

DEVELOPMENT AND APPLICATIONS
OF
CHROMATOGRAPHIC TECHNIQUE
IN
STUDIES OF PESTICIDES



COMPUTERISED

(A Thesis Submitted to the
University of Poona for the degree of
Doctor of Philosophy in Chemistry)

BY
ARUN NARHAR KADAM

National Chemical Laboratory, Poona 8 (India)

April, 82.

COMPUTERISED

TO MY WIFE

ACKNOWLEDGEMENTS

I take this opportunity to express my sincere thanks and sense of gratitude to Dr. B.B. Chatge, under whose able guidance the work presented in this thesis has been carried out.

The necessary help and co-operation rendered by my colleagues, particularly by Dr. B.M. Bhawal, Dr. Vijay Dhekne and Mr. Milind Shenvi are gratefully acknowledged. I am also grateful for the assistance received from analytical section (organic synthesis), microanalytical and spectroscopic sections of the laboratory.

I am extremely thankful to the Director, National Chemical Laboratory, Pune and Dr. R.B. Mitra, Dy. Director, National Chemical Laboratory, Pune for kindly permitting me to present this work in the form of a thesis.


(A.N. Kadam)

8th April, 1982.

CONTENTS

	Page
I GENERAL REMARKS ..	i.
II SUMMARY OF THE THESIS ..	ii
1. GENERAL INTRODUCTION ..	1
1.1 Gas chromatography ..	2
1.2 Organophosphorus Pesticides ..	19
1.3 Present Investigations ..	33
2. POLY (ETHYLENE GLYCOL) DI-CHLOROACETATES AS STATIONARY PHASES FOR GAS CHROMATOGRAPHY	41
2.1 Introduction ..	42
2.2 Experimental ..	49
2.3 Results and Discussion ..	64
2.4 Applications ..	88
3. THERMAL DECOMPOSITION OF DIMETHOATE ..	98
3.1 Introduction ..	99
3.2 Experimental ..	117
3.3 Results and Discussion ..	128
4. DEGRADATION OF IMIDAN ..	145
4.1 The Pesticide : Imidan ..	147
4.2 Thin Layer Chromatography ..	151
4.3 Hydrolysis studies ..	171
4.4 Effluent Treatment ..	203

I. GENERAL REMARKS

1. All the figure numbers, table numbers, structure numbers and references in a chapter refer to that particular chapter only.
2. All melting points and boiling points are uncorrected.
3. Infra Red spectra were recorded as liquid film or as nujol mull on Perkin Elmer Spectrometer (model 137B and 221) with sodium chloride optics.
4. Elemental analyses were carried out by micro-analytical section using conventional estimation methods.
5. Mass spectra were recorded on a CES-2-110B double focussing spectrometer using direct inlet system (wherever not mentioned).

II. SUMMARY OF THE THESIS

1. GENERAL INTRODUCTION

1.1 PRESENT INVESTIGATIONS: During process development of various organophosphorus pesticides, number of problems related to analytical methods and effluent treatment had to be solved. Several modern techniques such as Gas-Liquid Chromatography (GC), Thin Layer Chromatography (TLC) and Coupled Gas Chromatography/Mass Spectrometry (GCMS) were extensively employed for the purpose. During these studies, several new stationary phases were developed for GC. Alternative methods were also devised. Simultaneously ideal chemical methods for detoxication of the pesticides were studied.

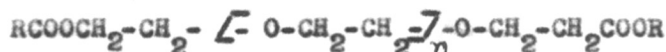
1.2 GAS CHROMATOGRAPHY: Principles of different chromatographic methods have been described. A brief account of GC technique has been given. Among various selective parameters for enhanced separation, types of detectors and liquid stationary phases were discussed with illustrations.

1.3. ORGANOPHOSPHORUS PESTICIDES: Different classes were described by giving examples. Their mode of action, activity, environmental decomposition and side effects have been mentioned in brief.

2. POLY(ETHYLENE GLYCOL)DI-CHLOROACETATES AS STATIONARY PHASES FOR GAS CHROMATOGRAPHY

Among numerous stationary phases for GC, poly (ethylene glycol) or Carbowax phases are very popular¹. Their esterification with acids yielded highly selective and thermally stable phases²⁴⁻²⁶.

In an attempt to increase its applicability, chloroacetic acids were condensed with Carbowax-20M which is the most preferred polyglycol²⁷⁻²⁹ to get phases A-D.



where R = CH₃-, (A)

= Cl·CH₂-, (B)

= Cl·CH₂-, (B)

= Cl₂·CH-, (C)

= Cl₃·C-, (D)

Following groups of samples were analysed on them (1) straight chain aliphatic alcohols (C₁ - C₇), (2) Compounds with varied polarities such as water, methanol, ethanol, chloroform, acetone, ethyl acetate and methylisobutylketone (MIBK) and (3) Chloromethanes.

Following groups of isomers were analysed (1) Propanols and butanols, (2) Xylenes, (3) Chlorobenzenes and (4) Chlorotoluenes.

Elution of alcohols observed to be according to the order of increasing boiling points³¹. Efficient separation of water-methanol, water-ethanol, chloroform-MIBK pairs have been achieved. Novel resolution of chloromethanes also has been obtained. A typical behaviour of chloro derivatives of aromatics has been observed. The retention phenomenon was discussed on the basis of electrostatic properties and hydrogen bonding.

No relation of number of chlorine atoms in the phase with retention data was observed. Eventhough a decrease in polarity of the phases was observed, they behave selectively towards chloro derivatives and polar compounds due to highly acidic protons in terminal chloroacetate groups.

The phase (C) found to be selective and ideal substitute for Carbowax-20 M due to lowest operational temperature³⁷ and greater resolution power. The same was employed to determine percentage of alcohols in aqueous solutions produced in some processes of organophosphorus pesticides^{30,40}.

3. THERMAL DECOMPOSITION OF DIMETHOATE

Among various organophosphorus pesticides, O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorothiolothiomate or Dimethoate³⁸ is most widely used for agricultural use. Many reports have described that

temperature is mainly responsible for its environmental degradation⁴⁵⁻⁵⁶. Additionally, its explosion at elevated temperature is the unique feature.

Dimethoate was thermally decomposed and separated into organic and inorganic parts which were studied to investigate the role of temperature. The latter found to contain oxides of phosphorus and charcoal. Presence of charcoal was very astonishing. The former was resolved into nine components by GC. Their percentages were determined using standard solution technique. Mass spectra obtained by GCMS were interpreted for structure elucidation. Some of them were identified as (i) O,S-dimethyl dithio-phosphate, (ii) O,O,S-trimethyl phosphorothiolothionate, (iii) O,S,S-trimethyl phosphorothiolethionate^{dithiolate}, (iv) O,O,S-trimethyl phosphorothiolate and (v) sulfoxide of compound ii. The data was co-related with studies in environmental degradation^{42,43,61,62}. It accounts impurities and various transformations in stored product.

Attempts were made to explain the explosion phenomenon e.g. polarisation of bonds and group migration in the cyclic intermediate due to electron rearrangements.^{66,67}

4. DEGRADATION OF IMIDAN

Introduction of an organophosphorus pesticide for an agricultural use necessitates evolution of prompt analytical tool for residue studies, chemistry of

transformations and industry oriented effluent treatment procedure. From this point of view these studies were taken up.

4.1 THE PESTICIDE: IMIDAN¹ (I). A brief account of preparation, activity and degradation was given.

4.2 THIN LAYER CHROMATOGRAPHY (TLC): Mainly TLC and paper chromatography with various solvent systems were employed to analyse Imidan degradation products^{5-8,17,18}. But they have restricted applications and are not suitable for general purposes. Therefore an handy but efficient two stage TLC technique has been developed for simultaneous analysis of Imidan and its metabolites: Imidoxon (II), N-hydroxymethyl phthalimide (III), phthalimide (IV), phthalamic acid (V) and phthalic acid (VI)⁵⁻⁸. It is suitable for studies in degradation in soil⁵, animal⁶ and plants as well as aquatic residue⁵ and decomposition on storage.

4.3 HYDROLYSIS STUDIES: Biological detoxication reactions have hydrolytic pathways⁴¹ and there also exists an identity relationship between these transformations and chemical hydrolysis^{18,40}. Therefore the latter has been thought to be appropriate method for artificial detoxication which can be utilised for effluent treatment.

Hydrolysis was carried at different conditions, using NaOH, Na_2CO_3 and HCl. NaOH cleaved the molecule at S-C linkage while protonation occurred at sulfur atom in P-S-C-N bridge, resulting in P-S cleavage. The latter reaction yielded N-mercaptomethyl phthalimide⁴³ (VII) which further hydrolysed to IV probably via bis (phthalimidomethyl) sulfide (IX)⁴³. Oxidative desulfuration also occurred simultaneously to get (II)⁴⁷. The weak nucleophile attacked phosphorus atom due to octet expansion of the latter and causes cleavage of P-S bond⁴⁹. It resulted in VII.N,N'-ethylene diphthalimide⁶³ (VIII) was further hydrolysis product which has been formed probably via IX. Compounds VII and VIII were reported for the first time as hydrolysis products. The TLC technique (4.2) found to be very useful for identification purposes.

The empirical data showed that the double bonding character of phosphoryl bonds⁴⁸ is partial and non-rigid. Behaviour of P-S-C-N bridge is uncertain but predominantly depends upon basic nature of reagents. The identity relationship^{18,40} was confirmed in this case but pertains to hydrolysis only using NaOH. In principle the reactions are useful to predict fate of a pesticide in environment .

4.4 EFFLUENT TREATMENT: Due to development of efficient analytical method (4.2) and chemistry of transformations (4.3), the studies are recommended for effluent treatment of waste water from the process industry. Because a need arises from acute problem to minimize water pollution⁵⁵.

