
</tr>
<tr>
<td>DW</td>
<td>Distilled water</td>
</tr>
<tr>
<td>eq.wt.</td>
<td>Equivalent weight</td>
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<tr>
<td>GA</td>
<td>Gallic acid</td>
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<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>hr</td>
<td>Hour</td>
</tr>
<tr>
<td>IAA</td>
<td>Indole-3-acetic acid</td>
</tr>
<tr>
<td>IBA</td>
<td>Indole-3-butyric acid</td>
</tr>
<tr>
<td>id</td>
<td>Internal diameter</td>
</tr>
<tr>
<td>IPA</td>
<td>Indole-3-propionic acid</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>MA</td>
<td>Malic acid</td>
</tr>
<tr>
<td>MEA</td>
<td>Maleic acid</td>
</tr>
<tr>
<td>MOA</td>
<td>Malonic acid</td>
</tr>
<tr>
<td>m/v</td>
<td>Mass by volume</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting point</td>
</tr>
<tr>
<td>m</td>
<td>Metre</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>meq/g</td>
<td>Milliequivalent per gram</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>mM</td>
<td>Millimole</td>
</tr>
<tr>
<td>mV</td>
<td>Millivolt</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>M</td>
<td>Molarity</td>
</tr>
<tr>
<td>mol.wt.</td>
<td>Molecular weight</td>
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<tr>
<td>ng</td>
<td>Nanogram</td>
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<tr>
<td>nm</td>
<td>Nanometre</td>
</tr>
<tr>
<td>1-NPAA</td>
<td>1-Naphthaleneacetic acid</td>
</tr>
<tr>
<td>2-NPAA</td>
<td>2-Naphthaleneacetic acid</td>
</tr>
<tr>
<td>2-NXAA</td>
<td>2-Naphthoxyacetic acid</td>
</tr>
<tr>
<td>N</td>
<td>Normality</td>
</tr>
<tr>
<td>OA</td>
<td>Oxalic acid</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>ppt</td>
<td>Parts per trillion</td>
</tr>
<tr>
<td>PAA</td>
<td>Phenoxyacetic acid</td>
</tr>
<tr>
<td>RW</td>
<td>River water</td>
</tr>
<tr>
<td>SA</td>
<td>Salicylic acid</td>
</tr>
<tr>
<td>TW</td>
<td>Tap water</td>
</tr>
<tr>
<td>TA</td>
<td>Tartaric acid</td>
</tr>
<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>2,4,5-Trichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume by volume</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight by weight</td>
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ABSTRACT

A new indicator and test paper has been developed for the semi-quantitative determination of carbaryl in traces in water. A chromatographic paper impregnated with sodium hydroxide (test paper) spotted with a carbaryl solution is dipped in 2,6-dichloroquinone-4-chloroimine (Gibb's reagent) in benzene, it turns blue with violet tinge. The colour formation is based on the hydrolysis of carbaryl to give 1-naphthol which reacts with 2,6-dichloroquinone-4-chloroimine to produce indophenol that turns blue with violet tinge in alkaline medium. The test paper can be successfully used for the detection and semi-quantitative determination of carbaryl at ppm level in water.

Sequential thin-layer chromatographic behaviour of some carboxylic pollutants has been studied on calcium sulphate coating in simple organic solvents such as acetone, benzene, carbon tetrachloride, chloroform, dioxan, ethyl acetate and propanol. Alkaline bromophenol blue is used to locate the test materials on the chromatograms. Herbicides such as 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, trichloroacetic acid and plant growth regulators such as indole-3-acetic acid, gallic acid, 1-naphthaleneacetic acid have been separated from one another and from several other carboxylic pollutants.

Separation and detection of some phenoxyacid herbicides such as 4-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, phenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid and trichloroacetic acid and plant growth regulators such as benzoic acid, cinnamic acid, gallic acid, indole-3-acetic acid, indole-3-propionic acid, 1-naphthaleneacetic acid, 2-naphthaleneacetic acid and 2-naphthoxyacetic acid have been achieved by ion-pair
reversed-phase thin-layer chromatography. Calcium sulphate coating containing an ion-pair reagent cetrimide and oil such as coconut oil, olive oil, paraffin oil and silicone oil is used. Alkaline bromophenol blue is used as a locating reagent. Ternary and quaternary separations of the compounds of the same group have been achieved successfully by this technique which are not possible by sequential and normal-phase thin-layer chromatography.

A selective spot test for the detection of trichloroacetic acid has been developed. It is based on the formation of yellowish-green fluorescent salicylaldazine under UV light. Trichloroacetic acid gives off chloroform on heating with aqueous sodium hydroxide. Chloroform so formed reacts with phenol to give salicylaldehyde which condenses with hydrazine to produce salicylaldazine. Hydrazine in presence of alkaline phenol is used as a fluorescence reagent. This test can also be successfully used for the detection of persistence of trichloroacetic acid in different soils.
CHAPTER - 1

GENERAL INTRODUCTION
1.1 INTRODUCTION

The presence of the following organic pollutants in water has been detected: amines, detergents, fertilizers, hydrocarbons, lignins, organic acids, phenols, phthalate esters, polychlorinated biphenyls (PCBs), pesticides, proteins, soaps and tannins. In the United States about 700 organic pollutants were determined in various drinking water supplies (1). These pollutants mix up in natural water, thereby polluting public water supplies in many ways including accidental fall-out of sprays from agricultural treatments, surface run-off from agricultural land, discharge of urban and domestic wastewater and sewage, fall-out from the atmosphere, release of industrial effluents and road run-offs.

The regular consumption of water containing above substances will certainly have devastating consequences such as skin trouble, sterility, lesions of the liver, vomiting, loss of weight, jaundice, intestinal swelling, abdominal pain, gradual poisoning and cancer. It has been reported (2) that at least 60% of the total cancer cases in the United States would have been caused by environmental factors, mostly chemicals. The potential hazards from the occurrence of carcinogenic organic pollutants in man's water supplies was noted by World Health Organization's Committee on the Prevention of Cancer. Therefore, to minimize any adverse effect, it is essential that proper testing procedures be used with a foreknowledge of the limitations of the analytical determination.

The analysis of water pollutants is carried out in the following stages.

1.1.1 Preconcentration or enrichment of organic pollutants in water.
1.1.2 The removal of interfering substances from the preconcentrated analyte—usually referred to as clean up procedure.

1.1.3 Detection, separation and determination of organic pollutants together with metabolites and breakdown products, in the cleaned up extract.

1.1.1 PRECONCENTRATION TECHNIQUES

In India the present concentration of pesticides such as aldrin, dieldrin, total DDT, heptachlor and heptachlor epoxide and lindane is 0-28, 55-240, 280-906, 0-72, and 47-100 ppt in river water (Ganga, Hooghly, Yamuna); nil, 0-32, 0-194, nil and 0-6 ppt in canal water (Kanpur); 0-26, 33-54, 48-2000, 33-38 and 15-116 ppt in well water (Calcutta, Delhi, Kanpur) and nil, nil, 0-218, nil and 0-81 ppt in hand-pump water (Kanpur). In the United States, Mississippi and Missouri rivers contain the suggested maximum permissible amount of chlordane (0.003 ppm), dieldrin (0.017 ppm) and endrin (0.001 ppm) respectively. In United Kingdom, Yorkshire river contains BHC (19-214 ppm), total DDT (2-43 ppm) and dieldrin (3-2840 ppm) respectively. The data described above show that the existing concentration of organic pollutants in water is low and it is generally out of the lower limit of detection of the analytical methods. Therefore a suitable pre-concentration method is coupled with the available analytical technique. A number of preconcentration techniques have been reported in the literature but no single method has been found to be adequate for concentrating all the organic pollutants in water. The choice of technique or combination of techniques of preconcentration depends on factors such as volatility and solubility of the test material, the degree of concentration required and the nature of the test to be used.

Techniques used for the preconcentration of organic pollutants
in water are divided into two classes.

1.1.1.1 Concentration techniques.
1.1.1.2 Isolation techniques.

1.1.1.1 Concentration Techniques

In concentration techniques water is removed in order to concentrate the dissolved analyte. The following concentration techniques have been used.

1.1.1.1.1 Freeze concentration

This technique is applicable at the level of millilitre volumes. As commercial equipment is not available it has not been used as a routine method. Preconcentration of organic pollutants by this technique decreases with increasing concentration of inorganics in the solution. It was applied to preconcentrate m-cresol from aqueous solution of 310 ppm (3-6) with 80% recovery and 20-fold volume reduction. Carbamate pesticides (7) were also preconcentrated from acetonitrile or acetone solutions by freezing out technique.

1.1.1.1.2 Lyophilization

It is also known as freeze drying process. Commercial lyophilizers are available in the market and they are capable of processing many litres of water per day. It gives poor results for volatile organic pollutants. It does not produce good results in presence of inorganics. This process is used largely in the area of biochemistry. It was also used in analysing organochlorine pesticides (8).
1.1.1.3 Evaporation

It is a simple and successful method for analysing inorganics. It was used for the detection of iron and non-volatile organic pollutants of high m.p. in river water. It becomes a unequivocal tool on coupling with spectrometry for the analysis of organic pollutants in water (9, 10). It was used to preconcentrate carbaryl and PCBs (11, 12) from raw water.

1.1.1.4 Distillation

The vacuum distillation alike lyophilization is more advantageous process and it has been used to concentrate organic pollutants in water (13). However, the vacuum distillation is more laborious than lyophilization and laboratory-scale equipment can be used to reduce the volumes of several litres per day. Steam distillation is useful in separating water insoluble organic pollutants. Kuznetsova and Chumakova (14) developed an effective procedure for concentrating ethylene chlorohydrin in reservoir water. The analyte was distilled off from the acidified sample in the presence of 30% sodium chloride. The sensitivity of the method was 0.05 ppm with a relative standard deviation of less than 0.25. Acrylonitrile was concentrated from water sample by Ramstad and Nicholson (15). A modified Nielsen-Kryger steam distillation apparatus was presented by Veith and Kiwas (16). It provides an exhaustive distillation of pesticides and industrial chemicals from water, sediments and tissues. Less volatile pesticides such as carbaryl and parathion and aldrin were isolated from fat using simultaneous steam distillation and extraction apparatus designed by Figuerola et al. (17). Nash (18) reported that except toxaphene and methoxychlor, many pesticides gave recovery more than 75% from soil
and plant by steam distillation. Luke et al. (19) isolated five organo-
phosphorus pesticides from beef fat using the unitrex sweep co-distillation. 
Chang-yen et al. (20) used cyclic steam distillation for the extraction 
of organochlorine pesticides and PCBs.

1.1.1.5 Reverse-osmosis

It is used to remove the solvent through a membrane by the application 
of pressure greater than the osmotic pressure of the solution. As the 
analyte can not pass through the membrane, it is concentrated in the 
sample. Commercial units that can treat large volumes per day are available 
in the market and they are used for purification of water in some places 
(Madras in India). Cellulose acetate membranes rejected 90-97% of in-
organics or organic pollutants with mol. wt. more than 200. Kopfler 
et al. (21) have reported that cellulose acetate membrane rejected about 
85% of the total organic pollutants from Cincinnati, Ohio, drinking water. 
when reverse-osmosis is coupled with solvent extraction and dialysis, approxi-
mately 30-40% of organic pollutants are recovered from tap water (22). 
The advances in water and wastewater treatment by reverse-osmosis and 
ultrafiltration in China including membrane types and performances, manu-
factoring and applications, characteristics of the membrane materials, 
methods for membrane formation and problems in the application of reverse-
osmosis and ultrafiltration technology has been described by Zheng (23) 
in a review article with 178 references. The drawback of the process 
is that the membranes may either absorb the analyte or release contaminants 
into the test sample.
1.1.1.1.6 Ultrafiltration

It is used to filter water sample under pressure through a membrane. The membrane used can pass molecular constituents below a certain size e.g. 1000 mol. wt. and retain those above that size. This process is more selective than the reverse-osmosis because it can be used to concentrate only higher mol. wt. analyte. Commercial units are available in market and they are capable of filtering litre quantities per day. Milanovich et al. (24) reported that good recoveries of organic pollutants from water were obtained by both ultrafiltration and lyophilization but ultrafiltration gives better results for humic materials. Greve et al. (25) described another filtration technique by which endosulfan residues can be recovered (82-85%) from water.

1.1.1.2 Isolation Techniques

Isolation techniques are those by which analyte is taken out from water. These techniques are discussed below:

1.1.1.2.1 Liquid-liquid extraction (Liquid-liquid chromatography)

It is one of the extensively studied and very old process of considerable significance to the chemical industry. It is a widely used technique for concentrating pesticides from water. Hexane is the useful solvent for organochlorine pesticides and for many others chloroform is useful. Dyatlovitskaya et al. (26) extracted DDT, chlordane, aldrin and dieldrin from water with benzene. Pyrethrins were quantitatively recovered from water by extraction with light petroleum (27). Organochlorine and organophosphorus insecticides were extracted from water with benzene (28). Hillman (29)
discussed the extraction of insecticides from the water of Rhine River. Trace amounts of chlorinated hydrocarbon insecticides such as lindane, aldrin, DDT and its derivatives were extracted with toluene (30). Vaughan (31) determined 2,4-D and silvex at ppb level using liquid-liquid extraction. The average recovery of 2,4-D at 10 and 1 ppb was 91 and 120% respectively while for the silvex at the same concentration recovery was 85 and 110% respectively. To deal with large volumes of water sample continuous extractors are used. A countercurrent flow system based on the difference in density is commercially available. It is much promising and it has been used to remove organic pollutants from drinking water supplies (32). Some new simple devices such as teflon helex continuous liquid-liquid extraction apparatus for organic pollutants in drinking water had been designed and used (1, 33, 34). The analyte obtained by this method has low co-extractive levels and it is analysed with little clean up. The clean up is generally brought about by column (35, 36) and thin-layer (37) chromatography. Thirty seven pesticides including phosphoric acid esters, chloropesticides and other chloroorganics were preconcentrated from water by liquid-liquid extraction using n-heptane in a light phase rotation perforator. The average recoveries were found to be 80-115% (38). Hoke et al. (39) reported the importance of the liquid-liquid extraction in the determination of phenoxyacetic herbicides such as 2,4-D, 2,4,5-T and silvex. The analysis of wastewater for organophosphorus pesticides such as diazinon, azinphos-Me and fenthion by continuous flow methodologies in which continuous flow and flow-injection both were combined with liquid-liquid extraction and adsorption on resin. The former method was found to be more selective (40).
It is advisable that organic solvent and extraction parameters e.g. pH, ionic strength and temperature must be tailored before the procedure is used in the particular circumstances. In the extraction of water samples, usually emulsions are produced with non-polar solvents and this may be avoided by the use of drying agents such as anhydrous sodium sulphate or isopropyl alcohol. Significant portions of the organic solvent may adhere to the walls of the vessel requiring repeated washings which decrease the concentration factor. If impurities are present in the solvent they may be concentrated along with the sample constituents, e.g., traces of cyclohexane which are present in the best grades of methylene chloride solvent are concentrated with the organic analyte when the solvent is distilled off (41). The solvent used is contaminated by contact with plastics or rubber specially from bungs and caps. The contaminant may be concentrated with the analyte. Therefore all the solvents used must be sufficiently pure and great care must be taken to avoid contamination by contact with solid supports.

The following developments in the solvent extraction are noteworthy. Beroza and Bowman (42-45) have developed the idea of p-value for confirmation of insecticide identity and for clean up of insecticides at the ng level. The concept of using the p-value for determination of the parameters of solvent choice for quantitative liquid-liquid extraction of organophosphorus pesticides from water are reported by Suffet and Faust (46). They claimed that the p-value can be used to determine the quantitative extraction of the parent molecule, oxon and hydrolysis products of diazinon, parathion, malathion, fenthion, 2,4-D and 2,4,5-T (47). Suffet et al. (48) used p-value method as a guide to optimize the liquid-liquid extraction. The
p-value determined in distilled water is shown to be applicable to other types of water including river water, sea water, and secondary sewage effluent. As stated above, the solvent may form emulsion with water on shaking at room temperature and emulsion behaves differently from the pure solvent. The emulsion formation tendency decreases with increasing temperature and, therefore, the extraction may be more effective at higher temperature. Weil and Quentin (49) examined the four procedures for extracting chlorinated hydrocarbons from water. Samples of water containing 10 ppb of DDT, lindane, heptachlor epoxide, dieldrin and methoxychlor with an without 500 ppm of suspended matter were extracted with petroleum ether by three cold extraction methods and one hot extraction method using a special glass micro separator. The other extraction methods gave lower results. The solvent used for extraction may solidify on cooling. If the solvent is carefully selected, once the extraction is complete, a solid bead will be formed on cooling and the extract will be retained. The small bead of a few milligrams may be physically removed from water sample for determining the analyte.

A comparative study was made by Bruchet et al. (50) for continuous and batch liquid-liquid extraction for the analysis of pesticides in water. Chau et al. (51) reported that three basic criteria exist for developing liquid-liquid extraction techniques for water samples. These are choice of solvent, knowledge of water quality and type of liquid-liquid extraction procedure i.e. serial or continuous.

1.1.1.2.2 Solid-phase extraction (Liquid-solid chromatography)

In this process a solid material e.g. adsorbent or ion exchanger is used
in place of a liquid phase to retain the analyte. Then the analyte is eluted from the solid phase with a suitable solvent for analysis. The solids used with different procedures are discussed below:

1.1.1.2.2.1 Activated carbon

It is known since long and it has extensively been used for the removal of pigments, waxes and oils from different samples (52-55). It has also been used for the removal of organic and inorganic pollutants from wastewaters discharged from food plants, oil refineries, paper industries, sewerages, textile industries and drinking water (56-61). The sorption and desorption studies of parathion, malathion, cinnamic acid, 2-naphthaleneacetic acid, 2-naphthoxyacetic acid and TCA on activated charcoal have been carried out (62-64). Activated granular carbon filters were developed and widely used for removing organic pollutants from drinking water in European cities (65). Lagana et al. (61) proposed a method for the determination of fluoranthene, benzo (e) acephenanthrene, benzo (k) fluoranthene, benzo (def) chrysene, benzo (ghi) perylene and indeno (1,2,3-cd) pyrene in water samples using a short column packed with graphitized carbon black (GCB). The recovery of these compounds from GCB depends on their molecular structure but satisfactory results can be achieved by using toluene-benzene-acetonitrile (5:2:3). Vanderbrought and Van Grieken (66) used activated carbon for preconcentration of trace ions. C18 bonded silica gel in column was used for concentrating oxine (8-hydroxyquinoline) complexes formed in sea water samples (67). Neutral oxine forms complexes with metal ions that can be eluted with methanol. By this procedure the saline matrix is eliminated and concentration factors of about 200 are attained. In the West Germany laboratory experiments showed that activated carbon
filters were effective to retain the low concentration of pesticides (68). All model chlorinated pesticides were completely adsorbed when extracted with GCB (69). Organochlorine pesticides such as \( p,p'\)-DDD, \( o,p'\)-DDD, \( p,p'\)-DDT and \( p,p'\)-DDE were rapidly extracted from natural water samples at the ppt level by means of a small GCB cartridge (70). Bacaloni et al. (71) reported that GCB serves as an excellent sorbent material for chlorinated pesticides from water samples. GCB was used for preconcentrating herbicide residues in water by Mangani et al. (72). Preconcentration of organophosphorus (73) and chlorinated (74) pesticides by GCB has been reported. Rosen and Middleton (75) remarked that activated carbon adsorption gives low results by a factor of two. However, it shows good recovery (75-86%) for organochlorine pesticides including BHC, chlordane, DDT, aldrin, DDD and endrin at 2.5 to 5.0 ppm level. Eichelberger and Lichtenberg (76) reported that carbon adsorption is a useful method for the isolation and measurement of some organochlorine pesticides such as methoxychlor, lindane, endrin, dieldrin and heptachlor epoxide in water at 2 ppb level as it gives 65-90% recovery. However, it is less efficient for other pesticides such as chlordane, DDT and endosulfan because the recovery is only 20-45%.

Activated carbon samples have been developed that can process many thousand gallons of water (77). The drawback of the method is the poor recovery of the adsorbed organic pollutants. The usual way of recovery is the air-drying of the carbon and then the use of prolonged soxhlet extraction with chloroform and then ethanol (41). Super critical liquid carbon dioxide was evaluated as a solvent for organic pollutants adsorbed on activated carbon (78). Fuchs and Khun (79) determined the
following yields (grams of organic residue per kilogram of activated carbon) of the adsorbed organic pollutants from activated carbon-filters used to treat drinking water: chloroform, 2.5; dioxane, 4.4; ethanol, 9.6; acetone, 10.9; dimethyl formamide, 29.7.

1.1.1.2.2 Organic polymeric adsorbents

Recently XAD resins have been used for concentrating pesticides in water. Two types of XAD resins, (i) styrenedivinylbenzene copolymers (XAD-2), and (ii) an acrylic ester polymer (XAD-8) are available (41, 80, 81). Chlorinated pesticides are adsorbed from potable waters on Amberlite XAD-2 and they can be eluted with n-hexane (36). Adsorption studies of chlorinated pesticides were made on XAD-4 by Musty and Nickless (82). XAD-2 resins were used to concentrate methyl and ethyl parathion from run-off water (83). A multiresidue technique to extract organochlorine pesticides and PCBs from natural waters was developed by Berkane et al. (84). A XAD-2 column can be used to recover 94% fenitrothion from water. As fenitrothion is stable on the column for at least two weeks, the procedure can be used as a preservation technique. Rees and Au (85) reported that Amberlite XAD-2 cartridge could be used to concentrate and remove traces of organochlorines and PCBs from river water. Drevenkar et al. (86) reported that the sensitivity can be increased two orders for detecting total organophosphorus and carbamate pesticides in surface water by using XAD-4 for preconcentration. XAD-2 and XAD-7 were used to preconcentrate lindane and atrazine from river waters (87). XAD-4 resin was used for field concentration of organic pollutants in surface waters. Recoveries of most organochlorine, organophosphorus, organonitrogen, chlorophenol and chlorophenoxy acid pesticides were 50%, for the samples
spiked at the 10 and 0.1 ppb levels (88). Levesque and Mallet (89) determined aminocarb and its derivatives in water by isolation on Amberlite resins followed by gas chromatography. Bain and Ding (90) determined organochlorine pesticides in groundwater after preconcentration on XAD-2 resins and recovery range was found to be 90 and 111%. Xue (91) analysed water samples for organophosphorus and organochlorine insecticides following preconcentration on XAD-4 resin and instrumental analysis. The resin approach gave comparable result to liquid-liquid partition approaches. Rosenfeld et al. (92) used XAD-2 resin for simultaneous extraction and derivatization of organic acids. Moore et al. (93) investigated extraction efficiency on XAD-2, XAD-4 and XAD-7 resins for pesticides from water. It was best at pH 7.0. Maskarinec and Manning (94) evaluated the efficiency of XAD-4 resin for removing the pesticides from water for subsequent analysis. A limited number of pesticides including lindane, aldrin and p,p'-DDT were added to water and extracted by elution on XAD-4 resin.