●

CHAPTER ONE

GENERAL INTRODUCTION

●

1·1 GAS CHROMATOGRAPHY

1·2 ORGANOPHOSPHORUS PESTICIDES

1·3 PRESENT INVESTIGATIONS

●

1.1 GAS CHROMATOGRAPHY

1.1.1 Chromatographic Methods

Chromatography is defined as a method of separation where the components in a mixture are separated as a result of their partitioning between two immiscible phases.

Day¹ and Tswett² discovered the phenomenon which was explained as sorption-desorption interactions. In 1931 Kuhn and Lederer³ proved the usefulness of chromatographic technique as a powerful analytical tool by separating carotene and xanthophyll using displacement and frontal analysis. The first mathematical presentation was described by Wilson⁴ in 1940.

Depending upon the nature of a stationary phase, general chromatographic methods may be classified as follows. (1) Liquid-solid, (2) Gas-solid, (3) Liquid-liquid and (4) Gas-liquid. In liquid-solid chromatography, a mixture to be separated gets adsorbed on stationary particles. Due to the continuous elution of a solvent, the more soluble component is taken away by the mobile phase. Column⁵ and thin layer chromatography (TLC)⁶ belong to this class. The former contains a glass column filled with alumina or silica gel. A sample is loaded on the top and eluted using suitable solvent system. The latter consists of glass-plate coated

with a thin film of silica gel or any other suitable adsorbent. A mixture to be separated is spotted with the help of a capillary and a suitable solvent system is allowed to ascend in a presaturated solvent chamber. Then the plate is developed using iodine vapours, conc. H_2SO_4 or other developers. Ion exchange method⁷ is an industrially useful liquid-solid chromatography in which cation or anion exchange resins such as zirconium phosphate and polystyrene serve as stationary phases.

In gas-solid system⁸ samples like permanent gases and hydrocarbons having low molecular weights are separated by adsorption on an active solid surface such as charcoal, silica gel and molecular sieves.

In liquid-liquid chromatography the separation of components is based on the partition co-efficient which is a function of difference in distribution ratios of different components. Important members in this class are partition⁹ and paper chromatography. In the former, a column is filled with substances like silica gel, alumina or cellulose powder, and saturated with polar solvents like water and ethanol. Nonpolar solvents serve as mobile phases. A component having highest distribution ratio is eluted preferentially. In paper chromatography, components present as single molecules or ionic species are separated by elution

of solvent through the adsorbent i.e. thin layer of cellulose which is a part of paper such as Whatman filter paper No.1 or 2. The eluted spots are developed using suitable developing agents in different characteristic colours. The advance technique in this class is high pressure liquid chromatography (HPLC)¹⁰ in which a solvent under pressure is eluted through a column containing liquid phase coated ^{on} inert particles. The components are detected by refractive indices or UV method.

In gas-liquid chromatographic method¹¹, vapours of a sample are eluted through a column filled with particles of inert material on which thin film of liquid phase is coated; by mobile phase called as carrier gas. Components in the mixture are separated according to the partition co-efficient. The emerging vapours of enriched component are detected by a suitable device.

1.1.2 HISTORY

Several reports on techniques similar to gas chromatography appeared in the 1930's but the first publication as a separate technique appeared in 1946¹². The development in gas chromatography was accelerated due to the discovery made by James and Martin¹¹ based on the suggestions of Martin and Synge⁹. They also demonstrated it as a powerful and versatile analytical tool¹³. Philips et al.¹⁴ reported

usefulness of this technique over conventional chromatographic methods. Ray¹⁵ who is one of the pioneers in industrial applications of gas chromatography, reported separation of several organic compounds such as ketones, aldehydes, alcohols, esters, ethers, etc. By 1956 the technique was recognised all over the world¹⁶ and now-a-days it has been used in all the branches of chemistry.

1.1.3 THE INSTRUMENT (Fig. 1)

It essentially consists of following parts (Fig. 2).

- a) Carrier gas system
- b) Injector system
- c) Columns
- d) Detecting device
- e) Recorder

(a) Carrier gas system: It is a mobile phase in the chromatographic system. Gas under pressure is allowed to pass at a constant rate through the column via injector block and eluted out through the detector. Eventhough choice of a gas depends upon the availability and the cost, selectivity is mainly based on type of a detector to be used and precision of analysis. It should not react with the stationary phase and should be free from oxygen. Therefore purified and chemically inert gases having low molecular weights such as hydrogen, nitrogen and helium are used.

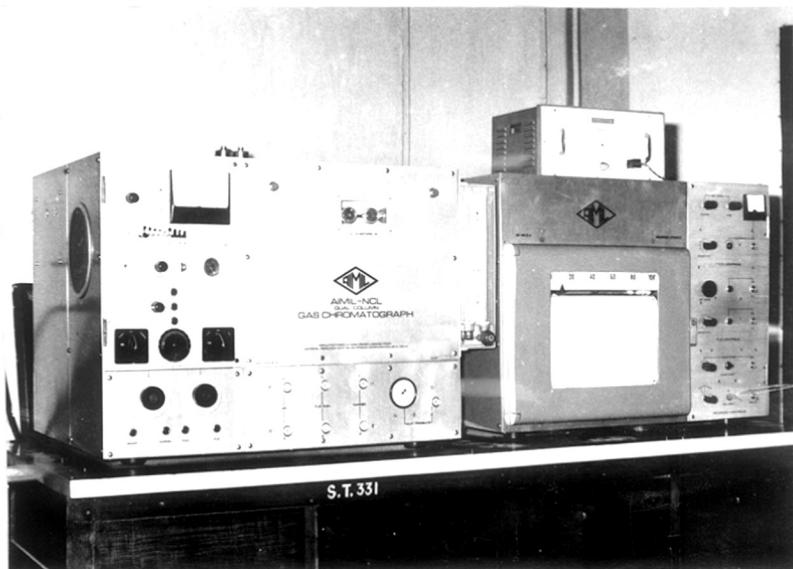
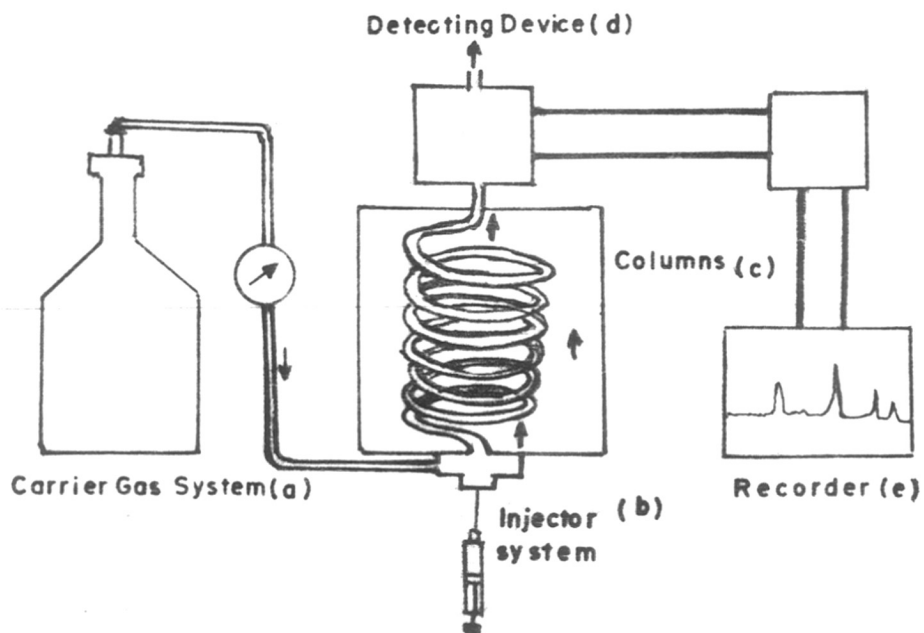


FIG. 1. GAS CHROMATOGRAPH (PHOTOGRAPH)



17
7

(b) Injector system: A sample injected by a syringe gets vapourised in a preheated metallic block and carried to column by the carrier gas. Many ancillary devices such as gas sampling device and pyrolyser are available. Sometimes on column injection is preferred where use of this part is avoided.

(c) Columns: Aluminium, copper, stainless steel or glass columns are in use. They are filled with material like celite coated with liquid stationary phase. Apart from conventional open tubular columns, glass capillary columns are also used for precision analysis¹⁷. Length and shape (coiled or U type) of columns vary according to the requirements. The columns are heated at a steady temperature in an oven. Solute vapours entering the column through the injector block gets partitioned between liquid stationary phase and mobile gas phase. Due to the continuous mass flow of gas, the enriched component fractions are eluted in the detector. Column is the important part of the instrument where separation of components takes place. Details about stationary phases are discussed in proceeding section.

(d) Detecting Device: Solute vapours are detected in this part. Working of the device is based on properties of solute such as thermal conductivity, ions formation, electron affinity, etc. Thermal conductivity detector (TCD), electron

capture detector (ECD) and flame ionisation detector (FID) are most common. Various types of detectors are discussed in proceeding section.

(e) Recorder: The detection of sample vapours is converted into an electrical signal which is amplified, and recorded by a potentiometric recorder. Now-a-days computerised print-out device (integrator) is in use.

1.1. 4 T H E O R Y

There are mainly three theories in practice to explain the separation phenomenon. They are rate theory¹⁸, plate theory¹⁹ and theory for capillary columns²⁰.

Plate theory^{9,19} is more simple. It is based on a hypothetical term 'theoretical plate'. It is defined as a section of the column in which vapours leaving the section has the composition that would be in equilibrium with average composition of liquid solute within the section. An enhanced separation of solute component between mobile and stationary phase is considered to be set at each plate. Thus the total separation depends upon separation at each plate and number of theoretical plates in the column.

Efficiency of a column is defined by a term 'Height Equivalent of the Theoretical Plate (HETP)'. It can be

obtained by a formula.

$$HETP = \frac{H}{D} \frac{L}{n} \text{ where } n = \text{theoretical plates}$$

L = length of column

and

$$n = 16 \left[\frac{t_R}{w} \right]^2 \text{ where } t_R = \text{retention time}$$

w = width of peak

Rate theory was explained on the basis of kinetic approach by Deemter¹⁸. The equation is

$$HETP = 2 \lambda dp + \frac{2 V D_{gas}}{u} + \frac{8 K'}{\pi^2 (1 + K')^2} \cdot \frac{df^2}{D_{liq}} \cdot u$$

The simplified form is

$$HETP = A + \frac{B}{u} + Cu \text{ where}$$

A = Multiple path effect

B = Molecular diffusion

C = Resistance to mass transfer

'A' can be reduced by reducing particle size, 'B' is proportional to diffusion co-efficient of the carrier gas. Therefore heavier gases like CO_2 tend to give best performance at low flow rate.

Keulmans²¹ has described comparative studies of both the theories. Rate theory is more realistic than plate theory.

Improvement in separation can be achieved using the data of the equation of rate theory²².

Performance of capillary columns can be expressed as

$$HETP = \frac{B}{u} + (C_L + C_G)u \quad \text{where}$$

C_L and C_G are resistances in the liquid and gas phase respectively.

Factors responsible for separation are: nature and flow of gas, temperature, length, type and internal diameter of column, nature and particle size of inert support, nature of stationary phase and type of detector.

1.1.5 SELECTIVE PARAMETERS

Among many parameters responsible for enhanced separation, stationary phases and detectors are highly selective due to numerous varieties available; according to the choice. They are described in this section.

(A) DETECTORS

Without a suitable and desired means of detection, the finest resolution of components would be useless. Therefore detector is very important in the chromatographic system.

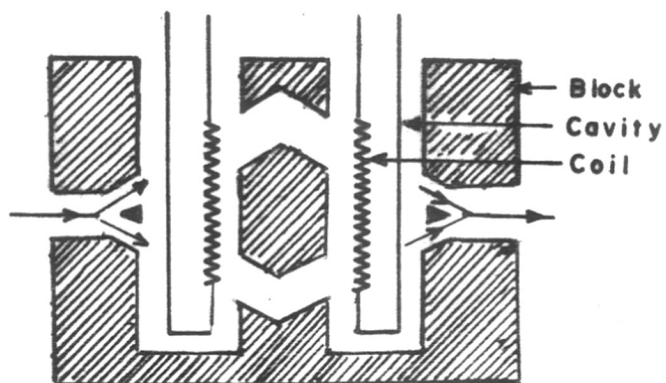
Popular detectors are described below.

(1) Thermal Conductivity Detector (TCD) (Fig. 3a): It was discovered by Shakespear²³. A very thin coil, fitted in the

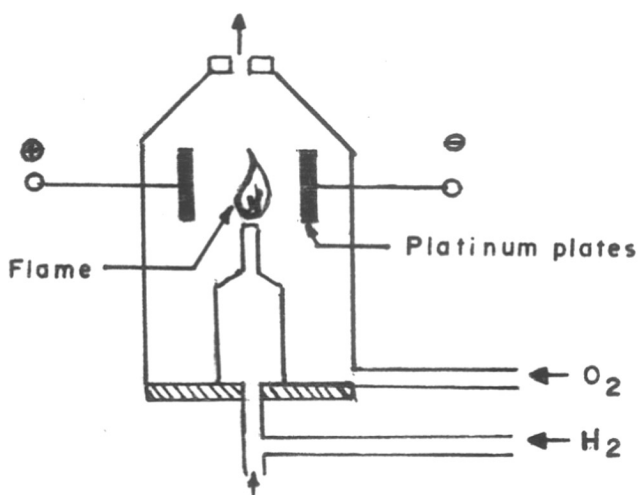
cavity in a metal box is heated electrically. It is connected to a bridge. An equilibrium of heat exchange between the gas and the block is set up and the bridge is balanced. When sample vapours enter the block, the equilibrium is disturbed causing imbalance in the bridge current and a signal is obtained. This is a very simple and most versatile detector²⁴.

(2) Flame Ionisation Detector (FID) (Fig. 3b): This sensitive detecting system was first suggested by Ryce and Bryce²⁵. An hydrogen flame is kept burning between two platinum electrodes. At high temperature the gas is ionised to set a steady current across the plates to which a steady potential is applied. When sample vapours enter the chamber, it burns forming ions which enhances the current multifold and supplied to the recorder.

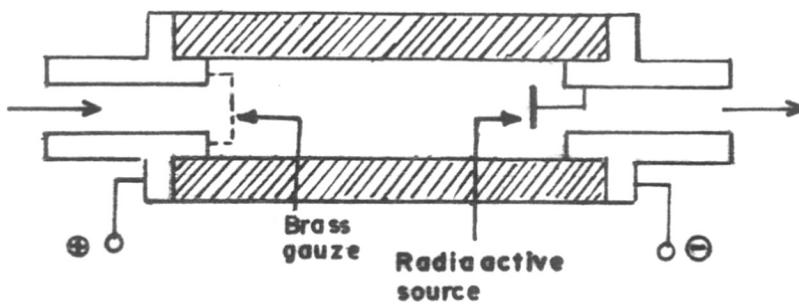
(3) Electron Capture Detector (ECD) (Fig. 3c): It was first reported by Pompeo and Otvos²⁶. This is one of the most sensitive detectors. It consists of two electrodes, one of them is with radio-active foil. Carrier gas molecules are ionised into long lived metastable atoms. The introduction of trace amount of organic impurities results in energy transfer through collision and travel to the electrode. The detector has sensitivity 2×10^{-12} moles²⁷.



a) Thermal Conductivity Detector



b) Flame Ionisation Detector



c) Electron Capture Detector

FIG. 3.

(4) Thermionic Detector (TID): It was developed by Kerman²⁸. Thermally ionised molecules of an alkali metal are present in the flame of hydrogen in the detector. If solute vapours contain phosphorus or halogens, an enhanced response is obtained. Nitrogen selective detector may also be prepared by proper choice of an alkali salt. The response of a compound containing ten carbon atoms and one phosphorus atom is 600 times greater than the response of conventional FID²⁹.

(5) Flame Photometric Detector (FPD): This sensitive detector is highly specific for phosphorus and sulfur over hydrocarbons. Hydrocarbons containing phosphorus or sulfur such as thiophene in benzene produce chemiluminescent species when combusted in a flame which is rich in hydrogen. When proper portion of the flame area is viewed by means of a fixed filter filter and photomultiplier tube or a spectrophotometer, they emit light of particular wave length. It was first described by Smitten³⁰. A suitable design has been reported by Brody and Chaney³¹. It is specially employed for pesticide residue analysis and to detect trace impurities of phosphorus and sulfur compounds^{27,32}.

(6) Electrochemical Detector: It is based on coulometric principle. It was first reported by Coulson and Cavanagh³³ for determination of halogens in combustible gas. A microgram quantity of the component ^{is} titrated with the internally

generated silver ion. A pesticide analyser using this detector was reported by Coulson et al.³⁴. Variety of coulometric detectors are available.

Many other detectors are also in use. As they have limited and specific uses, they have limited commercial value. Some of them are, palladium detector³⁵ based on permeability of alloy at high temperature, molecular weight detector³⁶ in which molecular weight is calculated by gas density balance, discharge detector³⁷ where presence of foreign component was detected by increase of current flow, flame emission detector³⁸, ultrasonic detector³⁹, helium ionisation detector⁴⁰.

(B) LIQUID STATIONARY PHASES

A choice of liquid stationary phase is made by its selectivity over other compounds, operational temperature and polarity. Polarity is defined as a retention ratio of a polar solute to that of one which is non-polar solute for which zero polarity is attributed⁴¹. Chovin and Lebbe⁴² have worked on polarity scale having butane as a standard. A system of retention indices was developed by Kovats⁴³ who predicted retention index on the basis of chemical structure of a solute. Rohrschneider⁴⁴ considered index difference of benzene as standard to characterise liquid phases. Tokacs⁴⁵ worked further to show that the interaction of solute and

stationary phase depends upon the quotients of their indices which is very useful term for computer programming. McReynolds⁴⁶ has recommended retention index difference of then standard substances-benzene, butanol, 2-pentanone, nitropropane, pyridine, 2-methyl-2-pentanol, 1-iodo-butane, 2-octyne, 1,4-dioxane and cis-hydrindane. Among many attempts to characterise polarity of stationary phases, system of Rohrschneider is most popular. These scales help for the selection of appropriate stationary phase for desired analysis. The scale shows that only a few phases are sufficient to carry out all types of analyses.

Liquid stationary phases are classified as non-polar, dilute, concentrated and specific stationary phases. A non-polar stationary phase has simple paraffinic linkages and useful for the analysis of hydrocarbons which are eluted in the order of increasing boiling points⁴⁷. Some important members in this class are squalane⁴⁸, Apizon greases particularly Apizon L, methyl silicones⁴⁹ (Fig. 4a) and surface bonded silicones⁵⁰.