Malcolm et al. (95, 96) found that about 50% of the total organic carbon of the average water could be concentrated on XAD-8 resin column. Aiken et al. (97) found that alike activated carbon total elution of the adsorbed substance on XAD resins is not possible. In the case of humic material it was found that 20% of the substances are adsorbed irreversibly on XAD-2 at pH 2.0 which can not be eluted with either alkaline solutions or organic solvents. Resin contaminates the analyte during chromatographic concentration. It can be avoided by proper cleaning of the resin. For example, XAD-2 resins are cleaned by successive extraction of the resin with methanol, diethyl ether, and acetonitrile in a soxhlet extractor. The clean resin must be stored under methanol and must be tested for contaminants prior to use. Cellulose columns (98) were used for adsorption
and recovery of some organophosphorus pesticides. Recoveries from the cellulose column were as follows: thimet 80%, Me-parathion 82%, malathion 87% and parathion 90%.

1.1.1.2.2.3 In-situ polymerized resin

A new in-situ polymerized resin process using open-pore polyurethane foam has been developed. These resins have high adsorption capacity on weight sorbent basis that is equivalent to that of activated charcoal. The resins have high affinity for phenols, PAHs, primary amines and nitrogen containing heterocyclic compounds. The adsorbed organic pollutants are easily eluted with a suitable solvent with good recovery. In-situ resin polymerization is readily adaptable for large scale use (99, 100). At this stage it seems that these resins possess a promising potential for water treatment.

1.1.1.2.2.4 Polyurethane foam

It has been used for preconcentration of organic pollutants found in air and water. There are limited examples of preconcentration of organic pollutants in water by polyurethane foam (PUF) while it has been proved to be a versatile material for trapping air borne organic pollutants (101). The common obstacles such as poor trapping efficiency, limited air or water flow, degradation of trapped pesticides, instability of trapping medium, and difficulty with the clean up of the recovered pesticides to permit its unambiguous quantitation do not arise with this method because of non-hygroscopic, semi-solid, non-volatile and relatively non-reactive nature of PUF. Gough and Gesser (102) used PUF for the recovery of phthalate esters from water. Saxena et al. (103) had shown that PUF plug can be used to concentrate traces of benzo (a) pyrene from drinking water.
The recovery was independent of flow rates over a wide range but increased to a great extent with increase in temperature of water. The retention of benzo (a) pyrene was found to be influenced by water pH, diameter of the column used for holding plugs and the nature of the material coated on the plug. Benzo (a) pyrene sorbed on the plug was stable and it was recovered quantitatively after storage of the plug for seven days at 4°C. If the plug was stored at room temperature (20°C) smaller amount of benzo (a) pyrene was found. Basu and Saxena (104) found that PUF plugs could preconcentrate benzo (a) pyrene, fluoranthene, benzo (j) fluoranthene, benzo (k) fluoranthene, indeno (1,2,3-Cd) pyrene and benzo (ghi) perylene (PAH family) from large volumes of finished and raw waters at a flow rate of 250 ± 10 ml/min at 60 ± 2°C. The retention of PAHs on the foams was 88% from finished water and 72% from raw water. By PUF procedure PAHs were detected in trace quantities in all the water supplies studied. Whereas the sum of the six representative PAHs in drinking water was small (0.9-15 ppt), the values found in raw water were as high as 600 ppt. Musty and Nickless (82) studied the extraction and recovery of organochlorines and PCBs from water using coated as well as uncoated porous PUF and found that in all the cases, increasing sample flow rate decreased the recoveries of organochlorines. PUF (105-107) was evaluated as a trapping agent for a number of pesticides including herbicides from environmental samples. Stalling and Huckings (108) used PUF for the detection and determination of toxaphene in water and fish. Ahmad et al. (63) found that PUF can be used to concentrate plant growth regulators and fungicides such as cinnamic acid, IAA, 2-naphthaleneacetic acid and 2-naphthoxyacetic acid from raw water. The acid sorbed on the plug can be eluted in good recovery (62-85%) with ethanol. PUF can also
be used to separate 2-naphthaleneacetic acid from acetic acid, benzoic acid, citric acid and TCA. There are some examples which show that PUF is very specific in nature and therefore it has limited applications in water analysis. Bedford (109) reported that PUF can not be used reliably to extract PCBs from turbid natural waters. The studies made by Webb (110) showed that PUF is of very limited utility in the isolation of organic pollutants from wastewater.

1.1.1.2.2.5 Inorganic adsorbents

Alumina, calcium phosphate, florisil, hydroxylapatite, magnesia and silica gel are used for preconcentration of organic pollutants in water. Activated alumina is used to concentrate fluorine from water (111). The adsorption capacity is 125 kg of fluorine per cubic metre of alumina. Dieldrin, endrin and p,p'-DDE can be concentrated by alumina columns (112). Muir and Grift (113) used florisil to extract and clean up nichlosamide in river water and in sediment. Recoveries were found to be 99 - 116% from river water and 73 - 126% from sediment. Lord et al. (114) used florisil column to extract carbaryl in poisoned honeybees prior to determination by HPLC. Eleeman and Karasek (115) investigated the retention behaviour of chlorinated chemicals including insecticide aldrin on florisil. Agostiano et al. (116) found that tenax is a useful adsorbent for the preconcentration of chlorinated pesticides from water and air. Bonded-phase silica columns were utilized to concentrate chlorinated pesticides from water (117).

1.1.1.2.2.6 Ion-exchangers

Ion-exchange resins have high exchange capacity as well as high stability in water at room temperature. Therefore the resins are mostly used
for water treatment. Inorganic ion-exchangers have low ion-exchange capacity and are prone to hydrolysis in natural water. They have high thermal stability and therefore they are used for water treatment at elevated temperatures. Junk and Richard (118) concentrated aliphatic acids, chloro and nitro phenols, substituted aliphatic, aromatic, sulphonic and phosphoric acids, and many neutral compounds from distilled water using strong anion-exchange resin. The average recovery efficiency was found to be 90%. It gives recovery nearly 100% for 1-40 ppm solutions of 2,4-D and tetrachlorophthalic acid in wastewater. Braid et al. (119) coupled ion-exchange method with other adsorption methods and obtained 70 - 90% recoveries of organic pollutants from water. Adsorption and elution of uranium and fission products on zirconium phosphate (120) and ammonium molybdophosphate (121) were studied. It was reported that titanium oxide (122) could be used to recover uranium from natural water. However utility of synthetic inorganic ion-exchangers has not been fully explored for preconcentration of pesticides from water. In comparison to other methods the extra advantage of ion-exchange beads lies in the fact that the preconcentrated analyte can be either detected and determined directly upon the beads (123, 124) or the analyte may be eluted from the column and then analysed (125, 126).

Ion-exchange chromatography is a simple and inexpensive technique that has been used since long for analysing organic pollutants in water. Haddad and Jackson (127) described ion-exclusion column chromatography for the preconcentration of low mol. wt. carboxylic acids.

1.1.1.2.3 Precipitation

In this process a specific substance is precipitated from solution and separat-
ed by filtration or centrifugation. It is one of the oldest chemical processes used for the separation and preconcentration. There has been a long search of specific precipitation reagents in order to minimize co-precipitation and to perform complete precipitation of the analyte. The former becomes less important if the end method of detection and determination is selective. The latter can be supplemented by the use of co-precipitation reagent in excess. As they are less sensitive their uses are limited in the area of preconcentration of organic pollutants in water. It is well known that humic acids are base soluble and alcohol insoluble (128, 129). Therefore humic materials may be precipitated from aqueous solutions by acidification with glacial acetic acid in presence of isoamyl alcohol. This method has been applied to fresh water and sea water samples in a cursory manner.

1.1.1.2.4 Impregnation materials

Many impregnation materials have been used for preconcentration of organic pollutants in water. Analytical potential of the adsorption material can be enhanced by impregnating it with another material. Uthe et al. (130) used PUF for the preconcentration of PCBs from water. Organochlorine pesticides were also preconcentrated from water samples through a column of PUF coated with a GC liquid phase. Subsequent washing with acetone and desorption of pesticides by hexane yielded the extractable organochlorine pesticides. Among the various GC liquid phases studied, DC-200 coated on PUF gave more than 90% recovery of most organochlorine pesticides at 1 ppb level (131).

1.1.1.2.5 Ion-retardation resin and Immobilizing ion-exchange site resins

These have been recently developed in order to improve the simplicity
and selectivity of adsorbents or ion-exchangers already known. Ion-retardation resin is synthesized by polymerization of acrylic acid inside Dowex-1 (Bio-Rad Laboratories). Anions and cations in equivalent amounts are adsorbed on the resin but can be eluted with water. Thus the process can be used for desalting an organic pollutant. For example, an aqueous solution of the organic pollutant is passed through a column containing ion-retardation resin and then the column rinsed off with pure water. The organic pollutant comes first and inorganic salts are retarded and they appear as a separate fraction following the organic materials. Immobilized ion-exchange sites can be arranged inside solid, water insoluble porous beads in different ways. Sekizuka et al. (132) swelled resin beads in organic solvent, treated with solution of chelating agents and then sprayed into cold water to shrink the pores. The beads so obtained can trap the ions reached by diffusion into the pores. This method gives material of very low exchange capacity (1-10 meq/g) and another weakness is the possibility of backing the metal chelate from the beads as it is not attached by a chemical bond. Branu et al. (133, 134) used reagents adsorbed into open cell polyurethane foam and sealed with plasticizers. This easily prepared material is an effective concentrating agent. Again the adsorption capacity is very low, for dithianon loaded foam the capacity was found to be 23.4 meq/g. A multipurpose chemical reaction, silylation has been developed recently. Chromatographic grade silica gel, silylated with ethylenediamine, ethylenediamine dithiocarbamate and ethylenediaminetetraacetic acid has been used for preconcentrating various organic pollutants in water (135).
1.1.2 CLEAN UP TECHNIQUES

An efficient extraction method which can exhaustively remove organic pollutants from the sample often yields considerable amounts of co-extractives, which are present in the sample matrix. These co-extractives usually interfere with the end analysis. For example in GLC the electron-capture (EC) detector is extremely intolerant to halogenated impurities, and in TLC fatty material tends to affect the rate of migration of the test material grossly. Other types of co-extractives can also interfere with TLC by reaction with the TLC material or the test material or by masking the visualizing agents. Similarly the co-extractives can affect spectrophotometric methods. Thus the identification as well as quantitation may be either inaccurate or impossible.

Different types of samples carry different interfering materials. For example, water samples from agricultural run-offs often contain many biocides such as organophosphorus, organochlorines, carbamates, herbicides etc. However, lake and river sediment samples in industrial areas contain sulphur, organosulphur, PCBs, phthalic esters etc. Animal tissue samples are rich in lipid content, while some food samples contain large amounts of fats and phospholipids. These are potential interfering substances when EC-GLC is used in the end analysis. However, most of them may be largely, if not completely removed by a proper combination of the following clean up procedures:

1.1.2.1 Liquid-liquid chromatography
1.1.2.2 Liquid-solid chromatography
1.1.2.3 Gel-permeation chromatography
1.1.2.4 High-performance liquid chromatography
1.1.2.5 Precipitation process

1.1.2.1 Liquid-Liquid Chromatography (LLC)

It is widely used for removing large amounts of fats, phospholipids and waxes as well as other polar impurities. In this method the extract is partitioned with an immiscible solvent of different polarity. Thus if the extract is in nonpolar solvents such as hexane, petroleum ether and benzene, it is partitioned with polar solvents such as acetonitrile. Alternatively, if the sample extract is in polar solvent it is partitioned with an immiscible nonpolar solvent. In either case, the nonpolar phase containing waxes and fats is discarded. The polar phase containing organic pollutants is diluted with water, and then sodium sulphate or sodium chloride is added. The addition salts out the organic pollutants from the polar phase and consequently facilitates partitioning of organic pollutant back into the newly added nonpolar phase. Solvents such as N,N-DMF-hexane (136), DMSO-petroleum ether (137) and acetone-petroleum ether were used for the partitioning of organic pollutants from fat. Dubchenko and Tsekhanovich (138) reported that the salting out effect (with 2.0 M magnesium chloride) increases the recovery of phosphamide and formothion from water samples partitioned with benzene-toluene.

1.1.2.2 Liquid-Solid Chromatography (LSC)

It is also used for the removal of fats, phospholipids, pigments, and other unwanted polar compounds. It is used to clean up sample extract prior to TLC or GLC analysis. Among the active adsorbents, florisil, alumina, activated charcoal and silica gel are most commonly used. Recently Muir and Grift (139) designed a method for determining nichlosamide in river water and sediment. The analyte was extracted, cleaned up
on florisil and analysed after methylation with methyl iodide by GC or HPLC. Recovery ranged from 99 - 116% for augmented waters and 73 - 126% for augmented sediments. Carbaryl was determined in food extracts by HPLC and it was necessary to clean up the food extract on a florisil column (140). Mc Garvey et al. (141) used florisil and Sep-PAK columns to clean up oxamyl in potato tubers (0.01 ppm). Carbaryl residues were determined in fruits or vegetables by extraction with methyl chloride and clean up on alumina-silver nitrate column prior to HPLC analysis (142). The plant growth regulator, 4-chlorophenoxyacetic acid, was determined in mung bean sprouts by EC-GC after silica gel column clean up (143). Carbaryl residues in apples were determined by silica gel TLC after charcoal clean up (144). Anderson and Anderson (145) used Amberlite XAD-7 column for cleaning up of IAA, IBA, gibberellic acid, zeatin and aspartic acid with ethanol as a solvent.

1.1.2.3 Gel Permeation Chromatography (GEPC)

It involves the removal of fats from fish and other fatty samples. In this method the separation mechanism is based mainly on the differences in the molecular size. Basically, molecules with higher mol. wt. are usually larger in size and are extracted by some of the gel pores. Thus they have a shorter distance to travel in column and are eluted first. Smaller molecules which can go through all the pores travel the longest distance and are eluted last. Recently this method has been used as a clean up tool for organochlorine compounds in onions prior to GC analysis (146). Automated GEPC has been successfully used to remove fat from food samples prior to organic pollutant analysis (147, 148). GEPC on Bio Beads SX-3 is a universal clean up method for many pesticides from fruits and vegetables (149).
1.1.2.4 High-Performance Liquid Chromatography (HPLC)

The use of HPLC as a clean up technique in organic pollutant analysis is still a relatively unexplored area. Recently a method was reported (150) for the HPLC clean up of environmental sample such as lindane in water prior to EC-GLC determination. Results indicate that interfering co-extractives such as fats, phospholipids and oils were completely removed and recovery of lindane was more than 90%. Aliphatics and polycyclic aromatics in hexane extracts of environmental samples were removed by HPLC on a nucleosil 100-5 column by using pentane and 20% methyl chloride in pentane as eluents (151). The use of HPLC for the clean up of organochlorines in fatty food extracts (152) and triglycerides (153) was also reported.

1.1.2.5 Precipitation Process

It involves elimination of interferences caused by sulphur and organosulphur compounds. Mercury or activated copper dust was used to precipitate sulphur and organosulphur compounds which are often found as co-extractives in the extracts of bottom sediments from lakes and rivers. The presence of these compounds will give a large solvent peak to aldrin peak in the chromatogram. Commonly used clean up techniques such as solvent partitioning and column chromatography are inadequate or ineffective for the complete removal of these interferences. Goerlitz and Law (154) reported that these interferences from sediment extracts could be removed by shaking a small drop (0.1 - 0.2 ml) of metallic mercury after column clean up. Schutzmann et al. (155) used copper aluminium alloy for removing sulphur from water samples.
Several instrumental and non-instrumental techniques have been used for the detection, separation and determination of organic pollutants in water. These techniques can be divided in the following groups. To show the relative utility of these techniques some recently reported papers are cited in each group.

1.1.3.1 Functional group analysis
1.1.3.2 Biological test methods
1.1.3.3 Chromatographic techniques
1.1.3.4 Spectrophotometric techniques
1.1.3.5 Electrochemical techniques
1.1.3.6 Radiochemical techniques

**1.1.3.1 Functional Group Analysis**

This technique involves the detection, separation and determination of a particular group or element in a compound. Spot test, colorimetry and volumetry are the readily available and largely used tools in this group. Spot tests are simple, sensitive and selective and they have been found to be extremely useful for the preliminary characterization of a test material.

A simple and inexpensive spot test for the detection of organic pollutants in water was developed by Qureshi et al. (156). Citric acid impregnated paper in the presence of acetic anhydride was used as the reagent. The limit of detection of organic pollutants such as amitrole, azobenzene, bavistin, calixin, 2,4-lutidine, nicotinic acid, 2-picoline and quinoline was found to be 0.40, 12.00, 3.20, 4.00, 100.00, 0.04 and 100.00 μg
respectively. The test was successfully applied in acidic, basic and saline waters. A new specific colorimetric spot test (157) for the detection of malathion residues in water was developed. In this test activated charcoal was used to recover and concentrate malathion from water samples. Then malathion was hydrolysed with potassium hydroxide to give potassium fumarate which gives a red colour with acetic anhydride on heating. Lower limit of detection was found to be 1 ppm.

A new technique (158), pressure capillary spot test was developed for the detection and determination of organic pollutants including plant growth regulators, IAA and gallic acid with lower limit of detection as 0.1 and 50 µg respectively. A capillary containing cotton plug impregnated with p-dimethylaminobenzaldehyde (p-DAB) and TCA was used as a detector. This technique was used for semi-quantitative determination of IAA in wheat shoots. A new capillary spot test for the detection of trace levels of organophosphorus pesticides in water, soil and citrus leaves was developed by Rathore et al. (159). In this test an equiproportional mixture of p-DAB and TCA was used as a detection reagent. The lower limit of detection of malathion, formothion, thiometon, dichlorvos, methyl parathion, dimethoate and phosphamidon in water was found to be 5, 25, 25, 8, 5, 30 and 9 ppm respectively. A sensitive and selective novel technique (160) was also developed in this laboratory for the detection and semi-quantitative determination of phenoxy herbicides. The herbicide was heated in presence of hydrated zinc sulphate to split off formaldehyde. The pressure was reduced in order to bubble the formaldehyde vapours in the reagent. One or two tiny crystals or chromotropic acid (sodium salt) in 0.20 ml of concentrated sulphuric acid was used as the reagent. This technique was successfully applied for the detection of 2,4-D acid, 2,4-D ethyl
ester and 2,4-D sodium salt in formulations, leaves, soil and water.

Specific detection of carbaryl on silica gel coated TLC plates by spraying 1% m/v cupric chloride in distilled water followed by 0.1% ammonium vanadate in concentrated hydrochloric acid diluted with ethanol or 0.5% m/v potassium hexacyanoferrate (III) in 0.5% m/v sodium hydroxide was reported by Padalikar et al. (161). The former reagent has relatively higher sensitivity (1 µg) for the breakdown products of carbaryl, 1-naphthol, whereas latter has same sensitivity for both carbaryl and 1-naphthol (10 µg).

A volumetric method (162) for the determination of malathion in emulsifiable concentrates was developed. Alkali cleavage of malathion gives 0,0-dimethylphosphorodithioic acid which forms a complex with Bi(III). The complex was extracted with chloroform and the bismuth left uncomplexed is titrated with the sodium salt of EDTA. Any free 0,0-dimethylphosphorodithioic acid, that may be present, interferes with the determination. This is rectified by the complex formation without hydrolysing the sample. For the determination of malathion one more simple, sensitive and safe volumetric method was reported (163). In this method potassium permanganate in alkaline medium was used as an oxidizing agent. The lower limit of detection was found to be 0.1 mg of malathion. A fast method (164) based on argentometric titration was developed for the determination of TCA directly on farms or in mobile laboratories. An aliquot of the sample solution containing sodium TCA is treated with a 5-10 fold amount of 30% sodium hydroxide solution and the mixture is heated for 2 hr using a reflux condenser. After cooling dilute nitric acid, and silver nitrate are added to the solution and the excess is titrated with ammonium thiocyanate in presence of ferroammonium aluminosilicates until a stable pink cinnamonic colour develops.
1.1.3.2 Biological Test Methods

These methods show the presence of toxically significant residues, for example, by inhibition of the enzyme cholinesterase in animals by certain classes of pesticides. As it is possible to use these methods without clean up they are useful as screening tests but are non-specific. The determination of organic pollutants by bioassay is based on the measurement of growth, death or some other physiological changes in animals, plants or microorganisms. Any organism which is susceptible to a pollutant (pesticide) may be used for the bioassay of its residues, but some organisms are more sensitive than others. The biological catalysts, enzymes, which participate in many chemical reactions occurring in living cells are widely used for pesticide analysis. The prime example of this method is the inhibition of cholinesterase by organophosphorus and carbamate insecticides. The techniques such as enzyme inhibition electrodes, enzymatic chromatography and enzyme colorimetry are applied in this area. Some of the selected recently reported papers are summarized below.

Guilbault and Kramer (165) prepared a column of immobilized horse serum cholinesterase in a flow system and developed an assay of organophosphorus pesticides. The fluorescence due to hydrolysis of a fluorescein or resorufin ester by the cholinesterase is monitored. When the enzyme is inhibited, no hydrolysis occurs and the fluorescence is no longer produced. As little as 1 ppb of parathion, systox or malathion can be analysed. A sensor for aspartame was prepared by chemical immobilization of L-aspartage on an ammonia-selective electrode. The sensor is stable for more than eight days. The probe is successfully used for the assay of aspartame in commercially available sweeteners (166). A new fiber-optic probe for the determination of glucose was developed. Glucose oxidase was immo-
bilized on a preactivated immunodyne membrane by direct application of the enzymatic solution to one side of the membrane. Then the membrane was placed around the common end of a bifurcated glass fiber optic bundle and immersed in the sample cell which contains the glucose sample, peroxidase, and a colourless dye. Glucose is quantified by the colour change when the reaction takes place (167). Guilbault and Nghe-Ngwainbi (168) used piezoelectric crystals coated with acetylcholinesterase or parathion antibodies as a detector for gaseous parathion, malathion, methyl parathion and disulfoton. The sensitivity was found to be in the range of 36-680 ppb.

An enzyme immunoassay for the determination of atrazine in water and soil was described by Bushway et al. (169). An atrazine antiserum is prepared by derivatizing atrazine at the 2-chloro position and covalently conjugating it to bovine gamma globulin using a modified carbodiimide cross-linking procedure. The coefficients of variation for atrazine in water samples at atrazine concentration of 4-50 ppb are reported to be 10.0-4.1% and in soil samples at 4-40 ppm are 16.3-8.4%. El Yamani et al. (170) developed automated system based on the use of a butyrylcholinesterase electrode for the detection of organophosphorus and carbamate pesticides at very low (ppb) concentrations. This technique was utilized for the routine monitoring of river water pollution. A hydrogen peroxide electrochemical sensor, coupled with immobilized lactate oxidase and covered with a cellulose acetate membrane, was applied in flow analysis of lactate in milk samples (171). Hydrogen peroxide produced by enzymic reaction was measured with a platinum electrode polarized at +650 mV versus Ag/AgCl. Milk samples were analysed and compared with a spectrophotometric reference method. The amperometric procedure is very simple and the short response time allows its use in assaying milk samples on dairy farms. Dreher and
Podratzki (172) developed an enzyme immunoassay (EIA) for the detection of endosulfan and its degradation products. The EIA is based on antibodies raised against the diol of endosulfan by immunizing rabbits with a keyhole limpet hemocyanin (KLH) endosulfandiol conjugate. With this method, endosulfan can be detected in aqueous solutions at a level of 3 ppb without any sample extraction procedure. The measuring range was found to be between 3 and 400 ppb.

TLC coupled with enzyme inhibition (173) was used for the sensitive and rapid detection of paraoxon. In this method, solutions of 0.5 mg/ml horse serum cholinesterase in either 50 mM sodium phosphate buffer (pH 7.7), or 10 mM sodium tetraborate buffer (pH 9.2), or 1:10 diluted human pool plasma in phosphate buffer were used. For the colour development the spray reagents used are: 1.5 mM Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid) pure], and 7 mM butylthiocholine in phosphate buffer or 15 mg of indoxyl acetate in ethanol (1 ml) and borate buffer (10 ml). The detection limit was found to be 0.3 ng. Bhaskar (174) developed a simple enzymatic method for field TLC detection and determination of fenitrothion in water with pig liver acetone powder as enzyme source. It was also reported that pig liver acetone powder is more advantageous than raw liver sources, owing to its assay procurement and instant use. For colour development, solutions of 1-naphthyl acetate in acetone followed by p-nitrobenzenediazonium fluoroborate in acetone were sprayed. The white spots appeared on the orange-red back ground. The lower limit of detection was found to be 1 ng.

Bhaskar and Kumar (175) developed colorimetric method for the determination of dimethoate and omethoate. Pig liver acetone was used as enzyme source and p-nitrobenzenediazonium fluoroborate as the chromo-
genic reagent. By this method 1-10 µg of dimethoate and 50-1000 ng of omethoate can be estimated.

1.1.3.3 Chromatographic Methods

Different chromatographic methods discussed below are used for the detection, separation and determination of organic pollutants in water.

1.1.3.3.1 Paper chromatography (PC)

This is the simplest method of identification and estimation. It has a reasonable degree of sensitivity and selectivity for chlorinated and organophosphorus insecticides and chlorophenoxy acid herbicides. The greater sensitivity and concurrent quantitative estimation possible with GC led to PC being used now mostly for confirmation of the relatively non-specific gas chromatography compounds. TLC has replaced PC in pesticides residue analysis because of its increased resolution and shorter development time.

Pain and Pal (176) separated IAA from honey by PC in propanol-ammonium hydroxide-water (10:1:1) or ethanol (70%). Ehrlich's reagent was used for locating the spots. Rathore et al. (177) separated tricarboxylic acids, amino acids and keto acids on papers impregnated with calcium sulphate and calcium carbonate in simple solvents such as acetone, ethanol and distilled water. Papers impregnated with hydroxides of aluminium and cadmium were also used to separate herbicides by Rathore et al. (178).

1.1.3.3.2 Thin-layer chromatography (TLC)

It is a simple and quick semi-quantitative analytical technique. It requires inexpensive apparatus for many routine applications. Though TLC is lacking the precise specificity of GLC, it is more precise and more sensitive than
PC. It readily provides much information which would have been difficult or impossible to get in any other way. When TLC is coupled with other sophisticated analytical techniques, it becomes a powerful tool and often plays a vital role in the separation of complex mixtures. There are, however, some limitations to this technique. For example, resolving power of TLC is limited because of chromatographic conditions e.g. mobile phase velocity is difficult to control.

It is well known that TLC has been, and is still being used for a wide variety of applications within the organic chemical industry. The use of TLC as a rapid and simple spot test either in the laboratory or in the field, is likely to remain with us for many years to come. Over the last decade, this technique has been widely applied in areas such as lipids, terpinoids, essential oils, vitamins, steroids, alkaloids, pharmaceuticals, drugs, antibiotics, clinical medicines, plant extracts, food additives, pesticides, proteins, amino acids, nucleic acids and sugars. Not only does TLC play an important part in the research, development and process and quality control of these materials, it also fulfills vital role in analytical schemes devised to unravel the composition of unknown mixtures. It is also useful in solving the problems concerned with conservation for the environment. In the case of organic pollutant analysis TLC has been used in many ways and several modes. Some of them are given below:

1.1.3.2.1 Normal-phase thin-layer chromatography (NP-TLC)

This is the simplest type of TLC in which a solvent system is allowed to develop the normal phase plates. This mode of development suffices for many applications, and it is commonly used for organic pollutants analysis.

Some carboxylie herbicides and plant growth regulators were separated
(179) by NP-TLC on glass plates coated with calcium sulphate plus activated charcoal, p-DAB, flyash, silica gel, starch, calcium nitrate and cupric sulphate. This technique may also be suitable for other acid pesticides. Garcia Lopez et al. (180) detected diazinon, malathion, dimethoate, ethion, methyl parathion and parathion on plates coated with silica gel in hexane-acetone (70:30) or benzene-chloroform (70:30). Ammonium molybdate (15%) mixed with nitric acid (2:1) was used for locating the spots. Matysik et al. (181) determined HCH, DDT, DDE, lindane, DDD and methoxychlor on plastic foil plates coated with silica gel in dichloromethane-n-heptane (2:9) solvent system. The detection limits were found to be 0.20 µg for HCH, DDT, DDE, lindane, 0.25 µg for methoxychlor and 1.00 µg for DDD. Srivastava and Reena (182) separated carbaryl, bendiocarb, carbofuran, baygon, ziram, zineb and aldicarb on plates coated with silica gel containing 1% zinc acetate. Benzene-ethyl acetate (50:10) was used as the solvent system. Dhillon et al. (183) developed a TLC method for the detection of aminophenols and aromatic amines. The plates coated with silica gel and impregnated with sodium nitrite were used. Benzene-ethanol (20:1) as a solvent system and 2% solution of sulphanilic acid in concentrated hydrochloric acid as a detector were used.

1.1.3.3.2.2 Reversed-phase thin-layer chromatography (RP-TLC)

Recently chemically bonded reversed phase TLC plates have made RP-TLC an important new tool. RP-TLC expands the scope of separations by TLC to include compounds traditionally not separable by NP-TLC because of their high polarity. RP-TLC invites the use of a nonpolar, usually hydrocarbonaceous stationary phase and a relatively more polar mobile phase.

Jost et al. (184) reported the retention behaviour of some substituted
benzoic acids on reversed phase pre-coated plates in the presence of ammoneium bromide and various tetraalkyl ammonium compounds with different alkyl chain length. The \( R_f \) values of the test compounds decrease in the absence as well as in the presence of ion-pair reagents with increasing water content of the solvent system. The \( R_f \) values of the strongly acidic substituted benzoic acids decrease with both increasing concentration of ion-pair reagent and increasing chain length of the tetraalkyl ammonium compounds. Wilson (185) noted the effect of solvent pH on the ion-pair RP-TLC of four organic acids (salicylic, gentisic, 2,5-dihydroxybenzoic, 2,6-dihydroxybenzoic acid). These acids were chromatographed on silica gel, paraffin impregnated silica gel and \( C_{18} \) bonded silica gel with a total of four ion-pair reagents. The \( R_f \) values of these acids were unaffected by solvent pH when tetra-n-butyl ammonium iodide and tetraheptyl ammonium bromide were used as ion-pair reagents. However when cetrimide and cetyltrimethyl ammonium bromide were used as ion-pair reagent a significant dependence of \( R_f \) values on the solvent pH was observed. Quantitative separation (186) of TCA from some carboxylic herbicides was achieved on barium sulphate or calcium sulphate coatings impregnated with coconut oil. Many ternary and quaternary separations were achieved using aqueous solvents. The lower limit of determination of TCA was found to be 400 μg.

1.1.3.3.2.3 Two-dimensional thin-layer chromatography (2-D-TLC)

In this method the analyte is spotted at the corner of a flat bed and a developer is allowed to migrate in one direction followed by another migration either of the same developer or a new developer at right-angle to the first. When the same developer is used successively in the two directions of a uniform adsorbent layer, all the spots align on a diagonal. Hence
the advantage of this method is very slight increase in resolution correspond-
ing to an increase by a factor of $\sqrt{2}$ in the distance of migration of the
spot. The system can be made more efficient, in order to resolve a number
of spots, by the use of the entire chromatographic area and appropriate
different developers for each direction. Similar analytical potential can
also be attained when the same developer is used successively at an ortho-
gonal angle to a chromatographic plate that has been coated with two
adsorbents.

Jost and Herbert (187) studied the applications of 2-D-TLC to amino
acids, bile acids, pesticides and sex hormones on precoated plates of CNF_{254s}.
Deleu et al. (188) employed 2-D-TLC for the identification of eleven urea
substituted herbicides from river water. Herbicides, metoxuron, fenuron,
monuron, isoproturon, chlorotoluron, diuron, methabenzthiazuron, nebourn
and buturon were separated on polygram SILG UV_{254} plates in ether -
toluene (1:3 or 2:1) and chloroform - nitromethane (2:1) solvent systems.
Rathore et al. (189) separated herbicides, fungicides and other organic
acids occurring in fruit juices by 2-D-TLC on calcium sulphate coated
glass plates. Chloroform, carbon tetrachloride, ethyl acetate, benzene
and distilled water were used as developers.

1.1.3.3.2.4 Sequential thin-layer chromatography (S-TLC)

In this technique the analyte is spotted as usual and a developer is allowed
to ascend a small part of the chromatogram. The plate is removed from
the chamber, it is allowed to dry in order to remove the developer and
then the second developer is allowed to ascend the distance covered by
the first developer as well as some additional fresh part of the chromatogram.
Thus a chromatogram can be developed in two to six different solvents.
The advantage of the technique is that ternary and quaternary separations can be achieved by S-TLC on the same plate while only binary separations are possible by NP-TLC.

Abou-Donia and Komeil (190) used S-TLC for the separation and determination of paraquat dichloride and five related compounds on glass fiber sheets impregnated with silicic acid using benzene - amyl alcohol - methanol - 1N hydrochloric acid (1:1:2:1) followed by methyl cyanide - water - ammonia (40:9:1). Riebel and Reillich (191) analysed methamidophos in potato tubers and foliage on silufol plates using hexane - acetone (1:1) followed by chloroform - acetone - methanol (1:1:1) solvent systems. Spots have been located by spraying a dilute swine liver homogenate and then alcoholic 2-naphthyl acetate. A mixture of eight herbicides (192) was analysed by S-TLC on silica gel Merck no. 5721 plates. The plates were first developed in hexane - acetone (3:1), dried in an air stream for 1 hr, and then developed again in hexane - acetone - ethanol (35:25:0.50).

1.1.3.3.3 Gas chromatography (GC)

The most versatile and sensitive method for organic pollutant analysis is undoubtedly GC.

Mehran et al. (193) separated organic pollutants from water by GC analysis coupled with capillary columns. Lahl et al. (194) demonstrated by GC analysis of drinking, surface and swimming pool water that TCA is generated by the reaction of lignisulphonic acid, humic acids and hypochlorite ion. Chmil (195) described a GC method for the determination of chlorophenoxyalkylcarboxylic acid and chlorophenols in water as their 2,2,2-trichloroethyl and pentafluorobenzyl ester derivatives. Ozaki et al.
(196) determined pesticides in river water and sediments by GC coupled with mass spectroscopy (MS) on a fused silica capillary column at 160°C or 200°C. Miyazaki et al. (197) determined the residues of chlordane in water, milk, fish and meat by this technique using stationary phases such as OV-210 (1%) + OV-275 (1%) and OV-210 (2%) at 175°C and 200°C. Bando et al. (198) detected IAA in serum by GC coupled with MS. Liill et al. (199) determined chlorophenoxy herbicides in wastewater and sludges by packed and capillary column with EC and MS detection. Lee et al. (200) analysed phenol and twenty one chlorinated phenols in natural water by the formation of pentafluorobenzyl ether derivatives and GC-ECD. Grover et al. (201) determined 2,4-D and dicamba in inhalation, dermal, hand wash and urine samples from spray applicators by GC.

1.1.3.3.4 High-performance liquid chromatography (HPLC)

HPLC and GC both are complementary and are efficient and highly selective techniques which need a small quantity of the sample that can be recovered after analysis. GC is better in speed and simplicity of the equipment while HPLC is preferable for analysing thermally unstable materials. HPLC continues to find a useful place as an analytical tool for the separation and determination of organic pollutants.

Archer and Stokes (202) detected residues of 1-naphthaleneacetic acid and related compounds in grapes by HPLC with fluorescence detector. A simple HPLC method (203) was reported for the determination of carbaryl deposited after aerial application. The carbaryl was collected on a filter paper, washed with acetone and the concentrated solution was chromatographed on a 5 um-ODS using methylcyanide - water as solvent and was determined at 267 nm. The carbaryl was detected in the range of 35-
3500 μg/m² within 200 m from the boundary of aerial application. She et al. (204) determined carbaryl in polluted river water by HPLC using a postcolumn catalytic reactor and fluorogenic levelling. Shiao and Hao (205) simultaneously determined gibberellic, IAA and abscisic acids by HPLC as their 1-bromo-2-acetonaphthene derivatives. Thakkar and Alone (206) detected traces of chlorophenoxy acids and their esters in water by HPLC using a UV detector. Chlorophenoxy acids and their esters were first partitioned with acetone and then extracted with methyl chloride - hexane (1:1) and chromatographed on micro particulate SiO₂ bonded with octadecyl-trichlorosilane using a mixture of isopropanol and water in a gradient elution system. The detection limits for chlorophenoxyacids and for their esters were found to be 0.3-0.9 and 0.5-1.0 ppb respectively. Akerblom (207) determined 2,4-D, MCPA, dichlorprop and mecoprop in water by HPLC using a precolumn packed with pellicular C₁₈ silica gel. The detection limits were found to be in the range of 0.005-0.01 ppm. The retention of these compounds by the precolumn was increased by increasing the ionic strength of water sample with sodium chloride.

1.1.3.4 Spectrophotometric Techniques

Spectrophotometry of organic pollutants does not achieve the sensitivity of TLC and GC techniques. It may not be able to distinguish between the parent compound, metabolites and hydrolysis products but can be used with chromatography as confirmatory technique.

1.1.3.4.1 Ultraviolet and visible spectrophotometry

Ultraviolet methods require a rigorous clean up of the extract to ensure that the final solution is free from any material that will absorb light in the region of the spectrum where organic pollutant content is to be
measured. It is commonly used in the determination of compounds which are difficult to chromatograph such as acids 2,4-D, 2,4,5-T and the ionic bipyridilium herbicides diaquat and paraquat. A distinct advantage of spectrophotometry in visible range is that it is readily adoptable to automated analysis.

Tsitovich et al. (208) determined several herbicides in natural water samples spectrophotometrically. 2,4-D is eluted with 2 M sodium chloride and determined by using butylrhodamine and a green filter. Dalapon is determined by UV spectrophotometry at 200-203 nm. TCA is eluted with 4 M sodium chloride and determined at 510 nm. Yolan (S-ethyl-N-hexamethyleneiminothiocarbamate) was eluted with sodium chloride in water-ethanol (1:1) and determined at 206 nm. Sessaiah and Mowli (209) described a simple and sensitive spectrophotometric method for the determination of malathion with methylene blue. The malathion was decomposed with alkali and the resulting dimethyldithiophosphate was extracted with methylene blue in chloroform. The absorbance of the organic layer was measured at 652 nm. A new simple, rapid and sensitive method was developed (210) for the determination of organophosphorus pesticides such as parathion, malathion, quinolphos and monocrotophos. The method involves the formation of 12-molybdophosphoric acid from orthophosphate and the reduction of excess of molybdate by succinyldihydrazine to give molybdenum blue. The dye extracted in butanol shows absorption at 780 nm. This method is free from the interferences of Mo, As and Cu. The molar absorptivity and Sandell's sensitivity were found to be $3.75 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ and 0.0008 µg respectively. Rathore et al. (211) determined citric acid in river water spectrophotometrically. The method is based on the red colour developed on heating the acid with acetic anhydride at 65°C in
presence of sodium acetate and phenyl acetate.

### 1.1.3.4.2 Fluorescence and phosphorescence

Selectivity and sensitivity of these methods are greater than ultraviolet and visible spectrophotometric methods. The sample should be free from naturally occurring biological materials which produce fluorescence.

Yuan and Cao (212) determined DDVP and related compounds by fluorescence method. These compounds react with o-dianisidine in the presence of sodium perborate to form fluorescent products, which can be measured by fluorimetry. A sensitive fluorimetric method (213) was developed for the determination of IAA, IBA and IPA. The method was based on the reaction of the above auxins with o-phthaldehyde in concentrated sulphuric acid.

Trautwein and Guyon (214) determined the pesticides, 2,4-D, 2-naphthoxyacetic acid and silvex by low-temperature phosphorimetry. Excitation and emission wavelengths were 284 and 480 nm for 2,4-D, 320 and 500 nm for 2-naphthoxyacetic acid and 290 and 464 nm for silvex. The linearity ranges were found to be 0.10-40.00 ppm for silvex, 0.30-60.00 ppm for 2-naphthoxyacetic acid and 0.50-80.00 ppm for 2,4-D respectively.

### 1.1.3.4.3 Infra-red spectrophotometry

It is useful in organic pollutant analysis because it is possible to obtain qualitative identification as well as quantitative determination with one physical measurement. Generally, microgram pure quantities are required to obtain a spectra. Better results can be obtained by infra-red spectrophotometry coupled with GC.
Eross-Kiss and Miesel (215) determined a number of pesticides without preliminary separations in some plant protectives by infra-red spectrophotometry. Chmil (216) identified organochlorine pesticides by infra-red spectrophotometry.

1.1.3.4.4 Densitometry

Herbicides residues (217) were analysed by spectro-densitometry coupled with TLC. The diffuse reflection electronic spectra of herbicides of different groups was obtained in UV region and the optimal wavelengths for scanning in routine analysis were determined. The substances studied are characterized by the intense band in the region of 220-280 nm. The place and the quantity of resolution of the band depends on the substituents present in the chromatophore group. Hitos (218) analysed some herbicides by TLC coupled with reflectance densitometer.

1.1.3.5 Electrochemical Techniques

Electrochemical techniques such as ion-selective electrode, potentiometry at small current, amperometry, coulometry and polarography are used in organic pollutant analysis. Guilbault et al. coupled these techniques with enzymatic sensors for analysing organic pollutants specially pesticides (165-168).

Polarography was applied for the detection and determination of several pesticides by measuring the diffusion current, i.e. height of the polarographic wave. Polak (219) determined dinoseb and DNOC in water by differential-pulse polarography with a relative standard deviation of 4.25% (20 determinations) with 95% recovery. Alak and Tuan (220) determined atrazine, prometryn and simazine by a differential - pulse polarography
using the dropping mercury electrode. The lower limit of detection was found to be 15 ppb. Khan (221) determined fenitrothion and methyl parathion by polarography in an ethanolic solution at pH 7.0. The method was applicable even in the presence of malathion which is not possible by any other techniques. The minimum concentrations for the polarograms recorded were 0.104 and 0.166 mg and half wave potentials, -0.95 and -1.0 V for the fenitrothion and methyl parathion respectively. Lechien at al. (222) determined 2,4-D and related residues in irrigation water by differential-pulse polarography. The lower limit of detection was found to be 30 ppb.

1.1.3.6 Radiochemical Techniques

In neutron activation analysis, radioactivity is induced into trace elements. The concentration of these trace elements (as low as 1 ppp) is determined by measuring the radiation so induced and comparing with standard samples. The technique has an excellent potential for trace pollutant analysis. It has limited uses because of high cost.

Radioactive isotopes have often been used in metabolism studies and in the development of analytical methods for routine residue determinations. The technique involves the introduction of a radioactive atom into the pesticide molecule. By tracing the radioactivity emitted, the progress of the pesticide, through the metabolism pathway or analytical method, may be closely followed. The method is quite sensitive (0.1 ppb for pesticides) and measurement is relatively independent of the colour and the chemical and physical states of the sample.

Isotope dilution analysis is a means of measuring the yield of a non-quantitative process and it also enables an analysis to be performed where no quantitative isolation procedure is known. A known weight of
a radioactive compound is added to an unknown mixture containing that compound. They mix so that they are chemically indistinguishable. A small amount is isolated and determined chemically and radioactively. The proportion of radioactive compound to the total gives the dilution and hence the original concentration.