Dilute stationary phases have non-polar structure with some polar grouping. It dissolves polar as well as non-polar solutes. It is the biggest class of phases. The dilute phases mainly include silicone derivatives like phenyl silicones, haloalkyl and cyanoalkyl silicones (Fig. 4b). Aromatic derivatives such as nitrobenzene, polymetaphenyl ether⁵¹ and

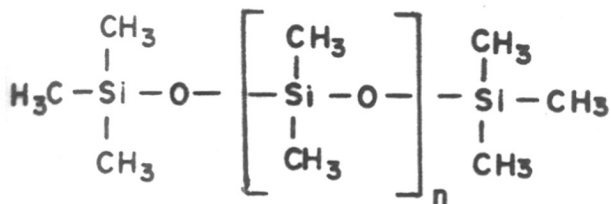
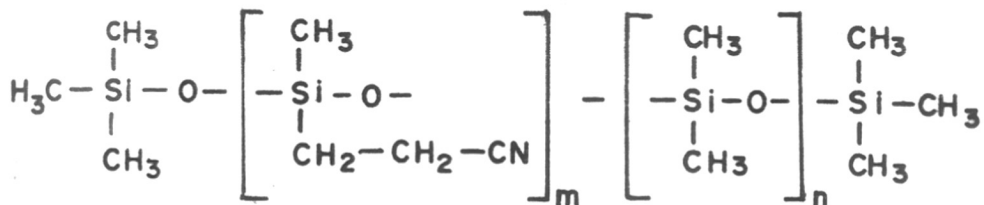
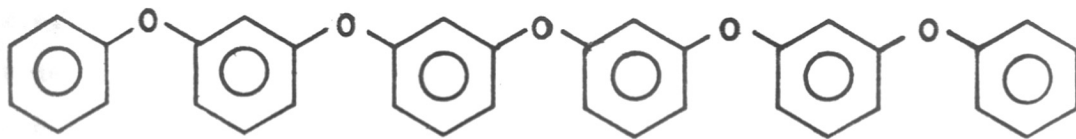
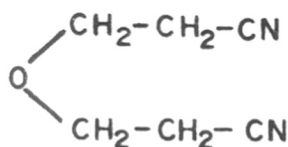
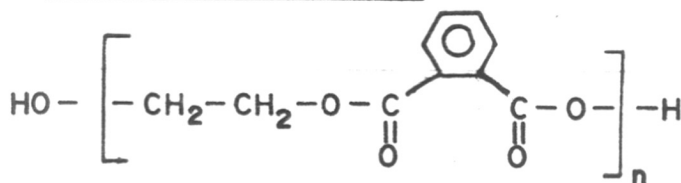
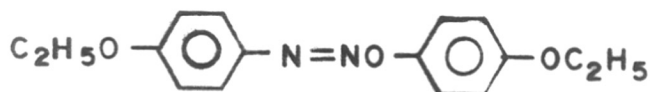
a) Methyl Siliconesb) Cyanoalkyl Siliconesc) 3,3' bis (m-phenoxyphenoxy) biphenyld) β, β' -Oxidipropionitrilee) Poly(ethylene glycol phthalate)f) p,p'-Azoxyphenetole

FIG. 4

3,3'-bis(m-phenoxyphenoxy) biphenyl⁵² (Fig. 4c) are also included in it. Esters of carboxylic, phosphoric acids: dinonyl and diphenyl phthalate, triphenyl phosphate are important in this class. Some selective phases included in this group are high molecular weight acids and alcohols like stearic acid, octadecyclic alcohol respectively.

The group of concentrated stationary phases have large proportion of molar volume, occupied by polar groups like -OH, -C≡N, halogens, etc. Water, diglycerol, d-sorbitol, acetyl butyl citrate, β,β' -oxydipropionitrile⁵³ (Fig. 4d) and triethanolamine are from this group. Formamide derivatives⁵⁴ are selective for isomeric separations. Most widely accepted phase is poly(ethylene glycol)⁶³ which is useful for all types of isomeric separations and variety of analyses. They are described elsewhere. Polyesters⁵⁶ prepared by condensing alcohols with dicarboxylic acids are also popular. Succinate⁵⁶, adipate⁵⁵ and phthalate⁵⁷ (Fig. 4e) polyesters are mostly in use.

Specific stationary phases form an important group. Silver nitrate with poly(ethylene glycol) in particular has very high selectivity⁵⁸. Trifluoroacetyl (TFA)-alcohol esters of optically active amino acids were used to separate amino acid enantiomers⁵⁹. Applicability of liquid crystals such as p,p'-Azoxyphenatole (Fig. 4f) has been reviewed by Kelker⁶⁰.

The phases were found to be very useful for isomeric separation⁶¹. Bentone-34⁶², which is prepared from a clay: bentonite and dimethyl dioctadecyl-ammonium salts are recommended for xylenes.

1.2 ORGANOPHOSPHORUS PESTICIDES

Practically pesticide is a general term which may be defined as a chemical substance used to kill harmful species to plants. The need of a pesticide has been necessitated when early civilization developed an art of cultivation and storage of foodgrains. Arsenic had been used to control garden insects in ancient China. In western countries sulfur, hellibore, light oil and tobacco were in use in pre-Christ era. Neem leaves have been utilised in storage of foodstuff since very old times in India.

For higher crop production and effective storage of surplus stock of foodgrains, more attention has been given to protect them from harmful species and the pesticide science has been evolutionised since then. Minerals like sulfur, phosphorus, lime and arsenic were tried at the very beginning. Then terpentine, whale oil, tar had been utilised. Synthetic compounds like ethylene oxide, methyl bromide, hydrocyanic acid, mercuric chloride and soap were used upto early forties. Naturally occurring pyrethrum, tobacco dust and other herbs were also widely used for this purpose.

By 1930 a new era in pesticide field has been started by introduction of synthetic organo-pesticides. Some of them are orthochlorobenzene, petanchlorophenol, beta-naphthol,

benzyl benzoate, azobenzene, nitrophenols, xanthenes, etc. Upto 1950 chlorinated pesticides were very exhaustively used all over the world as effective pest killers. Dichlorodiphenyltetrachloroethane (DDT) dominated the field during 2nd World War and post-war days. Gamma-benzene hexachloride, chlordane and toxaphene were also prominent among them. Due to highly toxic residue effects of long persistent chloro derivatives, they were slowly outdated.

A significant achievement in chemical warfare research in Germany during 2nd World War, is the development in organophosphates. Consequently a new class of pesticides i.e. organophosphorus pesticides has been evaluated. It has been described in detail as follows.

1.2.1 HISTORY

Phosphates are one of the essential constituents of protoplasm and play an important role in maintenance of life. Some of them are nucleic acids, nucleotides, co-enzymes, metabolic intermediates and phosphides. Many phosphorus derivatives are used in day-to-day life as lubricants, oil additives, plasticisers, pharmaceuticals and pesticides⁶⁴.

In 1905, Harden and Young⁶⁵ discovered the key role played by inorganic phosphates in alcoholic fermentation in which fructose diphosphate, a metabolic intermediate was isolated. Since then many organic phosphate esters have been

isolated from biological sources. Sir Todd and Cramer^{66,67} have studied phosphorylation reactions in naturally occurring esters. These esters are found to inhibit the activity of cholinesterases by phosphorylation at esteric site⁶⁸. Paraoxon, an oxo analogue of parathion was first demonstrated as synthetic anticholinesterase agent⁶⁹.

The relation between structure and activity of organophosphates was established for the first time by Helmstedt⁷⁰. Schrader⁷¹ suggested a general formula for compounds, having contact insecticidal activity. In 1941 a systemic insecticide, Schradan (OMPA) (Fig. 5a) along with other insecticides was introduced. Synthesis of parathion⁷² (Fig. 5b) was a great achievement in this field. Though it is highly toxic to mammals and insects, it helped in developing lesser toxic but promising derivatives. Some of them are Fenitrothion⁷³ (Fig. 5c) and chlorthion⁷⁴ (Fig. 5d). Other useful pesticides with low mammalian toxicity are Malathion (Fig. 5e) and Demeton (Fig. 5f).

1.2.2 CLASSIFICATION

Organophosphorus pesticides may be divided according to the potent groups in the structure. They are as follows:

1) Alkyl phosphates: Due to high mammalian toxicity and less stability this class has not been developed much. Very few compounds were prepared in this class. Paraoxon⁷⁴ (Fig. 6a) is one of them.

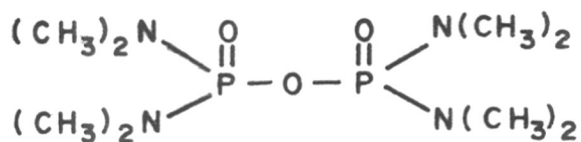
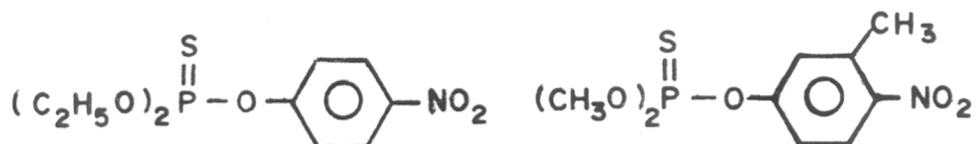
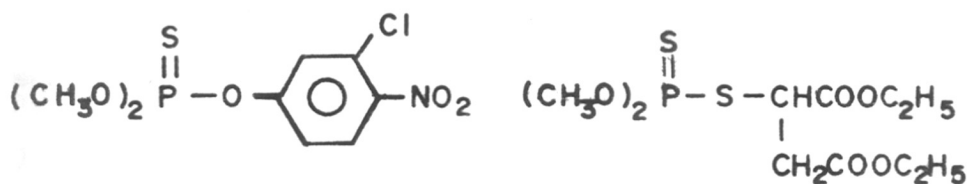
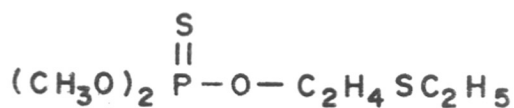
a) Schradan (OMPA)b) Parathionc) Fenitrothiond) Chlorthione) Malathionf) Demeton - O

FIG. 5.

- 2) Phosphorothionates: They are thiono ($P = S$) derivatives. Many useful pesticides belong to this class. They are stable and also have low mammalian toxicity. Some of the members are Phoxim, Salithion⁷⁶ (Fig. 6b), and Fenitrothion⁷³ (Fig. 5c).
- 3) Phosphorothiolates: Members of this class have $P = O$ and $P - SR$ linkages. Demeton-S⁷⁴ (Fig. 6c) is one of them.
- 4) Phosphoramidic acid derivatives: They have $P-N$ as important feature. Avenin⁷⁷ (Fig. 6d) belongs to this class.
- 5) Phosphorofluoridic acid derivatives: Compounds having $P-F$ and $P=O$ bonds are included in this group. Very few compounds of this class were employed for agricultural use. One of the members is Sarin⁷⁸ (Fig. 6e).
- 6) Pyrophosphoric acid derivatives: Very active studies were carried out at the beginning. The pesticides have less practical value except Schradan⁷⁴ (Fig. 5a).
- 7) Phosphonates: The structural feature of this class is $P-C$ bond with variable organic moieties. These compounds are used as insecticides, fungicides and plant growth regulators. Some insecticides in this class are EPN and trichlorfon⁷⁹ (Fig. 7a).
- 8) Phosphinate Esters: Series of $P-C$ linkages are present in this group of pesticides. Only one marketed pesticide i.e. Agvitor⁸⁰ (Fig. 7b) is known from this class.