Sandberg et al. (223) analysed IAA in pine needles by radioimmunoassay. Knopp and Nuhn (224) analysed 2,4-D and related pesticides by radioimmunoassay. An isotope dilution GC/MS technique for the determination of dicamba and 2,4-D as their pentafluorobenzyl bromide derivatives was reported by Lopez-Villa et al. (225). Guler (226) reported the distribution of 2,4-D herbicide in soil-plant ecosystem under field conditions by isotope tracer technique. The uptake of 2,4-D by plants was very low and most of the herbicide was left in the soil. Norwood et al. (227) determined TCA at ppm level by GC/MS using isotope dilution method.

On the basis of above discussion, it is clear that several instrumental and non-instrumental techniques are being used for organic pollutants analysis in water. Since GC, MS, HPLC, electrochemical and radioactive methods are costly and sophisticated, they are not available or have not been installed in most of the laboratories in the third world countries. Therefore, there is a growing interest in developing new, simple and inexpensive techniques for organic pollutant analysis.

The work presented in this thesis describes the efforts made to develop new, simple and inexpensive methods of organic pollutant analysis. A new indicator and test paper for semi-quantitative determination of carbaryl (Sevin) in water is discussed in Chapter 2. TLC studies of 2,4-D and related compounds are described in Chapter 3. Separation and detection
studies of some phenoxyacid herbicides and plant growth regulators by IP-RP-TLC are given in Chapter 4. A new fluorescence based spot test for the detection and semi-quantitative determination of TCA in water and soil is discussed in Chapter 5.

REFERENCES


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CHAPTER - 2

A NEW INDICATOR AND TEST PAPER FOR THE SEMI-
QUANTITATIVE DETERMINATION OF CARBARYL IN TRACES IN WATER
2.1 INTRODUCTION

There is a growing use of carbamate pesticides as they are highly effective against invertebrates that have developed resistance to the organochlorines and organophosphates. Carbaryl (Sevin) has been used world-wide more than all the remaining carbamates combined. It has very low toxicity (1) viz. acute LD₅₀ (female rat) 500 mg/kg and (male rat) 850 mg/kg and a rather broad spectrum of insect control. It is used for the control of insects on more than hundred different crops, including citrus, forage crops, forests, field crops, lawns, nuts, ornamentals, range lands, shade trees and other crops as well as poultry and pets.

Water is a valuable commodity, needed in ever increasing quantities, but not available on an unlimited basis. Pesticides mix up in natural water thereby polluting public water supplies in many ways (2). As to environmental protection, the number of locations where the contamination of water or wastewater has to be tested, increases steadily (3). Virtually all sectors of industry as well as sewage plants, boiler houses and swimming pools are being forced to control the quantity and nature of water impurities within well defined limits.

Macherey-Nagel (3) is marketing several indicators and test papers and test kits for the detection of acidity, alkalinity, cadmium, chloride, chlorine, chromate, copper, cyanide, detergent, hardness, hydrazine, iron, lead, magnesium, manganese, nickel, nitrate, nitrite, oxygen, pH, phenol, phosphate, silica, silicon, sulphate, sulphide, sulphite in drinking water and industrial wastewater. Some new simple and inexpensive methods for the detection of carboxylic acid pollutants in water have been reported from our laboratory (4-7). However, efforts have not been made to develop
indicators, test papers and test kits for the detection of pesticide residues in water. Therefore, it is thought worthwhile to develop new indicators and test papers for the detection of carbamate insecticides in water.

This chapter describes a new indicator and test paper for the detection and semi-quantitative determination of carbaryl at trace level in water. It is based on the alkaline hydrolysis of carbaryl to 1-Naphthol which gives a blue colour with 2,6-dichloroquinone-4-chloroimine (Gibb's Reagent).

2.2 EXPERIMENTAL

2.2.1 Apparatus and Materials

A Stahl apparatus with a universal applicator (adjustable thickness of the applied layer from 0.25-2.00 mm), glass plates (20 x 4 cm), glass jars (25 x 5 cm), Whatman No. 1 chromatographic paper strips (14 x 3.5 cm), watch glass (8 cm id), separating funnel (50 ml), graduated micro pipette (0.1 ml) with vacuupet control, temperature controlled electric oven (Tempo, India), water bath (Tempo, India), and densitometer (Systronics, India), were used.

Aluminium hydroxide Gel, 1-Naphthol (CDH, India); ammonium hydroxide, calcium sulphate, silica gel (E.Merck, India); calcium carbonate (GSC, India); 2,6-dichloroquinone-4-chloroimine (Sigma, USA); phenol (BDH, India); sodium hydroxide (Ranbaxy Ltd., India); aldrin 30% EC, benzenehexachloride 2% dust (Swastik Ltd., India); bavistin 50% dust (BASF India Ltd., India); carbaryl 5-10% dust (Kilpest, India); 2,4-D-ester 34% EC (Unique Farmaid Pvt. Ltd., India); dimethoate 30% EC (Rallis India Ltd., India); endosulfan 35% EC (Hoechst, India); malathion 50% EC (Cyanamid, India); methyl parathion 50% EC (Pesticides India, India); and phosphamidon 85% EC (Hin-
2.2.2 Preparation of Solutions

A suspension of carbaryl in acetone (0.1%) and all other solutions (1%) in ethanol were used. If a 1% solution could not be prepared, saturated solutions were used.

Carbaryl was extracted with diethyl ether from aqueous suspension of its dust (formulation) and standardized by the reported procedure (8).

A solution of 2,6-dichloroquinone-4-chloroimine in benzene (0.1%) was used as a reagent.

Aqueous sodium hydroxide solution (1%) was used for impregnating paper strips and TLC plates.

2.2.3 Preparation of Plates

The slurry was applied to the glass plates with the help of the applicator so that the thickness of the coating would be 0.50 mm. For the different coatings following slurries were used:

A- Calcium sulphate (30 g) in 70 ml of distilled water.
B- Calcium carbonate (25 g) in 60 ml of distilled water.
C- Silica gel G (20 g) in 50 ml of distilled water.
D- Aluminium hydroxide Gel (15 g) in 50 ml of distilled water.

The plates were first allowed to dry at room temperature and then at 110 \(^\circ\) C for 1 hr in an oven for activation. The TLC plates were impregnated with sodium hydroxide by spraying and then the solvent was removed.
at 110°C.

2.2.4 Preparation of Test Paper

The paper strips (14 x 3.5 cm) were divided in seven equal divisions (2 x 3.5 cm) and the centre of each division was marked in order to spot the test solution at the centre. The strips so obtained were impregnated in aqueous sodium hydroxide for 30 sec. The excess solution was drained off by placing the strips over a filter paper sheet and allowed to dry at the room temperature. The dried strips were collected in a hard paper box and used for testing carbaryl.

2.2.5 Environmental Samples

Water (river) containing dissolved oxygen 8.2-8.7 ppm, total hardness 122-141 ppm, total alkalinity 174-189 ppm, chlorides 9.2-9.9 ppm, sulphates 0.42-0.47 ppm, BOD 5.2-5.6 ppm, COD 6.5-6.7 ppm and having pH 8.7-8.8.

Water (tap) containing dissolved oxygen 8.0 ppm, total hardness 135-144 ppm, total alkalinity 261-268 ppm, chlorides 13-14 ppm, sulphates 14.0-18.5 ppm and having pH 7.5-7.6.

2.3 PROCEDURES

2.3.1 Detection on TLC Plates

A test solution was spotted on the TLC plate with the help of the pipette. The solvent was removed and the colour was developed on the point of application by spraying sodium hydroxide and the reagent. The colour so developed was recorded. To determine the lower limit of detection
the same volume (0.01 ml) of the different standard solutions of varying concentration of a particular test material was spotted on a TLC plate by maintaining a distance of 2 cm between the two spots and the solvent was removed, the colour was developed as above. The nature and the intensity of the colour were recorded.

2.3.2 Detection by Test Paper

The test solution (0.01 ml) was spotted on the test paper and the colour was developed by dipping the test paper in a watch glass containing the reagent. The lower limit of detection was determined by the procedure given in 2.3.1.

2.3.3 Detection in Solution

One drop of the test solution (1%) and equal volumes of sodium hydroxide solution and the reagent were taken in a micro test tube. The contents were warmed on a spirit lamp and the colour so developed was recorded.

2.3.4 Thin-Layer Chromatographic Detection

A known volume of a test solution was spotted on the TLC plate with the help of the pipette. The solvent was removed and the plate was developed in a particular solvent system. The plate was taken out from the jar after the solvent had ascended 10 cm. The solvent was removed and the spot was located by spraying sodium hydroxide solution and then the reagent. For tailing the front limit (Rf) and rear limit (RT) were measured, while for compact spot Rf value was calculated by the following expression:

\[ R_f = \frac{\text{Distance travelled by the substance (cm)}}{\text{Distance travelled by the solvent (cm)}} \]
The lower limit of detection of the test materials was determined by repeating the above procedure for the same volume (0.01 ml) of different standard solutions of varying concentration.

2.3.5 Paper-Chromatographic Detection

The detection and the determination of lower limit of detection on the test paper were performed by the procedure given in 2.3.4. The colour on the chromatogram was developed by dipping the test paper in a watch glass containing the reagent.

2.3.6 Semi-Quantitative Determination by Test Paper

The same volume (0.01 ml) of the five standard solutions of a particular test material equivalent to 25, 50, 75, 100 and 125 µg was spotted at five different and marked places on a test paper. An equal volume of (0.01 ml) of an unknown solution was spotted at the sixth place on the same test paper. The distance between two spots was kept to be 2 cm. The solvent was removed, and the colour of the spots was developed by dipping the test paper in a watch glass containing the reagent. Semi-quantitative determination was made on the basis of comparison of the intensity and nature of the colour of the spot.

2.3.7 Densitometric Determination by Test Paper

The same volume (0.01 ml) of different standard solutions of varying concentration of a particular test material were spotted on the test paper, solvent was removed and colour was developed as above. The absorbance of the coloured spots was read by the following procedure: The test paper was mounted on the glass plate and placed in a carriage of densitometer. The
carriage was inserted in the slot till the geared rack engages the pinion side. The absorbance with yellow filter was recorded by advancing the carriage mm by mm. A calibration curve was drawn by plotting the concentration (µg) verses absorbance of the spot and it was used to determine the concentration in a given sample.

### 2.3.8 Detection in River Water and Tap Water

Suspensions of carbaryl dust equivalent to 1 ppm carbaryl in river water (RW) and tap water (TW) were prepared separately. The suspension was shaken thoroughly and 20 ml of it were taken in a separating funnel, 5 ml of diethyl ether were added into it, and after thorough shaking the extract was collected in a beaker. Similarly four more fresh portions of 20 ml were extracted and all the five extracts were collected in the same beaker. The ether was removed by brief warming on the water bath and the residue was dissolved in 1 ml of ethanol. A small volume (0.01 ml) of the ethanolic solution was spotted on the test paper and the colour was developed as above.

### 2.4 RESULTS

The compounds listed in Table 2.1 give no colour on calcium sulphate or silica gel G plates by spraying the reagent alone. The colour produced on calcium sulphate plates by spraying (i) mixture of ammonium hydroxide (25%) and the reagent (1:1), (ii) mixture of sodium hydroxide and the reagent (1:1), (iii) sodium hydroxide followed by the reagent, (iv) the reagent followed by sodium hydroxide, are summarized in columns 1, 2, 3 and 4 respectively of Table 2.1. The colour developed on silica gel G coated plates by spraying (i) sodium hydroxide followed by the reagent and (ii) the reagent followed
by sodium hydroxide, are given in columns 1 and 2 respectively of Table 2.2. The results obtained on silica gel G coated plates preimpregnated with sodium hydroxide and sprayed with the reagent alone are given in column 3 of Table 2.2. The results obtained on aluminium hydroxide Gel coated plates by spraying (i) the reagent, (ii) sodium hydroxide followed by the reagent, (iii) the reagent followed by sodium hydroxide are given in columns 1, 2 and 3 respectively of Table 2.3. The compounds understudy (Table 2.1) do not produce any colour on calcium carbonate plate by spraying the reagent alone or any of the above reagent admixtures in different conditions. The lower limit of detection of carbaryl (1.2 µg) and 1-Naphthol (0.06 µg) along with other compounds by the above procedure is reported in Tables 2.2 and 2.3.

The results of detection on the test paper are summarized in Table 2.4. The lower limit of detection of carbaryl (1.2 µg) and 1-Naphthol (0.06 µg) along with other test materials is given in the parenthesis of Table 2.4.

The solution state spot test gives blue colour with violet tinge (BVT) for carbaryl, greenish blue (GB) for 2,4-D-ester, light blue (LB) for sodium salt of 2,4-D (2,4-D-Na), blue with violet tinge (BVT) for 1-Naphthol and blue (B) for phenol. The lower limit of detection in solution state for carbaryl and 1-Naphthol was found to be 2.0 µg and 0.5 µg respectively.

The results of semi-quantitative determination by the test paper are summarized in Table 2.5.

The $R_f$ values, nature of the spot and colour developed by thin-layer chromatography (TLC) and paper chromatography (PC) in different
solvents are given in Tables 2.6 and 2.7 respectively. The lower limit of detection of carbaryl, 2,4-D-ester, 2,4-D-Na, 1-Naphthol and phenol was found to be 12, 5.0, 25, 0.25 and 0.5 µg respectively by TLC and 0.6, 3.0, 2.5, 0.10 and 0.5 µg by PC in ethanol.

The results of densitometric determination of carbaryl and 1-Naphthol and their analytical parameters are given in Table 2.8. The following expressions are used for calculating the parameters.

\[
\text{C.V.} = \frac{\sigma \times 100}{\bar{X}} \quad \ldots \quad (2)
\]

\[
\mu = \text{average value}
\]

\[
N = \text{number of sets}
\]

Where

\[
x_1, x_2 \quad = \text{measured values}
\]

2.5 DISCUSSION

In order to develop indicator and test paper for the detection of carbaryl at trace level in water the following already known colour reactions, have been investigated. The commonly used chromogenic reagents are Tollens's reagent (9), diazophenol (10) and Alkaline Fast Blue B (11). These are general reagents and can not be used for the specific detection of carbaryl. Recently Padalikar et al. (12) have reported two chromogenic reagents: (i) ammonium metavanadate in conc. hydrochloric acid and (ii) alkaline potassium hexacyanoferrate (III) for the specific detection of carbaryl at trace level (1 µg) in biological fluid (minced visceral tissue). The reagents give violet colour with hydrolysis product of carbaryl, 1-Naphthol. The
<table>
<thead>
<tr>
<th>Test Material</th>
<th>Colour Developed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>NC</td>
</tr>
<tr>
<td>2,4-D-ester</td>
<td>NC</td>
</tr>
<tr>
<td>2,4-D-Na</td>
<td>NC</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>NC</td>
</tr>
<tr>
<td>Phenol</td>
<td>NC</td>
</tr>
</tbody>
</table>

Abbreviations used are: NC = no colour, B = blue, BVT = blue with violet tinge, GB = greenish blue.
<table>
<thead>
<tr>
<th>Test Material</th>
<th>Colour Developed (Lower Limit of Detection in µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>BVT (2.0)</td>
</tr>
<tr>
<td>4-Chlorophenoxyacetic acid</td>
<td>NC</td>
</tr>
<tr>
<td>2,4-Dichlorophenoxyacetic acid</td>
<td>NC</td>
</tr>
<tr>
<td>2,4-D-ester</td>
<td>GB</td>
</tr>
<tr>
<td>2,4-D-Na</td>
<td>LB</td>
</tr>
<tr>
<td>Indole-3-acetic acid</td>
<td>NC</td>
</tr>
<tr>
<td>Indole-3-propionic acid</td>
<td>NC</td>
</tr>
<tr>
<td>1-Naphthaleneacetic acid</td>
<td>NC</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>BVT</td>
</tr>
<tr>
<td>2-Naphthoxyacetic acid</td>
<td>NC</td>
</tr>
<tr>
<td>Phenol</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-Trichlorophenoxyacetic acid</td>
<td>NC</td>
</tr>
<tr>
<td>Trichloroacetic acid</td>
<td>NC</td>
</tr>
</tbody>
</table>

Abbreviations used are: LB = light blue and other abbreviations are defined in Table 2.1.
<table>
<thead>
<tr>
<th>Test Material</th>
<th>Colour Developed (Lower Limit of Detection in µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Aldrin</td>
<td>NC</td>
</tr>
<tr>
<td>Bavistin</td>
<td>B</td>
</tr>
<tr>
<td>BHC</td>
<td>NC</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>BVT (2.0)</td>
</tr>
<tr>
<td>Catechol</td>
<td>BVT</td>
</tr>
<tr>
<td>Cresol-red</td>
<td>NC</td>
</tr>
<tr>
<td>2,4-D-ester</td>
<td>GB</td>
</tr>
<tr>
<td>2,4-D-Na</td>
<td>LB</td>
</tr>
<tr>
<td>Detergent</td>
<td>NC</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>NC</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>NC</td>
</tr>
<tr>
<td>Lemon juice</td>
<td>NC</td>
</tr>
<tr>
<td>Malathion</td>
<td>NC</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>NC</td>
</tr>
<tr>
<td>Milk</td>
<td>NC</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>BVT</td>
</tr>
<tr>
<td>Phenol</td>
<td>B</td>
</tr>
<tr>
<td>Phosphamidon</td>
<td>NC</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>NC</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>RV</td>
</tr>
<tr>
<td>Soap</td>
<td>NC</td>
</tr>
<tr>
<td>Soil extract</td>
<td>NC</td>
</tr>
<tr>
<td>Thymol</td>
<td>B</td>
</tr>
</tbody>
</table>

Abbreviation used are: RV = reddish violet and other abbreviations are defined in Table 2.1.
**TABLE 2.4 DETECTION ON TEST PAPER**

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Colour Developed (Lower Limit of Detection in µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>NC</td>
</tr>
<tr>
<td>Bavistin</td>
<td>B (20)</td>
</tr>
<tr>
<td>BHC</td>
<td>NC</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>BVT (1.2)</td>
</tr>
<tr>
<td>Catechol</td>
<td>BVT (0.5)</td>
</tr>
<tr>
<td>Cresol-red</td>
<td>NC</td>
</tr>
<tr>
<td>2,4-D-ester</td>
<td>GB (2.0)</td>
</tr>
<tr>
<td>2,4-D-Na</td>
<td>LB (20)</td>
</tr>
<tr>
<td>Detergent</td>
<td>NC</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>NC</td>
</tr>
<tr>
<td>Endosulfan</td>
<td>NC</td>
</tr>
<tr>
<td>Lemon juice</td>
<td>NC</td>
</tr>
<tr>
<td>Malathion</td>
<td>NC</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>NC</td>
</tr>
<tr>
<td>Milk</td>
<td>NC</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>BVT (0.06)</td>
</tr>
<tr>
<td>Phenol</td>
<td>B (1.0)</td>
</tr>
<tr>
<td>Phosphamidon</td>
<td>NC</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>NC</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>RV (0.5)</td>
</tr>
<tr>
<td>Soap</td>
<td>NC</td>
</tr>
<tr>
<td>Soil extract</td>
<td>NC</td>
</tr>
<tr>
<td>Thymol</td>
<td>B (0.5)</td>
</tr>
</tbody>
</table>

Abbreviations are defined in Tables 2.1 and 2.3
<table>
<thead>
<tr>
<th>Test Material</th>
<th>Colour Developed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 µg</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>LBVT</td>
</tr>
<tr>
<td>2,4-D-ester</td>
<td>VLGB</td>
</tr>
<tr>
<td>2,4-D-Na</td>
<td>VLB</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td>LBVT</td>
</tr>
<tr>
<td>Phenol</td>
<td>LB</td>
</tr>
</tbody>
</table>

Abbreviations used are: L = light, V = very, D = dark and other abbreviations are defined in Table 2.1.
### TABLE 2.6 \( R_f \) VALUES, NATURE AND COLOUR OF THE SPOTS ON ALUMINIUM HYDROXIDE GEL COATED PLATES

<table>
<thead>
<tr>
<th>Solvents</th>
<th>( R_f ) Values/Nature and Colour of the Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbaryl (^f)</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.0 ( S(BVT) )</td>
</tr>
<tr>
<td>Benzene</td>
<td>0-4 cm ( U(BVT) )</td>
</tr>
<tr>
<td>Carbon tetra-chloride</td>
<td>0.0 ( C(BVT) )</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0-5 cm ( U(BVT) )</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.0 ( C(BVT) )</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0-10 cm ( U(BVT) )</td>
</tr>
<tr>
<td>Hexane</td>
<td>0.0 ( C(BVT) )</td>
</tr>
<tr>
<td>Tap water</td>
<td>0-10 cm ( U(BVT) )</td>
</tr>
</tbody>
</table>

Abbreviations used are: \( C = \) compact, \( S = \) streak, \( U = \) unsymmetric and other abbreviations are defined in Tables 2.1 and 2.5.
### TABLE 2.7 R<sub>f</sub> VALUES, NATURE AND COLOUR OF THE SPOTS ON TEST PAPER

<table>
<thead>
<tr>
<th>Solvents</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; Values/Nature and Colour of the Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbaryl</td>
</tr>
<tr>
<td>Acetone</td>
<td>1.0 S(BVT)</td>
</tr>
<tr>
<td>Benzene</td>
<td>1.0 S(BVT)</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>1.0 C(BVT)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>7-10 cm U(BVT)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.0 C(BVT)</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1.0 S(BVT)</td>
</tr>
<tr>
<td>Hexane</td>
<td>0-7 cm U(BVT)</td>
</tr>
<tr>
<td>Tap water</td>
<td>8-10 cm U(BVT)</td>
</tr>
</tbody>
</table>

Abbreviations are defined in Tables 2.1, 2.5 and 2.6.
## Table 2.8: Reliability of Colour of Carbaryl and 1-Naphthol

<table>
<thead>
<tr>
<th>Amount (µg)</th>
<th>N</th>
<th>Optical Density (\overline{\mu} \pm \sigma)</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>0.86 ± 0.053</td>
<td>6.2</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>0.84 ± 0.060</td>
<td>7.2</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
<td>0.83 ± 0.057</td>
<td>6.8</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>0.81 ± 0.055</td>
<td>6.8</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0.80 ± 0.055</td>
<td>6.9</td>
</tr>
<tr>
<td>1-Naphthol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>0.90 ± 0.037</td>
<td>4.1</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>0.88 ± 0.041</td>
<td>4.7</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>0.86 ± 0.038</td>
<td>4.5</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>0.85 ± 0.036</td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.83 ± 0.031</td>
<td>3.8</td>
</tr>
</tbody>
</table>
specificity of the reagent is due to different Rf values of carbaryl and 1-Naphthol on silica gel G coated TLC plates in hexane-acetone (4:1). Feigl (13) has reported the following colour reactions for detection phenols as well as 1-Naphthol. 1-Naphthol reacts with chloroform in alkaline medium to give aldehyde which reacts with hydrazine in acidic medium to give aldehyde that gives yellow fluorescence in UV light. The same fluorescence is also obtained from the reaction product of 1-Naphthol and hexamethylene-tetramine. These reactions are less sensitive as their lower limit of detection is 12 µg. 1-Naphthol gives a brown chelate when heated with sodium cobaltinitrite. The lower limit of detection is 1 µg. 1-Naphthol absorbed on resin beads reacts with hydroxylamine to give aminophenol that reacts on heating under infra-red lamp with pentacyanoiron complex to give a blue product. The reaction is highly sensitive (0.25 µg). Five different procedures based on the formation of coloured indophenol (blue-red-green-brown) from 1-Naphthol, and nitrosophenol are given in Feigl's book. These reactions are ultra sensitive as their lower limit of detection is 0.1-5.0 µg. These procedures have not been tried for the detection of carbaryl so far. Amongst them the simple, sensitive and rapid procedure is the reaction of 1-Naphthol with 2,6-dichloroquinone-4-chloroimine to give a brown to yellow indophenol. The alkali and ammonium salts of these indophenols are blue. The indophenol reaction is trustworthy for the detection of volatile phenols because their vapours yield yellowish brown product with the reagent which turns blue when exposed to ammonia vapours. The test can also be accomplished as a solid body reaction if solid phenols are rubbed with 2,6-dichloroquinone-4-chloroimine. As the lower limit of the detection of this reaction in solution is 0.5 µg for 1-Naphthol, 2,6-dichloroquinone-4-chloroimine in presence of sodium hydroxide can be used
as an indicator for detecting the presence of carbaryl in water. Now this reaction is extensively studied in order to explore its utility in making test papers for detecting traces of carbaryl in water. The results discussed below show the importance of the reaction for detecting carbaryl.