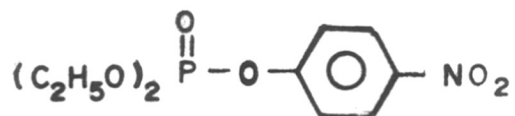
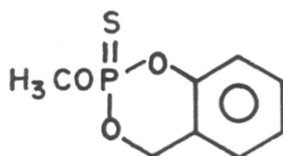
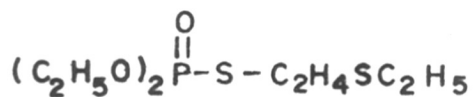
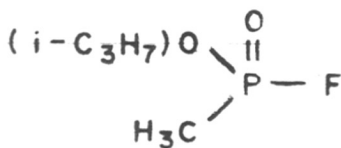
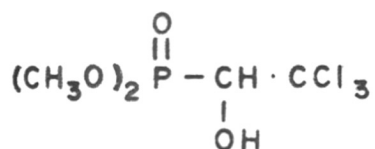
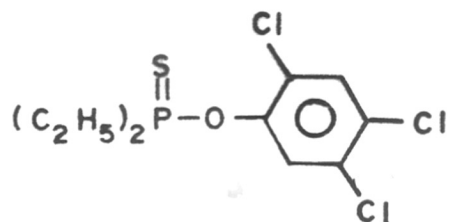
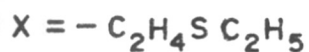
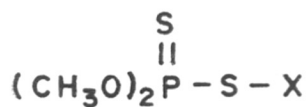
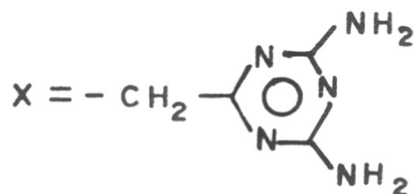
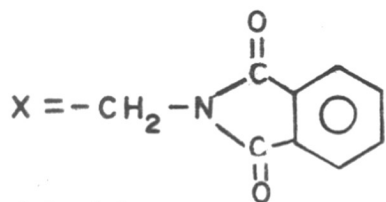
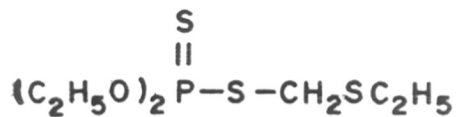
a) Paraoxonb) Salithionc) Demeton-Sd) Avenine) Sarin

FIG. 6

a) Trichlorfonb) Agvitorc) Thiometond) Menazonee) Imidanf) Dimethoateg) Phorate

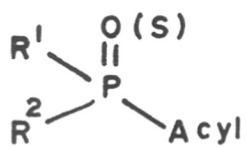
9) Phosphorothiolothionates: Members of this class are widely accepted due to low mammalian toxicity, low persistence and high insecticidal activity. More than forty compounds of this group were introduced in market. The compounds have both thiono ($P=S$) and thio ($P-SR$) linkages. The thio group can be further classified as carboxyesters, amines, heterocycles, sulfides, sulfoxides and amides. Some of the popular members are thiometon⁷⁴, menazone⁸¹, imidan⁸², dimethoate⁷⁵, phorate⁸³ (Fig. 7c-g) and Malathion⁷⁴ (Fig. 5e).

1.2.3 MODE OF ACTION

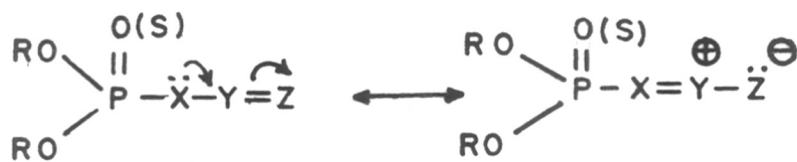
The pentavalent phosphorus esters or anhydrides have phosphorylating as well as alkylating properties. The insecticidal activity and mammalian toxicity of a compound is attributed due to the phosphorylation of acetylcholinesterase at the esteric-site⁶⁸ i.e. serin hydroxyl group. The enzyme inhibition reaction is similar to the alkaline hydrolysis. It also depends upon pH and temperature.

1.2.4 STRUCTURE ACTIVITY RELATIONSHIP

Schrader^{71,74} proposed a general formula in 1950 (Fig. 8a) It was modified by Clark⁸⁴ in 1964. According to the rule in P-XYZ system of a pesticide, XYZ may be H,C,N,O,S but Z should



a)



b)

FIG. 8.

be such that it is capable of accommodating electrons from the P-X bond (Fig. 8b). The most widely accepted relationship is between inhibition constant (K_1) and rate of hydrolysis⁸⁵. If phosphorus atom is attached with electron withdrawing group such as OR, OPh, SR and F, the activity is enhanced as the compound hydrolyses fast.

Some compounds are potent insecticides because of the presence of certain groups. The thiono derivatives are active by virtue of S-alkylation and also conversion to oxo analogue. For example paraoxon (Fig. 6a) is 10,000 folds active than parathion (Fig. 5b), while S-methyl analogue of malathion (Fig. 5e) is more active than the parent compound by the factor of 33. Based on electron donating capacity, amides are active in the order $-NH_2 > -NHR > -NR_2$ but no such rule can be predicted due to their abnormal behaviour.

Even though several enol phosphates are very potent anticholinesterase agents, the activity is not accounted due to hydrolysability but due to protonation. However, para substituted acetophenoxime phosphates are active due to the presence of electron releasing groups⁸⁶.

Steric factor is also very important for inhibition action. Molecules with smaller substituents are more active than one which has bulky substituents. For example methyl phosphate (Salioxon) has insecticidal activity but phenyl

phosphate is inactive. The compounds having structural resemblance with acetylcholine are found to be very active. The possible mechanism is sort of an antagonist behaviour e.g. Fenitrothion (Fig. 5c). Some pesticides show activity due to asymmetric phosphorus atom as acetylcholinesterase enzyme activity is highly stereospecific. Inhibition shown by Sarin (Fig. 6e) is due asymmetric phosphorus atom.

Even though many rules exist to define the relationship, no perfect prediction can be made about an activity of a compound. Therefore emphasis is given on preparation of compounds in large numbers and testing them.

1.2.5 METABOLISM

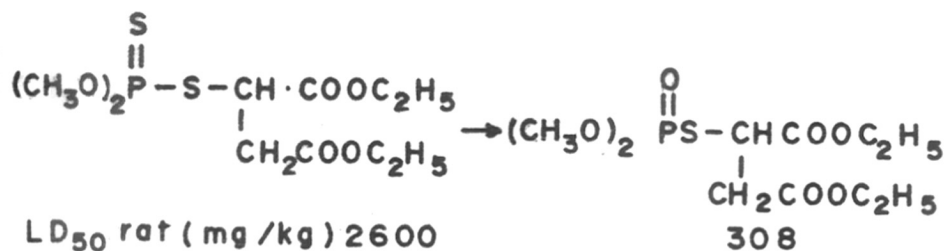
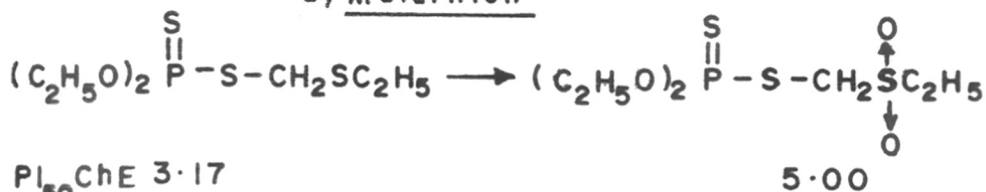
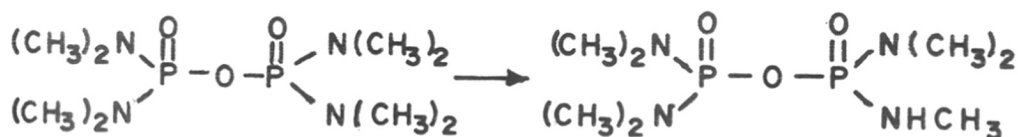
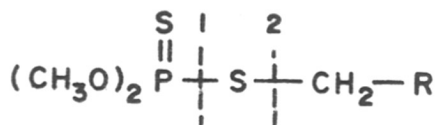
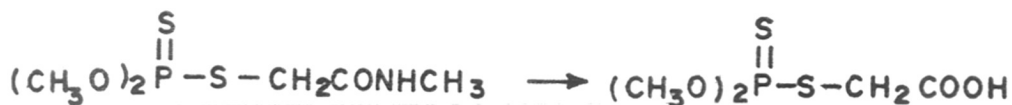
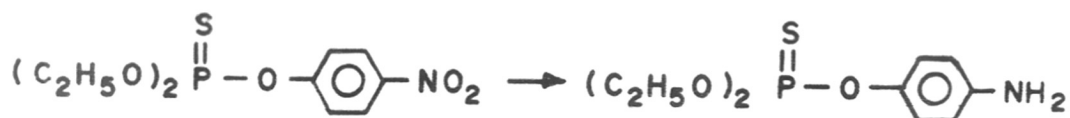
It is defined as chemical change occurred in foreign compounds in the body of organisms. As pesticides are less polar and lipophilic, they readily enter into the pests through the skin. Mainly two types of transformations take place. They are activation and detoxication. The former change means conversion to more active and polar form which is responsible for the increase of anticholinesterase activity. In detoxication process, the molecule is converted to lesser toxic forms so that it can be excreted out safely. Both the reactions take place simultaneously

and their competence determines toxicity of a pesticide.