Results given in Tables 2.1, 2.2 and 2.3 show that the colour reaction under study can be used for the detection of carbaryl, 2,4-D-ester, 2,4-D-Na, 1-Naphthol and phenol on TLC plates coated with calcium sulphate, silica gel G and aluminium hydroxide Gel. However colour does not appear on calcium carbonate coated plates. It is also clear that the colour is produced only when sodium hydroxide and the reagent are sprayed one by one and it is not produced when an admixture of the reagent with sodium hydroxide is sprayed (Table 2.1, column 2). Calcium sulphate coated plates can be used for the selective detection of carbaryl, 2,4-D-ester, 2,4-D-Na, 1-Naphthol and phenol as they give distinct colours. 2,4-D-Na gives colour on silica gel G coated plates while it gives no colour on calcium sulphate coated plates and 2,4-D acid does not give colour on any of the coatings. Therefore this differential behaviour of silica gel G and calcium sulphate can be utilized for the selective detection of 2,4-D-ester and 2,4-D-Na. It is also clear from Table 2.2 that the sensitivity of the reaction is the highest on silica gel G coated plates preimpregnated with sodium hydroxide than that on silica gel G alone. Table 2.3 (column 1) shows that bavistin, carbaryl, catechol, 2,4-D-ester, 2,4-D-Na, 1-Naphthol, phenol, resorcinol, and thymol give colour on aluminium hydroxide Gel coated plates by spraying the reagent alone while under this condition they do not give colour on calcium sulphate and silica gel G plates. However the reaction is more sensitive when the reagent was sprayed first and followed by sodium hydroxide. The results given in Table
2.4 show that the test paper understudy can be used for the selective and sensitive detection of bavistin, carbaryl, catechol, 2,4-D-ester, 2,4-D-Na, 1-Naphthol, phenol, resorcinol and thymol. Table 2.5 shows that the test paper can be used for the semi-quantitative determination of carbaryl, 2,4-D-ester, 2,4-D-Na, 1-Naphthol and phenol as the intensity of the colour increases with the increasing concentration of the test material. Table 2.6 shows that the selectivity of the reaction can be further raised by developing the chromatogram in different solvents. For example carbaryl, 2,4-D-ester, 2,4-D-Na, 1-Naphthol and phenol have $R_f$ values 0-5 cm, 0.0, 0.0, 0.0 and 1.0 respectively on aluminium hydroxide Gel coated plates developed in distilled water. Table 2.7 shows that PC gives better results than TLC i.e. carbaryl moves as a compact spot ($R_f = 1.0$), 1-Naphthol tails (0-10 cm) and the other test material do not move ($R_f = 0.0$) on PC in benzene. The data recorded in Table 2.8 show that the quantitative determination of carbaryl and 1-Naphthol can be done densitometrically on test papers. The results discussed above show that the following is the sequence of sensitivity of detection of carbaryl (I) and 1-Naphthol (II) on different phases:

I. Test paper (1.2 µg) $\supset$ silica gel G TLC (1.2 µg) $\supset$ aluminium hydroxide Gel (1.2 µg) $\supset$ solution state (2.0 µg).

II. Test paper (0.06 µg) $\supset$ silica gel G TLC (0.06 µg) $\supset$ aluminium hydroxide Gel (0.06 µg) $\supset$ solution state (0.5 µg).

The lower limit of detection is given in parenthesis. Hence it is clear that the detection by the test paper and the TLC is more sensitive than that in solution and the reaction understudy is more sensitive for 1-Naphthol than carbaryl. The dilution effect seems to be responsible
for the former and the partial hydrolysis of carbaryl to 1-Naphthol may be responsible for the latter. The lower limit of detection of the proposed method by the test paper for carbaryl is 1 ppm in river water as well as in tap water.

2.6 CONCLUSION

The test paper understudy can be used for the detection of traces of carbaryl in natural water. The method can also be used below 1 ppm (or 1 ppb in water) by raising the number of cycles of preconcentration (extraction).

REFERENCES


CHAPTER 3

SEQUENTIAL THIN-LAYER CHROMATOGRAPHY OF
2,4-D AND RELATED COMPOUNDS
3.1 INTRODUCTION

Herbicides are the chemicals (pesticides) used for killing weeds. They provide a more effective and economical means of weed control (1) than mechanical methods such as cultivation, hoeing and hand pulling. There are other locations such as industrial sites, roadsides, ditch banks, irrigation canals, fence lines, recreational area, railroad embankments and power lines where herbicides are used extensively. Plant growth regulators are the chemicals used to alter the growth of plants, blossoms, or fruits. Herbicides such as 2,4-D, 2,4,5-T and TCA are used selectively to kill most dicotyledonous (broad-leaf) plants while synthetic auxins such as IAA, 1-naphthaleneacetic acid and 4-chlorophenoxyacetic acid are used as plant growth regulators. 2,4-D, 2,4,5-T, TCA and 1-naphthaleneacetic acid are toxic and their LD₅₀ values (2) are 370 mg, 500 mg, 5000 mg and 1000 mg/kg respectively. The environmentally deleterious effects of the herbicides on aquatic organisms (3), their phototoxicity to plants (4), to fish (5), pollen grains (6) and to rats (7) have been studied.

The herbicides and plant growth regulators are mixed up in natural water either by accidental fall out of sprays from agricultural treatment or by surface run-off from agricultural land, thereby, they are considered as water pollutants. These pollutants are difficult to analyse by the frequently used and highly sensitive technique, GC, due to their non-volatile nature. HPLC has proved to be a good alternative to clean up and determine acid herbicides in different samples with great accuracy (8-11) but it is a sophisticated and expensive technique which requires technically skilled operators. Therefore, simple and commonly used methods (12) such as spectrophotometry and TLC have been proved to be readily available.
tools for their analysis. Our previous work (13) shows that these pollutants can be separated by NP-TLC on calcium sulphate in common solvents such as acetone, benzene, carbon tetrachloride, chloroform, dioxan, distilled water, ethyl acetate and propanol. Abou-Donia and Komeil (14) have claimed that the newly developed S-TLC is a rapid and fast method for analysing the complex mixtures.

Therefore, an attempt has been made to explore the utility of calcium sulphate for separation and determination of the above mentioned pollutants by S-TLC. As a result, many new ternary separations which were not possible by NP-TLC on calcium sulphate have now been achieved. The results obtained are summarized in this Chapter.

3.2 EXPERIMENTAL

3.2.1 Apparatus and Materials

A Stahl apparatus with a universal applicator (adjustable thickness of the applied layer 0.25 to 2.00 mm), glass plates (20 x 4 cm), glass jars (25 x 5 cm), micro pipette (0.1 ml) with vacuum control, hot air electric drier, temperature controlled electric oven, centrifugal machine and Bausch and Lomb spectronic-20 spectrophotometer were used.

Acetone (BDH, India); benzene (S.M. Chemicals, India); calcium sulphate, carbon tetrachloride, chloroform, dioxan, ethyl acetate, propanol (E.Merek, India), benzoic acid, bromophenol blue, 4-chlorophenoxyacetic acid, cinnamic acid, citric acid, 2,4-D, gallic acid, IAA, maleic acid, malic acid, malonic acid, 2-naphthaleneacetic acid, 2-naphthoxyacetic acid, oxalic acid, phenoxyacetic acid, salicylic acid, tartaric acid, TCA, 2,4,5-T (Sigma, USA) and all other chemicals used were of analytical grade.
3.2.2 Preparation of Solutions

All the test solutions (1%) were prepared either in ethanol or in distilled water.

Chromogenic reagent for spectrophotometry of IAA was prepared by mixing 1 ml of aqueous ferrie chloride (0.5 M) and 50 ml of perchloric acid (35% v/v).

3.2.3 Preparation of Plates

A slurry obtained by mixing calcium sulphate (30 g) with distilled water (70 ml) was applied on the glass plates with the help of the applicator so that the thickness of calcium sulphate coating would be 0.50 mm. The plates were first allowed to dry at room temperature and then at 100°C in an oven for 1 hr.

3.2.4 Spotting of Test Solutions

Test solutions were spotted on the plates with the help of a fine bore capillary. The solvent was removed by hot air drying. For NP-TLC the plate was developed upto the length of 10 cm in a single solvent. For S-TLC studies the plate was developed upto a length of 5 cm in the first solvent, it was taken out from the jar, the solvent was removed as above and then the plate was again developed upto a length of 10 cm in the second solvent.

3.2.5 Visualization of Chromatograms

The acids understudy were visualized on TLC plates by spraying 0.1% ethanolic alkaline bromophenol blue solution.
3.2.6 Measurement of $R_f$ Values

For tailing, the front limit (RI) and rear limit (RT) were measured while for compact spots $R_f$ values were calculated as in Chapter 2.

3.2.7 Qualitative Separations

To achieve the qualitative separations of acids, one of the acids was spotted and the solvent was removed and then the second acid was spotted and the solvent was removed again and so on. The plates so developed were dried and acids were visualized by spraying the above chromogenic reagent.

3.2.8 Quantitative Separation of IAA from Other-Acids

Known volumes of IAA and other acids were spotted on the plates with the help of a micro pipette with vacuupet control. The solvent was removed, plates were developed and spots were located and demarcated. The demarcated portion of the plate for IAA was scratched, the coating was collected in a micro beaker (10 ml), and IAA was eluted by treating the scratched coating with 3 ml of methanol. The solid portion was removed by centrifugation and IAA was determined in the centrifugate by the following procedure.

3.2.9 Spectrophotometric Determination of IAA

In the above centrifugate, 2 ml of freshly prepared reagent was added rapidly drop by drop with continuous agitation. The solution so obtained was placed in the dark for colour development for 1 hr. Finally the absorbance was measured at 510 nm against a blank containing methanol (3 ml) and the reagent (2 ml).
3.3 RESULTS

Separations achieved by NP-TLC and S-TLC are recorded in Tables 3.1, 3.2 and 3.3. Some of the important separations are shown in Figures 3.1 to 3.8. Results of the quantitative separations of IAA from other acids are recorded in Tables 3.4, 3.5 and 3.6. The coefficient of variation (C.V.) was calculated by the expressions given in Chapter 2.

For the sake of convenience the following abbreviations are used:

BOA = benzoic acid, CIA = cinnamic acid, CPAA = 4-chlorophenoxyacetic acid, CA = citric acid, 2,4-D = 2,4-dichlorophenoxyacetic acid, GA = gallic acid, IAA = indole-3-acetic acid, MEA = maleic acid, MA = malic acid, MOA = malonic acid, 2-NPAA = 2-naphthaleneacetic acid, 2-NXAA = 2-naphthoxyacetic acid, OA = oxalic acid, PAA = phenoxyacetic acid, SA = salicylic acid, TA = tartaric acid, TCA = trichloroacetic acid and 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.

a = acetone, b = benzene, c = carbon tetrachloride, d = chloroform, e = ethyl acetate, f = dioxan and g = propanol.

ab = a followed by b, ba = b followed by a and so on.

3.4 DISCUSSION

TLC is particularly useful for the analysis of herbicides containing carboxylic group because of their polar nature and insusceptibility to GC (15). Thus, it has been used for the detection, separation and determination of carboxylic herbicides (16, 17). Henzyká et al. (18) determined 2,4-D, dalapon, MCPA, dichlorprop, DNOC, dinoseb and TCA in water and sewage by TLC on silica gel G - kieselguhr G' (2:3) or silica gel G-H₃PO₄ (5 g of silica gel
<table>
<thead>
<tr>
<th>Carboxylic Pollutants</th>
<th>Separated From</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOA (1.0)</td>
<td>CA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a and e</td>
</tr>
<tr>
<td>CIA (1.0)</td>
<td>CA (0.0) or MEA (0-3 cm) or MA (0-7 cm) or MOA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a, b and e</td>
</tr>
<tr>
<td>CPAA (1.0)</td>
<td>CA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a and e</td>
</tr>
<tr>
<td>2,4-D (1.0)</td>
<td>CA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a and e</td>
</tr>
<tr>
<td>GA (1.0)</td>
<td>CA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a and e</td>
</tr>
<tr>
<td>IAA (1.0)</td>
<td>CA (0.0) or MEA (0-3 cm) or MA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a, b and e</td>
</tr>
<tr>
<td>MOA (1.0)</td>
<td>CA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>e</td>
</tr>
<tr>
<td>2-NPAA (1.0)</td>
<td>CA (0.0) or MEA (0.3) or MOA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>d and e</td>
</tr>
<tr>
<td>2-NXAA (1.0)</td>
<td>CA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a, c and f</td>
</tr>
<tr>
<td>PAA (1.0)</td>
<td>CA (0.0) or MEA (0-3 cm) or MA (0-7 cm) or OA (0.0) or TA (0-2 cm)</td>
<td>a, b and d</td>
</tr>
<tr>
<td>SA (1.0)</td>
<td>CA (0.0) or MOA (0.0) or OA (0.0)</td>
<td>e and e</td>
</tr>
<tr>
<td>TCA (1.0)</td>
<td>CA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a and e</td>
</tr>
<tr>
<td>2,4,5-T (1.0)</td>
<td>CA (0.0) or OA (0.0) or TA (0-2 cm)</td>
<td>a, b and e</td>
</tr>
</tbody>
</table>

Abbreviations are defined in the results section and $R_f$ values are given in parentheses.
### TABLE 3.2  BINARY SEPARATIONS OF SOME CARBOXYLIC POLLUTANTS BY S-TLC

<table>
<thead>
<tr>
<th>Carboxylic Pollutants</th>
<th>Separated From</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIA (1.0)</td>
<td>IAA (0-5 cm) or MEA (0-3 cm) or MOA (0.0)</td>
<td>cd, ed and db</td>
</tr>
<tr>
<td>IAA (6-10 cm)</td>
<td>CA (0-2 cm) or MEA (0-3 cm) or MOA (0.0)</td>
<td>db and gb</td>
</tr>
<tr>
<td>MA (7-10 cm)</td>
<td>CA (0-2 cm) or OA (0.0) or TA (0.0)</td>
<td>de and fe</td>
</tr>
<tr>
<td>MEA (8-10 cm)</td>
<td>CA (0-2 cm) or OA (0.0) or TA_T (0.0)</td>
<td>de and fe</td>
</tr>
<tr>
<td>MOA (7-10 cm)</td>
<td>CA (0-2 cm) or OA (0.0) or TA (0.0)</td>
<td>de and fe</td>
</tr>
<tr>
<td>2-NPAA (6-10 cm)</td>
<td>MEA (0-3 cm) or MOA (0.0)</td>
<td>db</td>
</tr>
<tr>
<td>2-NXAA (5-10 cm)</td>
<td>MA (0-3 cm) or MOA (0.0)</td>
<td>db</td>
</tr>
<tr>
<td>PAA (8-10 cm)</td>
<td>IAA (0-5 cm) or MEA (0-3 cm) or MA (0-5 cm) or MOA (0.0)</td>
<td>db, gb and gc</td>
</tr>
<tr>
<td>SA (1.0)</td>
<td>MEA (0-3 cm) or MOA (0.0)</td>
<td>db and gb</td>
</tr>
<tr>
<td>2,4,5-T (1.0)</td>
<td>MEA (0-3 cm)</td>
<td>gb</td>
</tr>
</tbody>
</table>

Abbreviations are defined in the results section and R\textsubscript{f} values are given in parentheses.
### TABLE 3.3 TERNARY SEPARATIONS OF SOME CARBOXYLIC POLLUTANTS BY S-TLC

<table>
<thead>
<tr>
<th>Carboxylic Pollutants</th>
<th>Separated From</th>
<th>Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA (0.0)</td>
<td>MEA (0.5) and 2-NPAA (1.0)</td>
<td>ed</td>
</tr>
<tr>
<td>OA (0.0)</td>
<td>MEA (4-5 cm) and 2-NXAA (9-10 cm)</td>
<td>gd</td>
</tr>
<tr>
<td>OA (0.0)</td>
<td>MA (0.5) and CIA (9-10 cm)</td>
<td>ed</td>
</tr>
<tr>
<td>OA (0.0)</td>
<td>MEA (0.5) and PAA (1.0)</td>
<td>ab</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>2-NXAA (0.5) and CIA (8-10 cm)</td>
<td>eb</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>IAA (3-5 cm) and SA (9-10 cm)</td>
<td>eb</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>MA (0.5) and SA (9-10 cm)</td>
<td>ec</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>MA (0.5) and CIA (1.0)</td>
<td>de</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>IAA (4-5 cm) and CIA (1.0)</td>
<td>ec</td>
</tr>
<tr>
<td>TA (0.0)</td>
<td>IAA (4-5 cm) and PAA (9-10 cm)</td>
<td>ec</td>
</tr>
</tbody>
</table>

Abbreviations are defined in the results section and $R_f$ values are given in parentheses.
Figure 3.1 Binary separation of some carboxylic pollutants by S-TLC.

Spot A - TA in 1, 2, 3, 4, 5 and 6.

Spot B - 2,4,5-T in 1, 2,4-D in 2, BOA in 3, TCA in 4, CPAA in 5 and GA in 6.
Figure 3.2  Binary separations of some carboxylic pollutants by S-TLC.

Spot A - TA in 7, 8, 9, 10, 11 and 12.

Spot B - CIA in 7, IAA in 8, 2-NPAA in 9, 2-NXAA in 10, PAA in 11 and SA in 12.
Figure 3.3  Binary separations of some carboxylic pollutants by S-TLC.

Spot A - OA in 1, 2, 3, 4, 5 and 6.

Spot B - 2,4,5-T in 1, 2,4-D in 2, BOA in 3, TCA in 4, CPAA in 5 and GA in 6.
Figure 3.4  Binary separations of some carboxylic pollutants by S-TLC.

Spot A - OA in 7, 8, 9, 10, 11 and 12.

Spot B - CIA in 7, IAA in 8, 2-NPAA in 9, 2-NXAA in 10, PAA in 11 and SA in 12.
Figure 3.5 Binary separations of some carboxylic pollutants by S-TLC.

Spot A - CA in 1, 2, 3, 4, 5 and 6.

Spot B - 2,4,5-T in 1, 2,4-D in 2, BOA in 3, TCA in 4, CPAA in 5 and GA in 6.
Figure 3.6  Binary separations of some carboxylic pollutants by S-TLC.

Spot A - CA in 7, 8, 9, 10 and 11.

Spot B - CIA in 7, 2-NPAA in 8, 2-NXAA in 9, PAA in 10 and SA in 11.
Figure 3.7  Ternary separations of some carboxylic pollutants by S-TLC.

Spot A - OA in 1, 2, 3, 4 and 5.

Spot B - MA in 1, IAA in 2 and 3 and MEA in 4 and 5.

Spot C - CIA in 1, 2 and 4 and PAA in 3 and 5.
Figure 3.8  Ternary separations of some carboxylic pollutants by S-TLC.

Spot A - TA in 1, 2, 3, 4 and 5.
Spot B - MEA in 1, 4 and 5 and IAA in 2 and 3.
Spot C - CIA in 1, 2 and 4 and PAA in 3 and 5.
**TABLE 3.4 BINARY QUANTITATIVE SEPARATIONS OF IAA FROM OA AND TA BY S-TLC**

<table>
<thead>
<tr>
<th>Carboxylic Pollutant</th>
<th>Separated From</th>
<th>Absorbance at 510 nm</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 μg of IAA</td>
<td>200 μg of TA</td>
<td>0.214 ± 0.005</td>
<td>2.32</td>
</tr>
<tr>
<td>50 μg of IAA</td>
<td>400 μg of OA</td>
<td>0.212 ± 0.009</td>
<td>4.64</td>
</tr>
</tbody>
</table>

Abbreviations are defined in results section.
### TABLE 3.5 TERNARY QUANTITATIVE SEPARATIONS OF IAA FROM OA AND CIA BY S-TLC

<table>
<thead>
<tr>
<th>Carboxylic Pollutant</th>
<th>Separated From</th>
<th>Absorbance at 510 nm $\mu \pm \sigma$</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 $\mu$g of IAA</td>
<td>400 $\mu$g of OA and 400 $\mu$g of CIA</td>
<td>0.203 ± 0.008</td>
<td>3.99</td>
</tr>
<tr>
<td>50 $\mu$g of IAA</td>
<td>800 $\mu$g of OA and 800 $\mu$g of CIA</td>
<td>0.205 ± 0.013</td>
<td>6.68</td>
</tr>
<tr>
<td>50 $\mu$g of IAA</td>
<td>1200 $\mu$g of OA and 1200 $\mu$g of CIA</td>
<td>0.191 ± 0.007</td>
<td>3.95</td>
</tr>
<tr>
<td>50 $\mu$g of IAA</td>
<td>1600 $\mu$g of OA and 1600 $\mu$g of CIA</td>
<td>0.193 ± 0.012</td>
<td>6.50</td>
</tr>
</tbody>
</table>

Abbreviations are defined in results section.
### TABLE 3.6 TERNARY QUANTITATIVE SEPARATIONS OF IAA FROM TA AND CIA BY S-TLC

<table>
<thead>
<tr>
<th>Carboxylic Pollutant</th>
<th>Separated From</th>
<th>Absorbance at 510 nm</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µg of IAA</td>
<td>200 µg of TA and 400 µg of CIA</td>
<td>0.216 ± 0.008</td>
<td>3.75</td>
</tr>
<tr>
<td>50 µg of IAA</td>
<td>400 µg of TA and 800 µg of CIA</td>
<td>0.207 ± 0.008</td>
<td>3.91</td>
</tr>
<tr>
<td>50 µg of IAA</td>
<td>600 µg of TA and 1200 µg of CIA</td>
<td>0.205 ± 0.005</td>
<td>2.68</td>
</tr>
<tr>
<td>50 µg of IAA</td>
<td>800 µg of TA and 1600 µg of CIA</td>
<td>0.215 ± 0.010</td>
<td>4.83</td>
</tr>
</tbody>
</table>

Abbreviations are defined in results section.
G in 17 ml of 0.3% $\text{H}_3\text{PO}_4$ plates using light petroleum-isopropyl ether (1:2) as solvent. Ethanollic diphenylamine was used as a locating reagent. Thielemann (19) separated and identified carboxylic herbicidal substances on UV$_{254}$ sheets with benzene-acetone (2:3) or (4:2) as the solvent. Ethanollic Rhodamine B was used as the locating reagent. Ahmad et al. (13) claimed that calcium sulphate is an excellent TLC material. They used it to separate plant growth regulators such as BOA, CPAA, IAA, 2-NPAA, 2-NXAA and PAA from many other carboxylic acids present in plants, fruits and soil using common solvents.

The results obtained from the present studies show that some important binary separations of herbicides such as 2,4-D, TCA and 2,4,5-T (Figures 3.1, 3.3 and 3.5) which were not possible by NP-TLC can be achieved on calcium sulphate by the sequential development of chromatograms. It is also clear that the plant growth regulators such as CIA, BOA, IAA, GA, 2-NPAA, 2-NXAA and PAA can also be separated by S-TLC (Figures 3.2, 3.4 and 3.6 and Table 3.2). Ternary separations of IAA, MEA, MA, OA, PAA, SA and TA, shown in Figures 3.7 and 3.8 and summarized in Table 3.3, can be achieved successfully by S-TLC while they can not be separated by NP-TLC. The results of quantitative separations summarized in Tables 3.4, 3.5 and 3.6 show the versatility of S-TLC for analysing complex mixtures of IAA, OA, TA and CIA.

3.5 CONCLUSION

The results discussed above show that S-TLC can be used for separations of the mixtures which are not possible by NP-TLC. It is also obvious that calcium sulphate is an excellent TLC material for separating the mixtures of carboxylic pollutants in water.
REFERENCES


4. A. Man and E.S. Lim, Pertanika, 9, 23 (1986).


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CHAPTER - 4

SEPARATION AND DETECTION OF SOME PHENOXYACID HERBICIDES AND PLANT GROWTH REGULATORS BY ION-PAIR REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY
4.1 INTRODUCTION

As described in Chapter 3 that herbicides such as 2,4-D, 2,4,5-T, TCA and plant growth regulators such as BOA, CIA, IAA, GA and 1-NPAA are toxic, they mix-up in water causing water pollution. They are difficult to analyse by GC due to their non-volatile behaviour. TLC is a versatile tool for their analysis. S-TLC on calcium sulphate in simple solvents can be used for the binary and ternary separations of these water pollutants.

Literature survey shows that RP-TLC is a useful alternative to NP-TLC on silica gel G for the separation of polar compounds. Lewis and Wilson (1) studied the organic acids by IP-RP-TLC on both paraffin coated and C\textsubscript{18} bonded silica gel plates using six ion-pair reagents. Ruane et al. (2) also studied normalphase ion-pair thin-layer chromatography (NP-IP-TLC) and IP-RP-TLC to separate and determine hydroxybenzoic acids, genestic and salicylic acid with bifunctional, 1,3-bis (trimethylammonium) propane and 1,12-bis (trimethylammonium) dodecane as ion-pair reagents. The chromatographic properties of 132 compounds were studied in two ion-pair reagent systems on RP-TLC plates (3). Hui and Taylor (4) used RP-IP-HPLC technique for the determination of histamine and its metabolites in rat urine using 1-pentane sulphonic acid as an ion-pair reagent. RP-IP-HPLC was found to be very useful in quantitative analysis of choline in plant extracts and choline esters in pharmaceutical preparations (5).

Therefore, now the efforts have been made to study the chromatographic behaviour of some carboxylic acid herbicides and plant growth regulators by IP-RP-TLC on calcium sulphate in distilled water. Cetrimide (tetradecyltrimethylammonium bromide) solution along with an oil is used as IP-RP reagents. The results obtained are discussed in this Chapter.
4.2 EXPERIMENTAL

4.2.1 Apparatus and Materials

A Stahl apparatus with a universal applicator, glass plates (20 x 4 cm), glass jars (25 x 5 cm), hot air electric drier, separating funnel (50 ml), graduated micro pipette (0.1 ml) with vacuupet control and temperature controlled electric oven were used.

Bromophenol blue, herbicides, plant growth regulators (Sigma, USA); calcium sulphate (E.Merck, India); cetrimide, olive oil (Shalaks Chemicals, India); silicone oil, TCA (CDH, India); coconut oil (Tata product, India) and all other chemicals used were of analytical grade.

4.2.2 Preparation of Solutions

All the test solutions (1%) were prepared either in ethanol or in distilled water.

4.2.3 Preparation of Plates

The following slurries were applied on the glass plates with the help of the applicator so that the thickness of the coating would be 0.50 mm. The plates were first allowed to dry at room temperature and then at 80°C in an oven for 30 min.

A - Calcium sulphate (30 g), 1% ethanolic solution of cetrimide (5 ml) and coconut oil (2 ml) in 70 ml of distilled water.

B - Calcium sulphate (30 g), 1% ethanolic solution of cetrimide (5 ml) and olive oil (2 ml) in 70 ml of distilled water.
C - Calcium sulphate (30 g), 1% ethanolic solution of cetrimide (5 ml) and paraffin oil (2 ml) in 70 ml of distilled water.

D - Calcium sulphate (30 g), 1% ethanolic solution of cetrimide (5 ml) and silicone oil (2 ml) in 70 ml of distilled water.

4.2.4 Spotting of Test Solutions

Test solutions were spotted on the plates with the help of a fine bore capillary. The solvent was removed by hot air drying. Then the plates were developed up to the length of 10 cm in a solvent.

4.2.5 Visualization of Chromatograms

The compounds understudy were visualized on TLC plates by spraying 0.1% ethanolic alkaline bromophenol blue solution.

4.2.6 Measurement of R_f Values

For tailing, the front limit (RI) and rear limit (RT) were measured and recorded while for compact spots R_f values were calculated as in Chapter 2.

4.2.7 Detection and Determination of Lower Limit of Detection of Herbicides in Water

A known volume of distilled water was dosed with a known volume of standard ethanolic solutions (1%) of CPAA, 2,4-D, 2,4,5-T and PAA. The herbicides were extracted from the dosed water (50 ml) in diethyl ether (5 ml). The volume of the extract was reduced to 1 ml by warming at water bath. A small volume (0.01 ml) of the condensed extract was spotted on the TLC plate (Coating B) with the help of a micro pipette. The plate was developed in distilled water, solvent was removed and the herbicides
were detected on the plate by spraying ethanolic alkaline bromophenol blue. Petroleum ether and isopropyl ether were also used to extract herbicides from the dosed water for detection. The lower limit of the detection was determined by spotting same volume (0.01 ml) of different standard solutions of varying concentration.

4.3 RESULTS

Ternary and quaternary separations achieved on different coatings in distilled water are recorded in Tables 4.1 and 4.2. Some of the important ternary and quaternary separations are shown in Figures 4.1 and 4.2. Results of detection by IP-RP-TLC of some herbicides in water are recorded in Table 4.3.

The following abbreviations are used: BOA = benzoic acid, CIA = cinnamic acid, CPAA = 4-chlorophenoxyacetic acid, 2,4-D = 2,4-dichlorophenoxyacetic acid, GA = gallic acid, IAA = indole-3-acetic acid, IPA = indole-3-propionic acid, 1-NPAA = 1-naphthaleneacetic acid, 2-NPAA = 2-naphthaleneacetic acid, 2-NXAA = 2-naphthoxyacetic acid, PAA = phenoxyacetic acid, TCA = trichloroacetic acid and 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid.

4.4 DISCUSSION

The results obtained show that calcium sulphate coating impregnated with cetrimide alone cracked on drying. The coating impregnated with cetrimide and an oil was free from cracks and stable. It was also observed that $R_f$ values of the herbicides understudy on calcium sulphate alone coatings were higher than those obtained on calcium sulphate containing cetrimide and the oil coatings. Results recorded in Tables 4.1 and 4.2 show that
TABLE 4.1 TERNARY SEPARATIONS OF SOME CARBOXYLIC POLLUTANTS BY IP-RP-TLC

<table>
<thead>
<tr>
<th>Carboxylic Pollutants</th>
<th>Separated From</th>
<th>Coatings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPAA (0.0)</td>
<td>2,4-D (3-6 cm) and PAA (9-10 cm)</td>
<td>D</td>
</tr>
<tr>
<td>CIN (0.0)</td>
<td>2,4-D (5-7 cm) and GA (1.0) or TCA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>CIN (0-2 cm)</td>
<td>CPAA (5-7 cm) and TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>CIN (0.0)</td>
<td>2,4,5-T (3-5 cm) and GA (9-10 cm) or 2-NXAA (1.0) or TCA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>CIN (0.0)</td>
<td>BOA (4-6 cm) and 2-NXAA (1.0) or PAA (1.0) or TCA (1.0) or GA (1.0)</td>
<td>C and B</td>
</tr>
<tr>
<td>2-NXAA (0.0)</td>
<td>CPAA (4-5 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2-NXAA (0-2 cm)</td>
<td>BOA (3-5 cm) and GA (1.0) or PAA (9-10 cm)</td>
<td>B</td>
</tr>
<tr>
<td>2-NXAA (0.0)</td>
<td>IAA (3-5 cm) and GA (1.0) or PAA (9-10 cm) or TCA (1.0) or BOA (1.0)</td>
<td>B and D</td>
</tr>
<tr>
<td>2-NXAA (0.0)</td>
<td>2,4-D (0.5) and BOA (1.0) or CIN (1.0) or TCA (1.0)</td>
<td>D</td>
</tr>
<tr>
<td>1-NPAA (0.0)</td>
<td>CPAA (5-6 cm) and GA (1.0) or PAA (9-10 cm)</td>
<td>B</td>
</tr>
<tr>
<td>1-NPAA (0.0)</td>
<td>2,4-D (5-7 cm) and 2-NXAA (1.0) or TCA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>1-NPAA (0.3)</td>
<td>BOA (5-6 cm) and GA (1.0) or 2-NXAA (0.9) or PAA (1.0)</td>
<td>A, C and D</td>
</tr>
<tr>
<td>2-NPAA (0.0)</td>
<td>CPAA (3-5 cm) and TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2-NPAA (0.0)</td>
<td>BOA (0.3) and PAA (9-10 cm)</td>
<td>A, B and D</td>
</tr>
<tr>
<td>2-NPAA (0.0)</td>
<td>2,4-D (0.2) and PAA (9-10 cm)</td>
<td>A, B and D</td>
</tr>
<tr>
<td>Compound</td>
<td>Description</td>
<td>Notes</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>2-NPAA (0.0)</td>
<td>IAA (6-7 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-T (0.0)</td>
<td>CPAA (3-5 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-T (0.0)</td>
<td>2,4-D (2-3 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B and D</td>
</tr>
<tr>
<td>2,4,5-T (0-2 cm)</td>
<td>BOA (5-6 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>A and B</td>
</tr>
<tr>
<td>2,4,5-T (1-2 cm)</td>
<td>IAA (0.7) and GA (1.0) or PAA (9-10 cm) or TCA (1.0)</td>
<td>B and D</td>
</tr>
<tr>
<td>2,4,5-T (0.0)</td>
<td>IPA (5-6 cm) and GA (1.0) or PAA (9-10 cm) or TCA (1.0)</td>
<td>A</td>
</tr>
</tbody>
</table>

Abbreviations are defined in the results section and $R_f$ values are given in parentheses.
## TABLE 4.2 QUATERNARY SEPARATIONS OF SOME CARBOXYLIC POLLUTANTS BY IP-RP-TLC

<table>
<thead>
<tr>
<th>Carboxylic Pollutants</th>
<th>Separated From</th>
<th>Coatings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN (0.0)</td>
<td>BOA (2-3 cm) - IAA (0.8) and 2-NXAA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>1-NPAA (0.0)</td>
<td>2,4-D (0.3) - BOA (0.6) and GA (1.0) or PAA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>1-NPAA (0.0)</td>
<td>2,4-D (0.3) - PAA (7-8 cm) and 2-NXAA (1.0) or TCA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>1-NPAA (0.0)</td>
<td>BOA (0.2) - IPA (0.6) and PAA (1.0)</td>
<td>A</td>
</tr>
<tr>
<td>2-NPAA (0.0)</td>
<td>2,4-D (0.2) - CPAA (5-6 cm) and GA (1.0) or PAA (9-10 cm)</td>
<td>B</td>
</tr>
<tr>
<td>2-NPAA (0.0)</td>
<td>2,4-D (2-3 cm) - IAA (5-7 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2-NXAA (0.0)</td>
<td>2,4-D (0.2) - CPAA (4-5 cm) and PAA (9-10 cm)</td>
<td>B</td>
</tr>
<tr>
<td>2-NXAA (0.0)</td>
<td>2,4-D (2-3 cm) - IAA (5-6 cm) and GA (1.0) or PAA (9-10 cm)</td>
<td>A</td>
</tr>
<tr>
<td>2,4,5-T (0.0)</td>
<td>BOA (2-3 cm) - IPA (5-6 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>A</td>
</tr>
<tr>
<td>2,4,5-T (0.0)</td>
<td>2,4-D (2-3 cm) - BOA (0.6) and TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-T (0.0)</td>
<td>2,4-D (2-3 cm) - CPAA (7-8 cm) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-T (0-1 cm)</td>
<td>2,4-D (0.3) - IAA (0.7) and GA (1.0) or PAA (1.0) or TCA (1.0)</td>
<td>B</td>
</tr>
</tbody>
</table>

Abbreviations are defined in the results section and R_f values are given in parentheses.
Figure 4.1  Ternary separations of some carboxylic pollutants by IP-RP-TLC.

Spot A - 2,4,5-T in 1 and 2, 1-NPAA in 3 and 4, and 2-NPAA in 5 and 6.

Spot B - CPAA in 1 and 3, 2,4-D in 2 and 4, IAA in 5 and IPA in 6.

Spot C - PAA in 1 and 2, TCA in 3 and 4 and GA in 5 and 6.
Figure 4.2 Quaternary separations of some carboxylic pollutants by IP-RP-TLC.

Spot A - 2,4,5-T in 1, 2 and 6, 2-NXAA in 3 and 2-NPAA in 4 and 5.

Spot B - 2,4-D in 1, 2, 3, 4, 5 and 6.

Spot C - CPAA in 1, 2, 3, 4, 5 and 6.

Spot D - BAA in 1 and 5, TCA in 2 and 4 and GA in 3 and 6.
<table>
<thead>
<tr>
<th>Herbicides Present in Water</th>
<th>Extractant</th>
<th>Lower Limit of Detection (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA, CPAA, 2,4-D and 2,4,5-T</td>
<td>Diethyl ether</td>
<td>20</td>
</tr>
<tr>
<td>PAA, CPAA, 2,4-D and 2,4,5-T</td>
<td>Isopropyl ether</td>
<td>40</td>
</tr>
<tr>
<td>PAA, CPAA, 2,4-D and 2,4,5-T</td>
<td>Petroleum ether</td>
<td>60</td>
</tr>
</tbody>
</table>

Abbreviations are defined in results section.
the ternary and quaternary separations of the herbicides and plant growth regulators understudy can be achieved successfully. Figures 4.1 and 4.2 show the novelty of the method viz. quaternary separations of phenoxyacid herbicides (2,4,5-T from 2,4-D-CPAA and PAA) can be achieved successfully. Table 4.3 shows that the detection of herbicides on IP-RP-TLC is a sensitive method as the lower limit of detection is 20 μg.

4.5 CONCLUSION

The results discussed above indicate that IP-RP-TLC is a more convenient and simpler method to separate and detect the complex mixtures of herbicides in water.

REFERENCES


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CHAPTER - 5
A SELECTIVE FLUORESCENCE SPOT TEST FOR THE
DETECTION OF TRICHLOROACETIC ACID IN SOIL AND WATER
5.1 INTRODUCTION

Trichloroacetic acid (TCA) is an effective soil sterilant used to control weed, seedling grasses and some other broadleaf weeds in sugar beets and sugarcane (1). It acts by precipitation of protein within the cells (2). It is used alone or in mixture with other herbicides (2,4-D, dalapon and propazine) in carrot (3), fruit tree nurseries (4), winter oil seed rape (5), peanut and maize (6). It is toxic to plants and animals at higher concentration (LD$_{50}$ 5000 mg/kg). Its concentration in flora and fauna increases day by day due to its repeated usage. Therefore, there is a growing interest in methods of analysis for monitoring TCA in environmental samples. GC was used to determine TCA in phosphate fertilizers and tap water by Qiong (7) and Sugino et al. (8) respectively. The extraction of acidic herbicides from waters, derivatization of the acids with diazomethane, and analysis by GC with EC, halogen-mode Hall detectors was described by Cessna et al. (9). Shiao and Hao (10) determined acid herbicides by RP-HPLC. Pastore and Lima (11) used o-phthalaldehyde-$H_2SO_4$ as a detection reagent for carboxylic herbicides on TLC plates. Organic acids have been separated and detected on thin layer chromatograms by spraying bromophenol blue (12). Phenoxyacid herbicides and plant growth regulators have been separated and detected by IP-RP-TLC (13). As most of the carboxylic herbicides are non-volatile, their conversion into volatile derivatives is necessary before analysing by GC. Therefore GC has limited applications for acid herbicides. GC and HPLC are costly as well as sophisticated methods, which are not available in most laboratories in developing countries.

Spot test analysis based on the fluorescence or colour formation is simple, inexpensive and extremely useful for the preliminary characterization
of TCA particularly, in place where sophisticated instrumentation is not available. Feigl (14) described a spot test for the detection of TCA. It is based on the formation of yellow-greenish fluorescent salicylaldazine under UV light. He postulated that TCA gives off chloroform on heating with aqueous sodium hydroxide. Chloroform so formed reacts with phenol to give salicylaldehyde which condenses with hydrazine to produce salicylaldazine. This test has not been used for analysing TCA in environmental samples so far. Therefore now an attempt has been made to extend Feigl's fluorescence test with modified procedures for analysing TCA in environmental samples. The results obtained are discussed in this Chapter.

5.2 EXPERIMENTAL

5.2.1 Apparatus and Materials

A temperature controlled heating mantle (Sunvik, UK), UV lamp (366 nm, Hanovia, England), vacuustier pump (Atlantis, India), temperature controlled water bath (Tempo, India), hot air electric drier, electric oven, graduated micro pipette (0.1 ml), petri dishes (8 cm id), watch glass (6 cm id), and glass capillaries (3 mm and 1.96 mm id) were used.

Hydrazine sulphate, sodium acetate (GSC, India); methanol (E.Merck, India); phenol, sodium hydroxide (BDH, India); trichloroacetic acid (CDH, India) and all other chemicals used were of analytical grade.

5.2.2 Preparation of Solutions

Aqueous or ethanolic solutions of the test substances (1%). were used. If a 1% solution could not be prepared, saturated solutions were used.
Hydrazine sulphate (10 g) and sodium acetate (10 g) were boiled in 100 ml of distilled water, cooled, filtered and then the clear solution was used as the fluorescence reagent.

Aqueous sodium hydroxide (20%) and ethanolic phenol (5%) were used.

5.2.3 Preparation of Indicator Paper

The above reagent solution (5 ml) was taken in a watch glass (6 cm id), and a disc (3 cm id) of filter paper (Whatman No. 1) was dipped into it for 30 sec. The excess solution was drained off by placing the paper over a filter paper sheet. Then it was allowed to dry at room temperature. The dried discs were stored in a hard paper box and used as indicator paper for the detection of TCA.

5.2.4 Preparation of Capillary Detector

A 1 cm long cotton plug was fixed in the capillary, the plug was impregnated with two or three drops of the above reagent solution and then the excess solvent was evaporated by hot air drying. Then, the capillary detector was ready for use.

5.2.5 Environmental Samples

Soil (fertile) was composed of sand 32.68%, silt 57.00%, clay 10.06%, organic matter 0.61%, CEC 16.00 meq/100 g and having pH 7.75.

Soil (pond) was composed of sand 61.00%, silt 25.00%, clay 14.00%, organic matter 2.2%, CEC 7.60 meq/100 g and having pH 9.2.
Soil (red) was composed of sand 70.82%, silt 16.70%, clay 12.80%, organic matter 0.28%, CEC 3.50 meq/100 g and having pH 5.7.

Water (river) contained dissolved oxygen 8.2-8.7 ppm, total hardness 122-141 ppm, total alkalinity 174-189 ppm, chlorides 9.2-9.9 ppm, sulphates 0.42-0.47 ppm, BOD 5.2-5.61 ppm, COD 6.5-6.7 ppm and having pH 8.7-8.8.