The reactions causing activation are as follows.

Oxidative desulfuration of thiophosphoryl sulfur atom yields corresponding oxo analogue. The product is multifold toxic than the parent compound (Fig. 9a). Oxidation of thioether moiety into sulfoxide and sulfones has been observed in vivo in plants, mammals and insects. Sulfones have highest anticholinesterase activity⁸⁷ (Fig. 9b). Oxidative demethylation takes place in amido group of a pesticide. For example in oxidised Schradan, activity increases to 10^5 (Fig. 9c)⁸⁸. Aliphatic hydroxylation and some rearrangement reactions are also responsible for activation of the pesticides.

The metabolic detoxication is mainly due to the cleavage of phosphorus ester bond. Some of the reactions are oxidative dearylation, enzymatic hydrolysis and O-dealkylation. In phosphorothiolothionates two types of cleavages take place. One is at P-S bond and the other is at S-C linkage. In most of the organisms both types of cleavages occur, but S-C fission is predominant (Fig. 9d), e.g. menazone⁸⁹ (Fig. 7d), dimethoate⁹⁰ (Fig. 7f) and imidan⁹¹ (Fig. 7e). Hydrolysis of amide and reduction of nonphosphorus moiety also detoxicate the pesticides. Amide in dimethoate hydrolyzes to corresponding acid⁹⁰ (Fig. 9e), while nitro group in parathion reduces to amine⁹² (Fig. 9f).

a) Malathionb) Phoratec) Schradand) Phosphorothiolothionatese) Dimethoatef) Parathion

1.2.6 SIDE EFFECTS

Many organophosphorus pesticides show a chronic neurotoxicity, affecting peripheral nervous system which leads to paralysis⁹⁵. They also show teratogenic signs which include lack of feathers, parrot beak, shortening and deformation of legs, etc.⁹⁴. Enzymes other than cholinesterase are also susceptible to organophosphates, producing synergistic activity⁹⁵. In certain cases complex changes of the molecular architecture of membrane bound enzymes may be induced. Due to the presence of organophosphorus compounds, normal plant metabolism is affected. It causes abnormality in coloration of fruits, ripening period and smell of fruits⁹⁶.

1.3 PRESENT INVESTIGATIONS

In the National Chemical Laboratory, Pune, a large group of scientists is currently engaged in research and process development work on various types of pesticides. Processes for several organophosphorus pesticides have been developed. During these developments and transfer of technology, number of problems related to analytical methods and effluents had to be tackled. Therefore several new stationary phases were synthesised and employed for gas chromatographic analysis as it is the most precise and quick analytical technique. Alternative analytical methods were also devised where gas chromatography is not suitable. Simultaneously various chemical methods for degradation of the organophosphorus pesticides to nontoxic forms were studied in order to evolve proper effluent treatment processes for the same.

The present investigations described in the following chapters is the part of such research work undertaken by the author.

REFERENCES

1. Day, D.T., Science, 17, 1007 (1903).
2. Tswett, M., Ber.Deut.Botan.Gas, 24, 316 (1906).
3. Kuhn, R. and Lederer, E., Chem.Ber., 64, 1349 (1931).
4. Wilson, J.N., J.Am.Chem.Soc., 62, 1583 (1940).
5. Heftman, E., Ed., Chromatography, Reinhold, New York, (1967).
6. Beyerinck, M.W., Z.Phys.Chem. 3, 110 (1889).
7. Way, J.T., J.Roy.Agric.Soc., Engl., 11, 68 (1850).
8. Cremer, E. and Prior, F., Z. Electrochem., 55, 66 (1951).
9. Martin, A.J.P. and Synge, R.L.M., Biochem. J., 35, 1358 (1941).
10. Huber, J.F.K. and Hulsman, J.A.R.J., Anal.Chem.Acta., 38, 305 (1967).
11. James, A.T. and Martin, A.J.P., Biochem. J., 50, 679 (1952).
12. Claesson, S., Arkiv. Kemi Mineral Geol., 23(A), 1 (1946).
13. James, A.T. and Martin, A.J.P., Analyst, 77, 915 (1952).
14. Griffiths, J., James, A.T. and Phillips, C., ibid., 77, 897 (1952).
15. Ray, N.H., J.Appl.Chem., 4, 21 & 82 (1954).
16. Desty, D.H., Ed., Vapor Phase Chromatography, Butterworths, London (1957).
17. Martin, A.J.P., Gas Chromatography, 2 (1957).
18. van Deemter, J.J., Zuiderwag, F.J. and Klinkenberg, A., Chem.Eng. Sci., 5, 271 (1956).

19. Glueckauf, E., Trans Faraday Soc., 51, 34 (1955).
20. Golay, M.J.E., Gas Chromatography, Butterworths, London, 139 (1960).
21. Keulemans, A.I.M. and Kwates, A., Proc. 4th World Petrol Congress, Colombo, Rome, 273 (1955).
22. Scott, R.P.W., Gas Chromatography, D.H. Desty, Ed., Butterworths, London, 6 (1958).
23. Shakespear, G.A., Proc.Phys.Soc.(London), 33, 163 (1921).
24. Littlewood, A.B., Gas Chromatography, 2nd Ed., Academic Press, New York, 1966.
25. Ryce, S.A. and Bryce, W.A., Nature, 179, 54 (1957).
26. Pompeo, D.J. and Otvos, J.W., U.S.Patent 2,641,710 (1953).
27. Stevens, R.K., O'Keeffe, A.E. and Ortman, G.C., Environ.Sci.Tech., 3, 652 (1969).
28. Karmen, A. and Giuffrida, L., Nature, 201, 1204 (1946).
29. Giuffrida, L., J.Assoc.Offic.Agr.Chemists, 47, 293 (1964).
30. van der Smissen, Carl Ernst, U.S. Patent 3,213,747 (1965).
31. Brody, Sam S. and Chaney, J.E., J.Gas.Chromatogr., 4(2), 42 (1966).
32. Stevens, R.K., J.Assoc.Offic.Agr.Chemists, 50, 123 (1967).
33. Coulson, D.M. and Cavanagh, L.A., Anal.Chem., 32, 1245 (1960).
34. Coulson, D.M., Cavanagh, L.A. DeVries, J.E. and Walther, B., J.Agric.Food Chem., 8, 399 (1960).

35. Lorelock, J.E., Simmonds, P.O., Shoemaker, G.R.
and Rich, S., J.Chromatogr. Sci., 8, 452 (1970).
36. Paul, D.G. and Umbreit, G.R., Research/Development, May (1970).
37. Hampton, W.C., J.Gas Chromatogr., 3, 217 (1965).
38. Grant, D.W., Gas Chromatography, D.H. Desty, Ed.,
Butterworths, London (1958).
39. Noble, F.W., Abel, K. and Cook, P.W., Anal.Chem., 36,
142 (1964).
40. Bourke, P.J., Dawson, R.W. and Denton, W.H.,
J.Chromatogr., 14, 387 (1964).
41. Maier, H.J. and Karpathy, O.C., *ibid.* 8, 308 (1962).
42. Chovin, P. and Lebbe, J., Separation Immediate et
Chromatographie, 1961, GAMS, Paris, 90 (1961).
43. Kovats, S.Z., E., Helv.Chim.Acta, 41, 1915 (1958).
44. Schomburg, G., J.Chromatogr., 14, 157 (1964).
45. Takacs, J., Szentirmai, Zs., Molnar, E.B. & Kralik, D.,
ibid., 65, 121 (1972).
46. McReynolds, O.W., J.Chromat.Sci. 8, 685 (1970).
47. Eggertsen, F.T. and Groennings, S., Anal.Chem., 30,
20 (1958).
48. Data Sub-committee of the G.C. Discussion Group of the
Institute of Petroleum, J.Gas Chromatogr., 4, 1 (1966).
49. Riedmann, M., Z. Anal.Chem., 260, 341 (1972).
50. Abel, E.W., Pollard, F.H., Uden, P.C. and Nickless, G.,
J.Chromatogr., 22, 23 (1966).

51. Musav, I.A., Sanin, P.I., Pakhomov, U.P. and Kurashova, E.H., *Neftekhimiya*, 9, 914 (1969).
52. Hammann, W.C., Schisla, R.M., Adams, J.S. & Koch, S.D., *J.Chem.Engg.Data*, 15, 352 (1970).
53. Tenney, H.M., *Anal.Chem.*, 30, 2 (1958).
54. Keulemans, A.I.M., Kwantes, A. and Zaal, P., *Anal.Chim.Acta*, 13, 357 (1955).
55. Craig, B.M. and Murty, N.L., *J.Am.Oil Chem.Soc.*, 36, 549 (1959).
56. Orr, C.H. and Callen, J.E., *J.Am.Chem.Soc.*, 80, 246 (1958).
57. Jones, C.E.R., ref. 20, page 401.
58. Bradford, B.W., Harvey, D. and Chakley, D.E., *J.Inst.Petroleum*, 41, 80 (1955).
59. Gil-Av, E., Feibush, B. and Charles-Singler, R., *Tetrahedron Lett.*, 10, 1009 (1966).
60. Kelker, H. and von Schivizhofften, E., *Advances in Chromatography- J.C. Giddings and R.A. Kelker*, ^{Ed.} 6, Marcel Dekker, New York, 247 (1968).
61. Kelker, H. and Winterscheidt, H., *Z.Anal.Chem.*, 220, 1 (1966).
62. White, D. and Cowan, C.T., *Trans. Fraday Soc.*, 55, 557 (1958).
63. Leary, J.J., Justice, J.B., Tsuge, S., Lowry, S.R., and Isenhour, T.L., *J.Chromat.Sci.*, 11, 201 (1973).

64. Fisher, E.B. and Van Vazer, J.R., Uses of Organophosphorus Compounds in Phosphorus and its Compounds, Vol. II, Van Vazer, J.R., Interscience, 1897 (1981).
65. Harden, H., Alcoholic Fermentation, Lognmans, 42 (1932).
66. Todd, A., Proc.Natl.Acad.Sci., 45, 1389 (1959).
67. Cramer, F., Angew.Chem., 72, 236 (1960).
68. Balls, A.K. and Jansen, E.F., Adv.Enzymol., 13, 321 (1952).
69. Gage, J.C., Biochem. J., 54, 426 (1953).
70. Holmstedt, B., Structure-Activity Relationship of Organophosphorus Anticholinesterase Agents in Cholinesterase and Anticholinesterase Agents, Springer-Verlag, 428 (1963).
71. Schrader, G., Die Entwicklung neuer Insektizide auf Grundlage Organischer Fluor-und Phosphor-Verbindungen, Monographie Nr. 62 zu Angewandte Chemie und Chemie-Ingenieur-Technik, Verlag Chemie, Weinheim (1952).
72. Schrader, G. and Kukenthal, (Farbenfabriken Bayer, A.G.) D.B.P. 811, 574 (1948).
73. Nishizawa, Y., Bull.Agric.Chem.Soc., Japan, 24, 744 (1960).
74. Schrader, G., Die Entwicklung neuer insektizider Phosphorsaure-Ester, Verlag Chemie, Weinheim, 281 (1963).
75. Cassaday, J.T., Hoegberg, E.I. and Gleissener, B.D., U.S. Patent 2,494,283 (1948).

76. Eto, M., Kinoshita, Y., Kato, T. and Oshima, Y.,
Agric.Biol.Chem. (Tokyo), 27, 789 (1963).
77. Melnikov, N.N., Residue Rev., 36, 303 (1971).
78. Saunders, B.C., Some Aspects of the Chemistry and Toxic
Action of Organic Compounds containing phosphorus and
Fluorine, Cambridge University Pres, London (1957).
79. Barthel, W.F., Giang, P.A. and Hall, S.A.,
J.Am.Chem.Soc., 76, 4186 (1954).
80. Schrader, G., Lorenz, W., Coelln, R. and Schlor, H.,
Belg.Patent 576,811 (1959).
81. Overberger, C.G., Michelotti, F.W. and Carbateas, P.M.,
J.Am.Chem.Soc., 79, 941 (1957).
82. Fancher, L.W., U.S. Patent 2,767,194 (1956).
83. Clark, E.L., Johnson, G.A. and Mattson, E.L.,
J.Agric.Food Chem., 3, 834 (1955).
84. Clark, V.M., Hutchinson, D.W., Kirby, A.J. and Warren, S.G.,
Angew.Chem.Int.Ed., 3, 678 (1964).
85. Aldridge, W.N. and Davison, A.N., Biochem. J., 51,
62 (1952).
86. Fukuto, T.R., Metcalf, R.L., Jones, R.L. and Myers, R.O.,
J.Agric.Food Chem., 17, 923 (1969).
87. Bowman, J.S. and Casida, J.E., *ibid.*, 5, 192 (1957).
88. Tsuyuki, H., Stahmann, M.A. and Casida, J.E.,
ibid., 3, 922 (1955).
89. Gage, J.C., Food Cosmet.Toxicol., 5, 349 (1967).

90. Morikawa, O. and Saito, T., Botyu-Kagaku, 31,
130 (1966).
91. McBain, J.B., Menn, J.J. and Casida, J.E.,
J. Agric. Food Chem., 16, 813 (1968).
92. Ahmed, M.K., Casida, J.E. and Nichols, R.E.,
ibid., 6, 740 (1958).
93. Smith, M.I., Elvove, E. and Frazier, W.H., U.S. Public
Health Rep., 45, 2509 (1930).
94. Roger, J.C., Upshall, D.G. and Casida, J.E.,
Biochem. Pharmacol., 18, 373 (1969).
95. Ohkawa, H. and Eto, M., Agric. Biol. Chem. (Tokyo), 33,
443 (1969).
96. Gabbiks, J., Bantung-Jurilla, M. and Friedmann, L.,
Proc. Soc. Exp. Biol. Med., 125, 1002 (1967).

●

CHAPTER TWO

POLY [ETHYLENE GLYCOL] DI-CHLOROACETATES
AS
STATIONARY PHASES
FOR
GAS CHROMATOGRAPHY

●

2·1 INTRODUCTION

2·2 EXPERIMENTAL

2·3 RESULTS AND DISCUSSION

2·4 APPLICATIONS

●

2.1 INTRODUCTION

2.1.1 THE PHASE: POLY (ETHYLENE GLYCOL)

Many times polymers are used as stationary phases in gas chromatography. Among them, poly(ethylene glycol) phases¹ (Fig. 1) top the list. They are prepared by polymerisation of ethylene oxide².

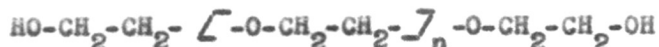


Fig. 1

Variety of polymers with different molecular weights could be obtained in the process depending upon the number of molecules combined. They are separated and named according to the range of molecular weights such as PEG-400, 1000, 1500 etc.

The phase is available in market under numerous trade names: polyethylene glycol 400, 600, 1600, 2000, 6000; carbowax series (Analabs); Ucon and carbowax series (Union Carbide Chemical Co.).

All poly(ethylene glycols) possess good polarity as stationary phases. They occupy intermediary positions in the table of McReynold's constants³.

The phase has etheric linkage between ethylene molecules, and hydroxyl groups at terminal positions. Two types of centres work as donors to solutes in the phase: the terminal hydroxyl oxygen and etheric oxygen. They principally determine retention of solute samples on the stationary phase¹. Hydrogen bonding remains to be the main attractive force for retention of solutes. The retention index found to be co-linear with hydroxyl concentration of the phase^{5,39}. So molecular weight of the phase greatly affects retention of a component. Consequently there is a wide choice of selectivity of these phases for appropriate analysis.

At the beginning of the development of gas chromatography, some polyglycols were evaluated for analytical applications by Adlrod⁴. Variety of compounds namely aliphatic and aromatic hydrocarbons, n-alkanols were analysed by him. Eventhough exhaustive use of polyglycols is being made, until 1974 critical examination of the phase remained to be evaluated. Castello⁵ thoroughly studied nature of the phase and derived many relations between characteristics of the phase and retention data. Some of them are; linearity of $\log V_g$ with number of carbon atoms in C_1-C_8 alcohols⁶, effect of molecular weight and temperature, calculations of activity co-efficient (γ). Chemistry of polyglycols also has been reported by Persinger²⁷.

Poly(ethylene glycol) phases are used for variety of analyses. Some of them are discussed here from the point of view of interactions between solutes and solvents.

As hydrogen bonding is mainly responsible for retention, there is no significant difference in behaviour of oxygenated compounds like aldehydes, ketones and ethers. Elution of particularly low molecular weight compounds is based on the order of boiling points⁷.

Retention of mainly aliphatic hydrocarbons is explained on the basis of inductive effect. Actually very small influence is exerted by methylene groups in the phase. The order of elution depends upon variation in inductive forces between the solvent and the solute. Adlord⁴ analysed aliphatic and aromatic hydrocarbons while Durrett⁸ has studied those hydrocarbons which pertain to aviation gasoline. At the latest n-alkanes and alkanols were analysed on carbowax bonded phase⁹. ^{hydrocarbons} Aromatic differ from aliphatic hydrocarbons in characteristic delocalization of electrons. This causes lessening of inductive effect in toluene and xylenes. So no pronounced difference among xylene isomers has been observed¹⁰. On the other hand, halogens have strong inductive effect. Additionally mesomeric effect (+M) imparts a partial double bond character to C-X bond. Consequently stronger interactions occur in elution of chloro-compounds^{6,11}.

Hydrogen atoms of amino groups are good donors which interact with the phase to retain amines. As retention wholly depends upon availability of amino hydrogen atoms, tertiary amines are eluted fast due to non-availability of hydrogen atoms. Therefore amines are selectively separated and eluted in the order as tertiary, secondary and primary¹².

Among variety of compounds, alcohols are mainly analysed on polyglycols. Number of workers have reported wide applicability of this phase. Smith⁷ has analysed alcohols with low molecular weights. Almost in the same period, Raupp¹³ critically studied the phase for the analysis of alcohols and recommended the same. Some of the other reports mainly deal with C₁ to C₅ alcohols^{4,5,9,11,14}. The phase also found to be useful to analyse volatile compounds from excess of alcohol¹⁵.

The phase is widely accepted for commercial use due to the characteristic behaviour. It is extensively used to analyse compounds in aqueous solutions such as blood¹⁶, wines and spirits¹⁷, urine¹⁸, pharmaceutical preparations¹⁹. They are also found useful in separation of isomers like carveols and carvomenthols²⁰. Cigarette smoke analysis²¹ has been found to give better results with this phase.

Strong bases can be separated using the same in capillary columns²². Additionally, acids also resolve efficiently on this phase when prior treatment of phosphoric acid is given. Many straight chain acids along with substituted acids were analysed on poly(ethylene glycols)²³.

2.1.2 DERIVATIVES OF THE GLYCOLS

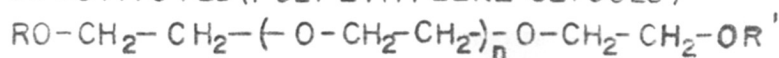
Many attempts were made to increase applicability of the phase by modifying its structure. Treatment of the free hydroxyl groups with different reagents yielded more polar and stable stationary phases²⁴⁻²⁶ (Fig. 2). Variation in selectivity and applicability of the phase can be achieved by this method. It has been found that substitution by long chain molecules favours the retention of hydrocarbons in comparison of alcohols. On the other hand, condensation of acids like tosyl, salicylic, terephthalic and glutaric acids results in phases which favour the retention of alcohols. Unfortunately very less theoretical information is available pertaining to the work.