Water (tap) contained dissolved oxygen 8.0 ppm, total hardness 133-144 ppm, total alkalinity 261-268 ppm, chlorides 13-14 ppm, sulphates 14.0-18.5 ppm and having pH 7.5-7.6.

5.3 PROCEDURES

5.3.1 Detection in Solution

A small volume of the ethanolic test solution (0.1 ml) was mixed with 2-3 drops of sodium hydroxide and phenol solution in a micro test tube. The mixture was heated at 100°C in a water bath for 2 min, cooled and then a drop of the reagent was added to it. The mixture was neutralized with acetic acid and then it was thoroughly shaken with diethyl ether (0.5 ml). The ethereal layer was spotted on a strip of filter paper, dried and exposed to UV light of 366 nm. A yellow-greenish stain on a purple background indicates the presence of TCA.

5.3.2 Detection by Indicator Paper

A drop of ethanolic test solution was mixed with a drop of sodium hydroxide and phenol solution in a micro beaker. The reaction mixture was evaporated to dryness, the indicator paper was placed on the mouth of the micro beaker and covered with a watch glass. The contents of the beaker were
heated at 180 ± 2°C for 5 min and the indicator paper was exposed to UV light of 366 nm. A yellow-greenish fluorescence indicates the presence of TCA.

5.3.3 Detection by Capillary Detector

A drop of ethanolic test solution was mixed with a drop of sodium hydroxide and phenol solution in a micro test tube, the contents were evaporated to dryness, the capillary detector was fixed in the mouth of a micro test tube with the help of a rubber stopper and the outer and of the capillary was connected with the vacuustier pump with the help of rubber tubing. The reaction mixture was heated at 180 ± 2°C for 5 min and then the capillary detector was exposed to UV light of 366 nm. A yellow-greenish fluorescence indicates the presence of TCA.

5.3.4 Detection of TCA in Soil

An ethanolic solution of TCA (1.0 ml of 0.1%) was applied dropwise with continuous shaking to 100 g of soil sample in a stoppered container. The treated soil was divided into five equal portions and each portion was kept in a glass petri dish with lid. After a fixed period of time, TCA was detected in the above soil samples by the following procedure: The treated soil (20 g) was taken in a glass column (10 mm id) with glass wool support and TCA was eluted with 20 ml of methanol at the rate of 0.5 ml/min. The solvent was evaporated by hot air and TCA was detected in the residue by the procedures mentioned above.

5.3.5 Detection of TCA in Water

Standard solutions of TCA of different concentrations were prepared in
distilled water. The lower limit of the detection was determined by detect-
in the TCA in 0.1 ml of the above solutions. Similarly, the lower limit
of detection was determined in tap water and river water.

5.3.6 Preconcentration and Detection in Water

A standard solution of TCA (1 g/l) was prepared in ethanol. Another
standard solution of TCA (0.4 ppm) was made by diluting the above solution
with distilled water. A 50 ml portion of this solution was taken in a
separatory funnel (100 ml) and TCA was extracted with diethyl ether (20 ml).
The aqueous layer was removed from the funnel and a fresh portion (50 ml)
of the above TCA solution was added. After thorough shaking, the aqueous
layer was removed from the funnel. Three more fresh portions (each of
50 ml) of the above TCA solution were treated similarly with the same
20 ml of diethyl ether in order to preconcentrate TCA. Finally the extract
was taken in a beaker and the solvent was removed. The residue so left
was dissolved in 1 ml of methanol and then TCA was detected in 0.1 ml
of it. Hence the lower limit of detection was found to be 10 μg/0.1 ml
or 0.4 ppm.

5.4 RESULTS

Several organic and inorganic compounds were detected by the procedures
understudy. The following compounds give positive response. The colour
of the fluorescence produced is given in first parenthesis and lower limit
of detection in μg by procedures 5.3.1, 5.3.2 and 5.3.3 are given in second
parenthesis at first, second and third places respectively.

Chloroform (YG) (100, 80, 200), 2,4-D (DY) (80, 80, 100), salicylic
acid (YG) (50, 50, 60), salicylaldehyde (YG) (30, 20, 30), salicylamide (BG)
Abbreviations used are: B = blue, D = dark, G = green and Y = yellow.

The following compounds do not give positive response and they do not interfere in the test.

**Acids**

Acetic acid, ascorbic acid, benzoic acid, cinnamic acid, citric acid, gallic acid, indole-3-acetic acid, indole-3-propionic acid, maleic acid, malic acid, malonic acid, oxalic acid and tartaric acid.

**Alcohols**

Butanol, ethanol, methanol and propanol.

**Amines**

Aniline, diethanolamine, diphenylamine and toluenediamine.

**Anhydrides**

Acetic anhydride, phthalic anhydride and succinic anhydride.

**Carbohydrates**

Dextrose, glucose, starch and sucrose.

**Carbonyl Compounds**

Acetone and acetophenone.
Esters

Ethyl acetate and ethyl formate.

Ether

Diethyl ether.

Heterocyclic compounds

Barbituric acid, indole and pyridine.

Hydrocarbons

Benzene, cyclohexane and toluene.

Pesticides

Aldrin, BHC, dalapon, 1-naphthaleneacetic acid, 2-naphthaleneacetic acid, 2-naphthoxyacetic acid and phenoxyacetic acid.

Phenols

Catechol, hydroquinone, phenol, pyrogallol and resorcinol.

Urea and Derivatives

Urea and thiourea.

Bicarbonate

Sodium bicarbonate.

Carbonates

Calcium carbonate and sodium carbonate.
Chlorides

Ammonium chloride and sodium chloride.

Nitrates

Ammonium nitrate and sodium nitrate.

Nitrite

Sodium nitrite.

Sulphates

Barium sulphate, calcium sulphate, magnesium sulphate and sodium sulphate.

Phosphate

Potassium hydrogen phosphate.

Miscellaneous

Bleaching powder, detergent, soap, lemon juice and tomato juice.

5.5 DISCUSSION

The fluorescence spot test under study is based on the Reimer-Tiemann formylation of phenol in aqueous alkali solution, followed by the formation of yellow-greenish fluorescent salicylaldazine through hydrazine. The reaction can be used for the detection of chloroform and chloroform producing compounds such as chloral and TCA. The lower limit of the detection is 50 μg. Since the reactive species are the alkaline phenolate solution and chloroform it also gives positive response with phenols using chloroform
### TABLE 5.1 LOWER LIMIT OF DETECTION OF TCA IN SOIL AND WATER

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Environmental Samples</th>
<th>Lower Limit of Detection (µg) and Fluorescence Observed by Different Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(5.3.1)</td>
</tr>
<tr>
<td>1.</td>
<td><strong>Soils</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soil (Fertile)</td>
<td>(40)-YG</td>
</tr>
<tr>
<td></td>
<td>Soil (Pond)</td>
<td>(30)-YG</td>
</tr>
<tr>
<td></td>
<td>Soil (Red)</td>
<td>(80)-YG</td>
</tr>
<tr>
<td>2.</td>
<td><strong>Waters</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water (Distilled)</td>
<td>(40)-YG</td>
</tr>
<tr>
<td></td>
<td>Water (River)</td>
<td>(50)-YG</td>
</tr>
<tr>
<td></td>
<td>Water (Tap)</td>
<td>(40)-YG</td>
</tr>
</tbody>
</table>

Abbreviations are defined in results section.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Contact Period</th>
<th>Fluorescence Observed</th>
<th>Soil(Fertile)</th>
<th>Soil(Pond)</th>
<th>Soil(Red)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>One Hour</td>
<td></td>
<td>YG</td>
<td>YG</td>
<td>YG</td>
</tr>
<tr>
<td>2.</td>
<td>Two Days</td>
<td></td>
<td>YG</td>
<td>YG</td>
<td>LYG</td>
</tr>
<tr>
<td>3.</td>
<td>Four Days</td>
<td></td>
<td>LYG</td>
<td>YG</td>
<td>VLYG</td>
</tr>
<tr>
<td>4.</td>
<td>Six Days</td>
<td></td>
<td>VLYG</td>
<td>VLYG</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Eight Days</td>
<td></td>
<td>-</td>
<td>VLYG</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations used are: L = light, V = very and other abbreviations are defined in results section.
and hydrazine sulphate as the reagent. This reaction was discovered in 1877 when organic pesticides were unknown. So it was not applied for the detection of pesticides in environmental samples.

Now the following procedures have been studied and developed in order to enhance the sensitivity and selectivity of the spot test and to apply it for the detection of TCA residual in environmental samples. Procedure (5.3.1) is similar to that used by Feigl and lower limit of detection of TCA is 40 µg. In procedure (5.3.2) the solvents were removed completely and then salicylaldehyde was evaporated to react with the indicator paper at the mouth of the micro beaker. In this case gas-solid reaction occurs and lower limit of identification is 30 µg. Hence it seems that the formation of salicylaldazine is more than that by procedure (5.3.1). It may be due to the absence of water in the reaction mixture. In procedure (5.3.3) the semi-dried reagent at the cotton plug reactes with salicylaldehyde and lower limit of identification is 50 µg that is comparable to that obtained by procedure (5.3.1).

The data recorded in results section show that chloroform, 2,4-D, salicylic acid, salicylaldehyde, salicylamide, 2,4,5-T, TCA and vanillin give yellow-greenish, dark-yellow, yellow-greenish, yellow-greenish, blue-green, dark-yellow, yellow-greenish and blue-green fluorescence respectively. Thus the selective detection of above compounds can be made in presence of several organics and inorganics listed in the results section. Possible interference due to chloroform can be eliminated by heating the test material to expell off solvent. The yellow-greenish fluorescence with salicylic acid and blue-green fluorescence with salicylamide and vanillin are in accordance to the results reported by Feigl i.e. salicylic ester saponifies with
sodium hydroxide to sodium salicylate that gives blue-violet fluorescence on indicator paper.

Table 5.1 shows that the test can be used for the detection of traces of TCA (30-50 µg) in some of the environmental samples such as soil and water. However, the test fails to detect TCA in leaves, lemons and seeds. It may be due to the consumption of TCA in precipitation of proteins or chlorosis.

Table 5.2 shows that the test can be used successfully for detecting persistence of TCA in different soils. It is also clear that TCA persists in fertile soil, pond soil and red soil for 6, 8 and 4 days respectively.

5.6 CONCLUSION

The test can be successfully applied for the detection of traces of TCA (30-50 µg/0.1 ml) in soil and water. It can be used to detect TCA at ppm level in water by coupling with a suitable preconcentration method (15) (extraction etc.). The capillary detector is very useful for detecting TCA without removing the matrix such as soil, organic matter etc.

REFERENCES


SEQUENTIAL THIN-LAYER CHROMATOGRAPHY OF 2,4-D AND RELATED COMPOUNDS

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SUMMARY

Separations and identification of carboxylic herbicidal substances such as 2,4-dichlorophenoxyacetic acid, trichloroacetic acid, 2,4,5-trichlorophenoxyacetic acid and plant growth regulators such as benzoic acid, cinnamic acid, indole-3-acetic acid, \( \beta \)-naphthalenesacetic acid, \( \beta \)-naphthoxyacetic acid, phenoxyacetic acid have been made by sequential thin-layer chromatography on calcium sulphate layer with acetone, benzene, carbon tetrachloride, chloroform, ethyl acetate, dioxan, propanol as solvents and bromophenol blue as detector.

INTRODUCTION

Plant growth regulators such as benzoic acid, cinnamic acid, indole-3-acetic acid and its derivatives, gallic acid, TCA and 2,4,5-T are difficult to analyse by largely used and highly sensitive technique, "Gas chromatography" due to their non-volatile nature. Therefore simple and commonly used methods such as spectrophotometry.
and thin-layer chromatography have been proved to be the readily available tool for their analysis. Amongst the above listed compounds 2,4-D, TCA and 2,4,5-T are toxic, their LD_{50} values are 370 mg,
5000 mg and 500 mg/kg respectively and they are being used as herbicides for destroying weeds.

Our previous work shows that these compounds can be separated by plain thin-layer chromatography (TLC) on calcium sulphate coating in common solvents, acetone, benzene, carbon tetrachloride, chloroform, dioxan, ethyl acetate, propenol etc. About-donia and Tomell have claimed that the newly developed sequential thin-layer chromatography (STLC) is a rapid and fast method of analysis of complex mixtures. Therefore an attempt has been made to explore the utility of calcium sulphate for the separation, detection and determination of herbicides by this procedure.

As a result many new ternary separations which were not possible by plain thin-layer chromatography on calcium sulphate, have now been achieved. The details of the procedure and the results obtained are reported in this paper.

EXPERIMENTAL

Apparatus

A sathal apparatus with a universal applicator (adjustable thickness of the applied layer from 0.0-2.00 mm), hot air electric drier, glass plates (20 x 4 cm), glass jars (25 x 5 cm), temperature controlled electric oven, centrifugation machine and spectrophotometer were used.

Chemicals

Acetone (BDH, India), benzene (J.M. Chemicals, India), calcium sulphate precipitated powder, carbon tetrachloride, chloroform,
2,4-D AND RELATED COMPOUNDS

Dioxan, ethyl acetate, propanol (E. Merck, India), benzoic acid, p-chlorophenoxyacetic acid, cinnamic acid, citric acid, 2,4-dichlorophenoxyacetic acid, gallic acid, indole-3-acetic acid, maleic acid, malic acid, malonic acid, p-naphthalenesuccinic acid, p-naphthoxyacetic acid, oxalic acid, phenoxyacetic acid, salicylic acid, tartaric acid, trichloroacetic acid, 2,4,5-trichlorophenoxyacetic acid (Sigma, USA) were used.

Preparation of solutions

Solutions (2%) of benzoic, p-chlorophenoxyacetic, cinnamic, 2,4-dichlorophenoxyacetic, gallic, indole-3-acetic, p-naphthalenesuccinic, p-naphthoxyacetic, phenoxyacetic, salicylic and 2,4,5-trichlorophenoxyacetic acids were prepared in ethanol. Solutions (2%) of citric, maleic, malic, malonic, oxalic, tartaric and trichloroacetic acids were prepared in distilled water.

Reagent for the spectrophotometric determination of indole-3-acetic acid was prepared by mixing 1 ml of 0.5M ferri chloride solution in 35% (v/v) perchloric acid. Prepared fresh reagent before use.

Preparation of plates

A slurry of calcium sulphate obtained by mixing calcium sulphate (30 g) with distilled water (70 ml) was applied on the glass plates with the help of applicator so that the thickness of calcium sulphate slurry would be 0.75 mm. The plates were first allowed to dry at room temperature and then in a temperature controlled electric oven at 110°C for one hour.

Detection of acids

The acids under study were located on the plates by 1% ethanolic, alkaline bromophenole blue solution.
Spotting of test solutions

Test solutions were spotted on the plates with the help of a fine capillary. The solvent was removed by hot air drying, the plate was developed up to the length of 5 cm in solvent A, the plate was taken out from the jar and A was removed as above, the plate was again developed up to the length of 10 cm in solvent B. B was also removed and then the spot was located with bromophenol blue by spray method. For trailing, the front limit (xI) and the rear limit (xII) were measured while for compact spot Rf values were taken as usual.

$$R_f = \frac{\text{Distance travelled by substance (cm)}}{\text{Distance travelled by solvent front (cm)}}$$

Qualitative separations

To achieve the qualitative separation of acids, one of the acids was spotted firstly and the solvent was removed, second acid solution was spotted and the solvent was removed and so on so far and then the plates were developed, dried and acids were located as above.

Quantitative separations

A known volume of standard acid solution was spotted on the plate with the help of a graduated micro pipette with vacuum control, the solvent was removed, plates were developed and spots were located as above. The demarcated area of the plate was scratched out, the acid was extracted with methanol (3 ml) and the solid was removed by centrifugation method. The acid in the centrifugate was determined spectrophotometrically by the following procedure.

In the extract (3 ml) containing indole-5-acetic acid, 2 ml of freshly prepared reagent were added dropwise but rapidly with continuous agitation, it was placed in dark for one hour for colour development. Finally the absorbance was measured at 510 nm against a blank containing methanol (3 ml) and reagent (2 ml).
Many separations have been achieved by using different sets of solvents. Some of the important separations are shown in photoplates 1 to 8 and other separations are given below. The first solvent that was allowed to ascend for 0-5 cm is listed first and the second solvent that was allowed to ascend for 0-10 cm listed at the second place.

**Binary separations by TLC:** Benzoic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0.2) in acetone, ethyl acetate; p-chloroanisoxycetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0.2) in acetone, ethyl acetate; adipic acid (1.0) from citric (0.0), maleic (0.3), maleic (0.0), oxalic (0.0), and tartaric (0.2) in acetone, benzene, ethyl acetate; 2,4-dichlorophenoxyacetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0.2) in acetone, ethyl acetate; gallic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0.2) in acetone, ethyl acetate; indole-3-acetic acid (1.0) from citric (0.0), maleic (0.0), malonic (0.0), oxalic (0.0) and tartaric (0.2) in acetone, benzene, ethyl acetate; malonic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0.2) in ethyl acetate; p-naphthaleneacetic acid (1.0) from citric (0.0), maleic (0.3), malonic (0.0), oxalic (0.0) and tartaric acid (0.2) in chloroform, ethyl acetate; p-naphthoxyacetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0.2) in acetone, carbon tetrachloride, i-propanol; phenoxyacetic acid (1.0) from citric (0.0), maleic (0.3), maleic (0.7), malonic (0.0), oxalic (0.0) and tartaric acid (0.2) in acetone, benzene, chloroform, salicylic acid (1.0) from citric (0.0), malonic (0.0), oxalic (0.0) and tartaric acid (0.2) in benzene, carbon tetrachloride, ethyl acetate; trichloroacetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0.2) in acetone, ethyl acetate; 2,4,5-trichlorophenoxyacetic acid (1.0) from citric (0.0), oxalic (0.0) and tartaric acid (0.2) in acetone, benzene, ethyl acetate.
Separation of tartaric acid (Spot A) from 1) 2,4,5-trichlorophenoxyacetic acid from 2) 2,4-dichlorophenoxyacetic acid from 3) benzoic acid from 4) trichloroacetic acid from 5) p-chlorophenoxyacetic acid and from 6) gallic acid (Spot B) on calcium sulphate coating developed in dioxan (5 cm) followed by ethyl acetate (10 cm).

Separation of tartaric acid (Spot A) from 7) cinnamic acid; from 8) indole-3-acetic acid; from 9) p-naphthaleneacetic acid; from 10) p-naphthoxyacetic acid; from 11) phenoxyacetic acid from 12) salicylic acid (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).
Photoplate No. 3
Separation of oxalic acid (Spot A) from 1) 2,4,5-trichloroacetic acid, from 2) 2,4-dichlorophenoxyacetic acid from 3) benzoic acid, from 4) trichloroacetic acid, from 5) p-chlorophenoxyacetic acid and from 6) gallic acid (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).

Photoplate No. 4
Separation of oxalic acid (Spot A) from 7) cinnamic acid, from 8) indole-3-acetic acid, from 9) 2-naphthaleneacetic acid, from 10) 2-naphthoxyacetic acid, from 11) phenoxycetic acid, and from 12) salicylic acid, (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).
Photoplate No. 5
Separation of citric acid (Spot A) from 1) 2,4,5-trichlorophenoxyacetic acid, from 2) 2,4-dichlorophenoxyacetic acid, from 3) benzoic acid, from 4) trichloroacetic acid from 5) p-chlorophenoxyacetic acid from 6) gallic acid (Spot B) on calcium sulphate coating, developed in dioxan (5 cm) followed by ethyl acetate (10 cm).

Photoplate No. 6
Separation of citric acid (Spot A) from 7) cinnamic acid, from 8) β-naphthaleneacetic acid, from 9) β-naphthoxyacetic acid, from 10) phenoxycetic and from 11) Salicylic acid (Spot B) on calcium sulphate coating, developed in chloroform (5 cm) followed by ethyl acetate (10 cm).
Photoplate No. 7

Ternary Separations: Oxalic acid (Spot A) from 1) malic acid (Spot B) and cinnamic acid (Spot C) in chloroform (5 cm) and ethylacetate (10 cm); from 2) indole-3-acetic acid (Spot B) and cinnamic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride; from 3) indole-3-acetic acid (Spot B) and phenoxyacetic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride (10 cm); maleic acid (Spot B) and cinnamic acid (Spot C) in acetone (5 cm) and benzene (10 cm); from maleic acid (Spot B) and phenoxyacetic acid (Spot C) in acetone (5 cm) and benzene (10 cm) on calcium sulphate coated plates.

Binary separations by TLC: Cinnamic acid (1.0) from indole-3-acetic (0-5), maleic (0-3), malonic acid (0.0) in carbon tetrachloride-chloroform, ethylacetate-chloroform, chloroform-benzene; indole-3-acetic acid (0-10) from citric (0-2), maleic (0-3) and malonic acid (0.0) in chloroform-benzene, propanol-benzene; maleic acid (8-10) from citric (0-2), oxalic (0.0) and tartaric acid (0.0) in chloroform-ethyl acetate, dioxan-ethyl acetate; malonic acid (7-10) from citric (0-2), oxalic (0.0) and tartaric acid (0.0) in chloroform-ethyl acetate, dioxan-ethyl acetate; malonic acid (7-10) from citric (0-2), oxalic (0.0) and tartaric acid (0-2) in chloroform-ethyl acetate, dioxan-ethyl acetate; β-naphthaleneacetic acid (6-10) from maleic (0-3), malonic acid (0.0) in chloroform-benzene; β-naphthoxy-acetic acid (5-8) from maleic (0-3) and malonic acid (0.0) in chloroform-benzene; phenoxyacetic acid (8-10) from indole-3-acetic (0-5), maleic (0-3),
Photoplate No. 8

Ternary Separations: Tartaric acid (Spot A) from 1) maleic acid (Spot B) and cinnamic acid (Spot C) in chloroform (5 cm) and ethyl acetate (10 cm); from 2) indole-3-acetic acid (Spot B) and cinnamic acid (Spot C) in ethylacetate (5 cm) and carbon tetrachloride (10 cm); from 3) indole-3-acetic acid (Spot B) and phenoxyacetic acid (Spot C) in ethyl acetate (5 cm) and carbon tetrachloride (10 cm); from 4) maleic acid (Spot B) and cinnamic acid (Spot C) in acetone (5 cm) and benzene (10 cm); from 5) maleic acid (Spot B) and phenoxyacetic acid (Spot C) in acetone (5 cm) and benzene (10 cm) on calcium sulphate coated plates.

Maleic (0.5) and malonic acid (0.0) in chloroform-benzene, propanol-benzene, propanol-carbon tetrachloride; salicylic acid (1.0) from maleic (0-5) and malonic acid (0-10) in chloroform-benzene, propanol-benzene; 2,4,5-trichloro phenoxyacetic acid (9-10) from maleic acid (0.5) in propanol-benzene.

Ternary Separations: Oxalic acid (0.0) from maleic acid (0.5) and 3-naphthaleneacetic acid (1.0) in ethylacetate-chloroform; oxalic acid (0.0) from maleic acid (4-5) and 3-naphthaleneacetic acid (9-10) in propanol-chloroform; tartaric acid (0.0) from 3-naphthaleneacetic acid (0.5) and cinnamic acid (9-10) in ethyl acetate-benzene; tartaric acid (0.0) from indole-3-acetic acid (3-5) and salicylic acid (9-10) in ethyl acetate-benzene; tartaric acid (0.0) from maleic acid (0.5) and salicylic acid (9-10) in ethyl acetate-carbon tetrachloride.
The results of quantitative separations are given in tables I, II and III. The analytical parameters are calculated by the following expressions:

\[
\sigma = \sqrt{\frac{(x_1 - \mu)^2 + (x_2 - \mu)^2 + \ldots}{N - 1}} 
\]

\[
C.V. = \frac{\sigma \times 100}{\mu} 
\]

where \( x_1, x_2, \ldots \) = measured values, \( \mu \) = average value and \( N \) = number of sets.

**Conclusion**

Thin-layer chromatography is particularly useful to herbicides containing carboxylic group because of their polar nature and insusceptibility to gas chromatography. Therefore it has been used.

**Table I.** Binary quantitative separations of indole-3-acetic acid (IAA) from oxalic acid (OA) and from tartaric acid (TA).

<table>
<thead>
<tr>
<th>Acid</th>
<th>N Separated from</th>
<th>Absorbance at 510 nm</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µg of IAA</td>
<td>6</td>
<td>200 µg of TA</td>
<td>0.215 ± 0.005</td>
</tr>
<tr>
<td>50 µg of IAA</td>
<td>6</td>
<td>400 µg of OA</td>
<td>0.212 ± 0.0098</td>
</tr>
</tbody>
</table>

Thickness of calcium sulphate layer = 0.75 mm, ethyl acetate 0-5 cm followed by carbon tetrachloride 0-10 cm.
### Table II. Ternary quantitative separations of indole-3-acetic acid (IAA) from oxalic acid (OA) and cinnamic acid (CTA).

<table>
<thead>
<tr>
<th>Acid</th>
<th>N</th>
<th>Separated from</th>
<th>Absorbance at 510 nm (μ &amp; s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IAA</td>
<td>OA and 400 μg of CTA</td>
<td>0.205 ± 0.0081 3.99</td>
</tr>
<tr>
<td>50 μg of IAA</td>
<td>6</td>
<td>800</td>
<td>0.205 ± 0.0081 6.68</td>
</tr>
<tr>
<td>50 μg of IAA</td>
<td>6</td>
<td>1200</td>
<td>0.197 ± 0.0075 3.45</td>
</tr>
<tr>
<td>50 μg of IAA</td>
<td>6</td>
<td>1600</td>
<td>0.193 ± 0.0126 6.50</td>
</tr>
</tbody>
</table>

Thickness of calcium sulphate = 0.75 mm, ethyl acetate 0-5 cm followed by carbon tetrachloride 0-10 cm.

### Table III. Ternary quantitative separations of indole-3-acetic acid (IAA) from tartaric acid (TA) and cinnamic acid (CTA).

<table>
<thead>
<tr>
<th>Acid</th>
<th>N</th>
<th>Separated from</th>
<th>Absorbance at 210 nm (μ &amp; s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IAA</td>
<td>TA and 400 μg of CTA</td>
<td>0.210 ± 0.0081 3.75</td>
</tr>
<tr>
<td>50 μg of IAA</td>
<td>6</td>
<td>800</td>
<td>0.207 ± 0.0081 3.91</td>
</tr>
<tr>
<td>50 μg of IAA</td>
<td>6</td>
<td>1200</td>
<td>0.205 ± 0.0055 2.58</td>
</tr>
<tr>
<td>50 μg of IAA</td>
<td>6</td>
<td>1600</td>
<td>0.215 ± 0.0104 4.93</td>
</tr>
</tbody>
</table>

Thickness of calcium sulphate = 0.75 mm, ethyl acetate 0-5 cm followed by carbon tetrachloride 0-10 cm.
for the detection, separation and determination of carboxylic herbicides. Zawadzka et al. have determined 2,4-D, dalapon, MCPA, dichlorprop, mecoprop, UNOC, dinoseb and TCA herbicides in water and sewage by TLC on silicic acid G-Kieselguhr G (2:3) or silicic acid G-H₂O₄ (5% of silicic acid in 17 ml of 0.3% H₂O₄ solution) layers with light petroleum-isopropyl ether (1:1) as solvent. Amanolic diphenylamine have been used as a location reagent. Thiemann has separated and identified carboxylic herbicidal substances on UV 254 sheets with benzene-acetone (2:3) or (4:1) as the solvent and 0.02% ethanolic toluidine B as the spray reagent. Bagalkot and Taylor have determined these pesticides in water by TLC on silicic acid G-Kieselguhr G (2:3) with perchloric acid as solvent and 0.5% AgNO₃ solution as spray reagent. Ahmed et al. have claimed that calcium sulphate is an excellent TLC material. They have separated plant growth regulators such as benzoic, cinnamic, indole-3-acetic, \( \beta \)-naphthaleneacetic, \( \beta \)-naphthoxyacetic and phenoxyacetic acids from many carboxylic acids present in plants, fruits and soils. The results obtained from the present study show that the separations of 2,4-D, TCA and 2,4,5-T shown in photoplates 1, 3 and 5 which were not studied by Ahmed et al. can also be achieved on calcium sulphate by the sequential development of chromatograms with common organic solvents. It is also clear that separations of plant growth regulators such as cinnamic, benzoic, indole-3-acetic, gallic, \( \beta \)-naphthaleneacetic, \( \beta \)-naphthoxyacetic, and phenoxyacetic acids shown in photoplates 2, 4 and 6 and mentioned in the result section which are not possible by PTLC can be achieved by TLC. Ternary separations of indole-3-acetic, maleic, malic, oxalic, phenoxyacetic, salicylic and tartaric acids shown in photoplates 7 and 8 and mentioned in result section can be achieved successfully by TLC while they are not possible by PTLC. Hence it is obvious that TLC on calcium sulphate gives better results than PTLC for separating complex and multicomponents systems.
ACKNOWLEDGMENTS

We thank Council of Scientific and Industrial Research, New Delhi for the financial assistance.

REFERENCES


Separation and Detection of Some Phenoxyacid Herbicides and Plant Growth Regulators by Ion-Pair Reversed-Phase Thin-Layer Chromatography

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(Received 2 September 1987, in final form 12 December 1987)

Separation and detection of phenoxyacid herbicides such as 4-chlorophenoxyacetic, 2,4-dichlorophenoxyacetic, phenoxyacetic, 2,4,5-trichlorophenoxyacetic, trichloroacetic acids and plant growth regulators such as benzoic, cinnamic, gallic, indole-3-acetic, indole-3-propionic, α-naphthaleneacetic, β-naphthaleneacetic, β-naphthoxyacetic acids have been made by ion-pair reversed-phase thin-layer chromatography on calcium sulphate coating impregnated with an ion-pair reagent cetrimide and different oils such as coconut oil, olive oil, paraffin oil and silicon oil using distilled water as a solvent and bromophenol blue as a detector.

KEY WORDS Phenoxy acids, plant growth regulators, phenoxyacid herbicides, reversed phase TLC, TLC.

INTRODUCTION

Phenoxyacid herbicides, such as 2,4-D and 2,4,5-T and trichloroacetic acid (TCA) are widely used to control dicot weeds in the crops and forests whereas plant growth regulators like benzoic, cinnamic, indole-3-acetic, gallic and α-naphthaleneacetic acids are used to
control the plant growth in crops. These herbicides and plant growth regulators mix up in natural water either by accidental fallout of sprays from agricultural treatment or by surface run-off from agricultural land. Work has been done to study the environmentally deleterious effects of the herbicides on the aquatic organisms, their phototoxicity to plants, to fish, pollen grains and to rats.

The mentioned compounds are difficult to analyse by the frequently used and highly sensitive technique of gas-chromatography due to their non-volatile nature. High performance liquid chromatography (HPLC) has proved to be a good alternative to clean-up and determine acid herbicides from different samples with great accuracy but is a sophisticated and expensive technique which requires technically skilled operators. These compounds have been separated by thin-layer chromatography (TLC) on calcium sulphate coating in our laboratory by previous workers. Reversed-phase thin-layer chromatography (RP-TLC) is a useful alternative to normal-phase thin-layer chromatography (NP-TLC) on silica gel for the separation of polar compounds.

Lewis and Wilson studied the organic acids by ion-pair reversed-phase thin-layer chromatography (IP-RP-TLC) on both paraffin-coated and C18 bonded silica gel plates using six ion-pair reagents. Hui and Taylor studied reversed-phase ion-pair high-performance liquid-chromatography (RP-IP-HPLC) procedure for the determination of histamine and its metabolites in rat urine using 1-pentane sulphonic acid as an ion-pair reagent. Ruane et al. have also studied normal-phase ion-pair thin-layer chromatography (NP-IP-TLC) and RP-IP-TLC of hydroxy benzoic acids, genestic acid and salicylic acid with bifunctional (bolafarm) bis trimethylammonium ion-pair reagent.

Literature survey shows that attempts have been made to use IP-RP-TLC in organic acids, peptides, amino acids and nitrogen bases. This paper describes the results on calcium sulphate coating using an ion-pair reagent cetrimide (tetradecyltrimethylammonium bromide) impregnating with oils such as coconut, paraffin, olive and silicon oils. Ternary and quaternary separations of phenoxyacid herbicides and plant growth regulators have been achieved using distilled water as a solvent. Efforts have been made to detect phenoxyacid herbicides from water samples after extraction with ethers.
EXPERIMENTAL

Apparatus
A Stahl apparatus with a universal applicator (adjustable thickness of the applied layer from 0.25-2.00 mm), hot air electric drier, glass plates (20 x 4 cm), glass jars (25 x 5 cm), separating funnel, graduated micro pipette with vaccupet control and temperature controlled electric oven were used.

Chemicals
Calcium sulphate precipitated powder (E. Merck, India), cetrimide, olive oil (Shalaks Chemicals, India), silicon oil (Central Drug House, India), coconut oil (Tata product, India), benzoic acid, 4-chlorophenoxyacetic acid, cinnamic acid, 2,4-dichlorophenoxyacetic acid, gallic acid, indole-3-acetic acid, indole-3-propionic acid, α-naphthaleneacetic acid, β-naphthaleneacetic acid, 4-chloro-α-naphthaleneacetic acid, 4-chloro-β-naphthaleneacetic acid, 4-chloro-β-naphthoxyacetic acid, phenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid (Sigma, USA), trichloroacetic acid (CDH, India) and all other reagents of analytical grade were used.

Preparation of solutions
Solutions (1%) of benzoic, 4-chlorophenoxyacetic, cinnamic, 2,4-dichlorophenoxyacetic, gallic, indole-3-acetic, indole-3-propionic, α-naphthaleneacetic, β-naphthaleneacetic, 4-chloro-α-naphthaleneacetic, 4-chloro-β-naphthaleneacetic, phenoxyacetic and 2,4,5-trichlorophenoxyacetic acids were prepared in ethanol. Solution (1%) of trichloroacetic acid was prepared in distilled water.

Preparation of plates
Plates of 0.5 mm thickness have been prepared by a slurry obtained by mixing calcium sulphate (30 g), 1% ethanolic solution of cetrimide (5 ml), oil (2 ml) and distilled water (DW) (70 ml). The plates were first allowed to dry at room temperature and then at 80°C for half an hour in an oven.

Four different coatings made to prepare thin-layer chromatographic plates are as follows:
Coating A: Calcium sulphate (30 g) + 1% ethanolic solution of cetrimide (5 ml) + coconut oil (2 ml) + DW (70 ml).

Coating B: Calcium sulphate (30 g) + 1% ethanolic solution of cetrimide (5 ml) + olive oil (2 ml) + DW (70 ml).

Coating C: Calcium sulphate (30 g) + 1% ethanolic solution of cetrimide (5 ml) + paraffin oil (2 ml) + DW (70 ml).

Coating D: Calcium sulphate (30 g) + 1% ethanolic solution of cetrimide (5 ml) + silicon oil (2 ml) + DW (70 ml).

Procedure

Test solutions were spotted on the plates with the help of fine capillary. The solvent was removed by hot air drying, the plates were developed up to 10 cm in the solvent and then dried and acids were detected with 0.1% ethanolic alkaline bromophenol blue. For tailing, the front limit (RI) and the real limit (RT) were measured, while for compact spot $R_f$ values were taken as usual,

$$R_f = \frac{\text{Distance travelled by substance (cm)}}{\text{Distance travelled by solvent (cm)}}.$$

Ternary and quaternary separations were achieved by spotting mixtures of three or four acids in water and detected by the same procedure. Phenoxy acid herbicides were extracted from water samples with diethyl ether, isopropyl ether and petroleum ether and quaternary separations were achieved successfully on coating B.

RESULTS

Tables 1 and 2 show that many ternary and quaternary separations have been achieved on different coatings. Some of the important separations are shown in photo print Nos. 1 and 2. Diethyl ether was the best extractant in comparison to petroleum ether and isopropyl ether due to herbicide's higher solubilities in the former. Recovery figures are given in Table 3.
### Table 1  Ternary separations

<table>
<thead>
<tr>
<th>Acid</th>
<th>Separated from</th>
<th>Coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPX (0.0)</td>
<td>2,4-D (3-6) and PHX (9-10)</td>
<td>D</td>
</tr>
<tr>
<td>CIN (0.0)</td>
<td>2,4-D (5-7) and GA (1.0)/TCA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>CIN (0-2)</td>
<td>CPX (5-7) and TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>CIN (0.0)</td>
<td>2,4,5-T (3-5) and GA (9-10)/β-NPX (1.0)/TCA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>CIN (0.0)</td>
<td>BN (4-6) and β-NPX (1.0)/PHX (1.0)/TCA (1.0)/GA (1.0)</td>
<td>C and B</td>
</tr>
<tr>
<td>β-NPX (0-1)</td>
<td>CPX (4-5) and GA (1.0)/PHX (1.0)/TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>β-NPX (0.0)</td>
<td>2,4-D (0-5) and BN (1.0)/CIN (1.0)/TCA (1.0)</td>
<td>D</td>
</tr>
<tr>
<td>β-NPX (0-2)</td>
<td>BN (3-5) and GA (1.0)/PHX (9-10)</td>
<td>B</td>
</tr>
<tr>
<td>β-NPX (0.0)</td>
<td>IAA (3-5) and GA (1.0)/PHX (9-10)/TCA (1.0)/BN (1.0)</td>
<td>B and D</td>
</tr>
<tr>
<td>α-NPA (0.0)</td>
<td>CPX (5-6) and GA (1.0)/PHX (9-10)</td>
<td>B</td>
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<tr>
<td>α-NPA (0.0)</td>
<td>2,4-D (5-7) and β-NPX (1.0)/TCA (1.0)</td>
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<td>α-NPA (0.3)</td>
<td>BN (5-6) and GA (1.0)/β-NPX (0.9)/PHX (1.0)</td>
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</tr>
<tr>
<td>β-NPA (0.0)</td>
<td>CPX (3-5) and TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>β-NPA (0-1)</td>
<td>BN (0.3) and PHX (9-10)</td>
<td>A, B and D</td>
</tr>
<tr>
<td>β-NPA (0.0)</td>
<td>2,4-D (0.2) and PHX (9-10)</td>
<td>A, B and D</td>
</tr>
<tr>
<td>β-NPA (0.0)</td>
<td>IAA (6-7) and GA (1.0)/PHX (1.0)/TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-T (0-1)</td>
<td>CPX (3-5) and GA (1.0)/PHX (1.0)/TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-T (0-1)</td>
<td>2,4-D (2-3) and GA (1.0)/PHX (1.0)/TCA (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>2,4,5-T (0-2)</td>
<td>BN (5-6) and GA (1.0)/PHX (1.0)/TCA (1.0)</td>
<td>B and D</td>
</tr>
<tr>
<td>2,4,5-T (1-2)</td>
<td>IAA (0.7) and GA (1.0)/PHX (9-10)/TCA (1.0)</td>
<td>A and B</td>
</tr>
<tr>
<td>2,4,5-T (0-1)</td>
<td>IPA (5-6) and GA (1.0)/PHX (9-10)/TCA (1.0)</td>
<td>B and D</td>
</tr>
</tbody>
</table>

Abbreviations used: Benzoes acid BN, 4-chlorophenoxyacetic acid CPX, cinnamic acid CIN, 2,4-dichlorophenoxyacetic acid 2,4-D, gallic acid GA, indole-3-acetic acid IAA, indole-3-propionic acid IPA, α-naphthaleneacetic acid α NPA, β-naphthaleneacetic acid β NPA, γ-naphthaleneacetic acid γ NPA, β-naphthoxyacetic acid β-NPX, phenoxycetic acid PHX, trichloroacetic acid TCA, 2,4,5-trichlorophenoxyacetic acid 2,4,5-T.
Table 2 Quaternary separations

<table>
<thead>
<tr>
<th>Acid</th>
<th>Separated from</th>
<th>Coating</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN (0.0)</td>
<td>BN (2-3)—IAA (0.8) and β-NPX (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>α-NPA (0.0)</td>
<td>2,4-D (0.3)—BN (0.6) and GA (1.0)/PHX (1.0)</td>
<td>B</td>
</tr>
<tr>
<td>α-NPA (0.0)</td>
<td>2,4-D (3.5)—PHX (7-8) and β-NPX (1.0)/TCA (1.0)</td>
<td>C</td>
</tr>
<tr>
<td>α-NPA (0.0)</td>
<td>BN (0.2)—IPA (0.6) and PHX (1.0)</td>
<td>A</td>
</tr>
<tr>
<td>β-NPA (0.0)</td>
<td>2,4-D (0.2)—CPX (5-6) and GA (1.0)/PHX (9-10)</td>
<td>B</td>
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<td>β-NPA (0.0)</td>
<td>2,4-D (2-3)—IAA (5-7) and GA (1.0)/PHX (1.0)/TCA (1.0)</td>
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<td>β-NPX (0.0)</td>
<td>2,4-D (2-3)—IAA (5-6) and GA (1.0)/PHX (9-10)</td>
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<tr>
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<tr>
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<tr>
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<td>B</td>
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</table>

Abbreviations are defined in Table 1.

Table 3 Recovery figures

<table>
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<tr>
<th>Sl. No.</th>
<th>Extractant</th>
<th>Herbicides present in water sample</th>
<th>Lower limit of detection (μg)</th>
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<td>Di-ethyl ether</td>
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<td>Petroleum ether</td>
<td>PHX, CPX, 2,4-D, 2,4,5-T</td>
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</tr>
</tbody>
</table>

Abbreviations are defined in Table 1.

DISCUSSION

Ion-suppression reversed-phase high-performance liquid-chromatography (IS-RP-HPLC) analysis of gibberillins and its conjugates on glycosides and glucosyl esters using as a C<sub>18</sub> support was done by Jensen et al. Reversed-phase ion-pair high-performance
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Figure 1 Ternary separation achieved on coating B. Spot A—2,4,5-Trichlorophenoxyacetic acid in 1 and 2; α-Naphthaleneacetic acid in 3 and 4; β-Naphthaleneacetic acid in 5 and 6. Spot B—4-Chlorophenoxyacetic acid in 1 and 3; 2,4-Dichlorophenoxyacetic acid in 2 and 4; Indole-3-acetic acid in 5; Indole-3-propionic acid in 6. Spot C—Phenoxyacetic acid in 1 and 2; Trichloroacetic acid in 3 and 4; Gallic acid in 5 and 6.

Liquid-chromatography (RP-IP-HPLC) has been found very useful in quantitative analysis of choline in plant extracts and choline esters in pharmaceutical preparations. The chromatographic properties of 132 compounds were studied in two ion-pair reagent systems on reversed-phase thin-layer plates. The retention power of polar ionic compounds is achieved using ion-pair reagent and some applications of the use of such ion-pair reagent compounds in RP-TLC have been described by Bieganouskal et al. and Grossini-Starazza et al.

In our laboratory calcium sulphate has been developed as an excellent TLC material, acids used to separate plant growth regulators, phenoxy acids and carboxylic acids. Therefore an attempt is made
to expose the utility of IP-RP-TLC in separating and detecting these compounds from water samples. The coatings of calcium sulphate impregnated with cetrimide cracked on drying. Addition of 2 ml of coconut/olive/paraffin/silicon oil prevented the cracking of the coatings. However, the $R_f$ values of 4-chlorophenoxyacetic, 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acids significantly decreased in the presence of the oils, indicating that ion-pair formation had occurred, e.g. $R_f$ values of 4-chlorophenoxyacetic, 2,4-dichlorophenoxyacetic and 2,4,5-trichlorophenoxyacetic acid decreased from 1.0 to 5-6, 1.0 to 3-4 and 1.0 to 0.0 respectively.

The coatings A, B, C and D impregnated with coconut, olive, paraffin and silicon oil respectively were found to be very useful for ternary and quaternary separations. Results indicate the novelty of the method in ternary and quaternary separations of the same group.
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of compounds like 2,4-D, 2,4,5-T, CPX and PHX from water samples. Separation of plant growth regulators is also possible. The best results were obtained on coating B.

Thus it seems that IP-RP-TLC on calcium sulphate provides a more convenient and simpler method to separate and detect these compounds in multi-component systems in comparison to NP-TLC.

Acknowledgement

We are grateful to the Council of Scientific and Industrial Research, New Delhi, for the award of financial assistance to one of the authors, S. K. Saxena.

References

2. A. Man and E. S. Lim, Pertanika 9(1), 23 (1986).