2.1.3 PRESENT INVESTIGATIONS

As hydrogen bonding is mainly responsible for retention phenomenon in poly(ethylene glycol) and its derivatives, it is greatly affected by inductive effect of neighbouring groups.

NO	R	R'	Nature Of substituted Comp.
1	$C_{16}H_{33}O -$	$HO -$	Poly(ethylene glycol) hexa-decylether 47
2	$C_9H_{19} - \text{C}_6\text{H}_4 - O -$	$HO -$	Poly(ethylene glycol) nonyl-phenylether (Tergitol (NPX))
3	$C_{17}H_{35}COO -$	$HO -$	Poly(ethylene glycol) monostearate (Ethofat 60/25)
4	$CH_3 -$	$CH_3 -$	Methoxypoly(ethylene glycol)
5	$CH_3 - \text{C}_6\text{H}_4 - SO_3 -$	$CH_3 - \text{C}_6\text{H}_4 - SO_3 -$	Poly(ethylene glycol) ditosylate
6	$\text{C}_6\text{H}_4(\text{OH})COO -$	$\text{C}_6\text{H}_4(\text{OH})COO -$	Poly(ethylene glycol) disalicylate
7	$HOOC - \text{C}_6\text{H}_4 - COO -$	$HOOC - \text{C}_6\text{H}_4 - COO -$	Poly(ethylene glycol) diterephthalate
8	$C_{17}H_{33}COO -$	$C_{17}H_{33}COO -$	Poly(ethylene glycol) dioleate
9	$\text{O}_2\text{N} - \text{C}_6\text{H}_3(\text{O}_2\text{N})COO -$	$\text{O}_2\text{N} - \text{C}_6\text{H}_3(\text{O}_2\text{N})COO -$	Poly(ethylene glycol) bis 3,5-dinitrobenzoate
10	$HOOC - \text{C}_6\text{H}_3(\text{NO}_2)COO -$	$HOOC - \text{C}_6\text{H}_3(\text{NO}_2)COO -$	Poly(ethylene glycol) di-2-nitroterephthalate (FFAD)
11	$\text{O}_2\text{N} - \text{C}_6\text{H}_3(\text{O}_2\text{N})COO -$	$C_{17}H_{35}COO -$	Poly(ethylene glycol) 3,5-dinitrobenzoate monostearate
12	$HOOC - (CH_2)_3 - COO -$	$HOOC - (CH_2)_3 - COO -$	Poly(ethylene glycol) diglutarate

FIG. 2. SUBSTITUTED (POLY ETHYLENE GLYCOLS)



Therefore it may be interesting to prepare the derivatives which possess groups that can exert strong inductive forces. Therefore acetate, monochloroacetate, dichloroacetate and trichloroacetate derivatives were proposed. They possess number of chlorine atoms in increasing order and number of hydrogen atoms in decreasing order. So they are typical models to study influence of inductive forces. Therefore it was thought to condense chloroacetic acids with poly (ethylene glycol) and to evaluate them by analysing various types of organic compounds. Carbowax-20M was chosen because it is the most preferred phase among poly (ethylene glycols)²⁷.

2.2 EXPERIMENTAL

Details of raw materials, methods of preparation of phases, instrumental parameters and retention data have been described in this part.

2.2.1 RAW MATERIALS

Carbowax-20M: White solid, varian aerograph WalHut Greek/California (Cat. No.82-1115), m.p.50-55°C.

IR : 3580, 1970, 1760, 1630, 1600, 1345, 925, 825, 713 cm^{-1}

Elemental Analysis : C, 54.94%; H, 9.34%.

Acetic Acid: Glacial, Analytical grade, Poona Chemical Laboratory, Pune (India), Assay (Acidimetric) 99.6%, b.p.115°C-119°C.

Monochloroacetic acid: BDH, 99%, Product No.27667, Batch No.BAW8/450,3109, b.p.189°C.

Dichloroacetic acid: Koch-Light Laboratories Ltd., Coinbrook Bucks, England (Batch No.79456), b.p.192°C-194°C.

Trichloroacetic acid: It was available in laboratory stock as white crystalline material in sealed tube, m.p.58°C.

Purity of above acids was examined as follows. They were esterified using diazomethane and the esters were

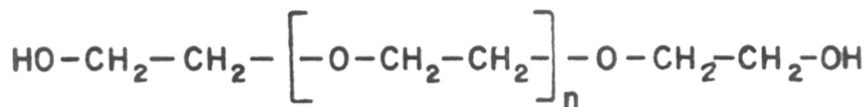
analysed by gas chromatography (Carbowax-20 M (5%) on chromosorb W, column temp. 80°C , thermal conductivity detector at 250°C). All samples were recorded as single peak pure compounds ($>99\%$).

2.2.2 METHOD OF PREPARATION (Fig. 3)

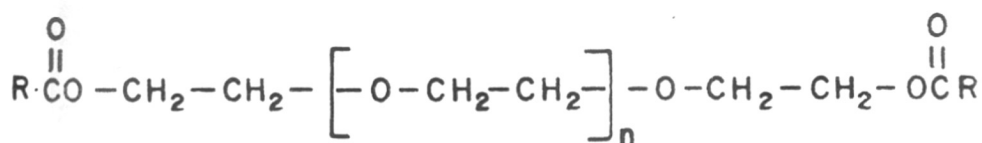
Carbowax-20M (1 g) was added to acetic acid (10 ml) in a 50 ml round bottom flask. Para-toluene sulphonic acid (PTS, 0.1 g) was added to it. The mixture was heated at 120°C for 2 hr and at 140°C for further 4 hr. The dark syrupy liquid was cooled and diluted with chloroform (100 ml). The organic layer was washed with water (100 ml) initially and with aqueous solution of sodium carbonate (10%, 300 ml) in three portions. Then it was washed with water till washings were neutral to litmus. The chloroform layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. A brown coloured material was obtained.

It was characterised by elemental analysis and infrared spectroscopy.

Note: During washing the organic layer, the carbonate solution forms an emulsion which was difficult to break. It was only destroyed after standing for about one hour and by repeated extraction of aqueous layer. Every time only a clearly separated organic layer was taken out.



POLY (ETHYLENE GLYCOL) or CARBOWAX-20M, (X)



POLY (ETHYLENE GLYCOL) DI - ACETATES

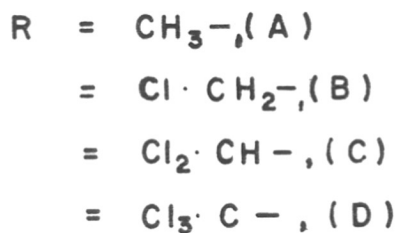


FIG. 3

Details of individual compounds were given below:

1) Poly(ethylene glycol)di-acetate (A): Brown solid,
m.p. 34° - 36° C.

IR : 1725 cm^{-1} ($>\text{C}=\text{O}$), 1210 cm^{-1} , 835 cm^{-1} (Fig. 4).

Elemental
Analysis : C, 49.10; H, 8.32%. ?

2) Poly(ethylene glycol)di-monochloroacetate (B)

Pale yellow solid, m.p. 52° - 53° C

IR : 1750 cm^{-1} ($>\text{C}=\text{O}$), 832 cm^{-1} (Fig. 5).

Elemental
Analysis : C, 53.40%; H, 9.21%; Cl, 0.98%.

3) Poly(ethylene glycol)di-dichloroacetate (C): Dark red,
thick liquid, m.p. $(-4^{\circ}) - (-2^{\circ})$ C.

IR : 1740 cm^{-1} ($>\text{C}=\text{O}$), 802 cm^{-1} .

Elemental
Analysis : C, 53.14%; H, 9.19%; Cl, 20.02%.

4) Poly(ethylene glycol)di-trichloroacetate (D)

Red gummy solid, m.p. 31 - 32° C.

IR : 1725 cm^{-1} ($>\text{C}=\text{O}$), 780 cm^{-1} .

Elemental
Analysis : C, 52.85%; H, 9.15%; Cl, 3.97%.

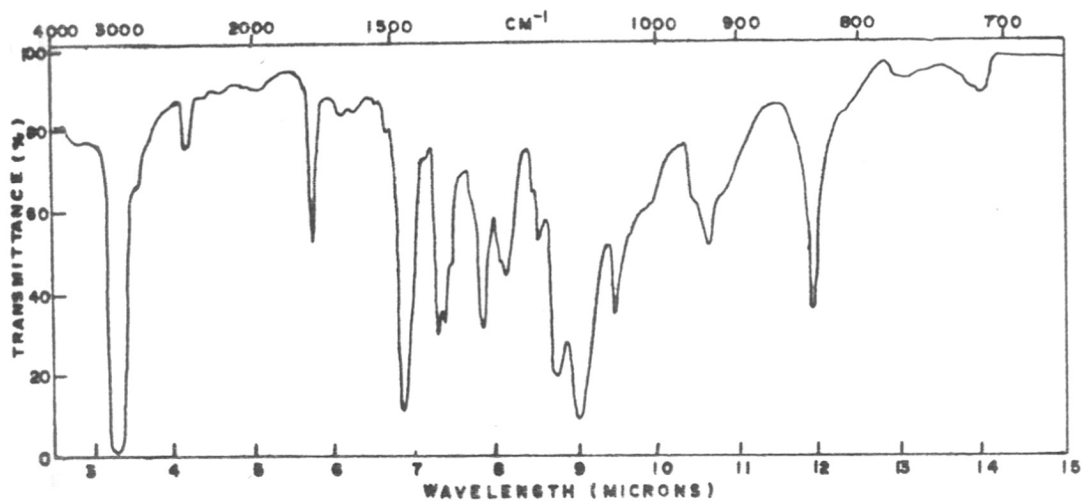


FIG 4 IR SPECTRUM OF POLY (ETHYLENE GLYCOL) DI-ACETATE,(A)

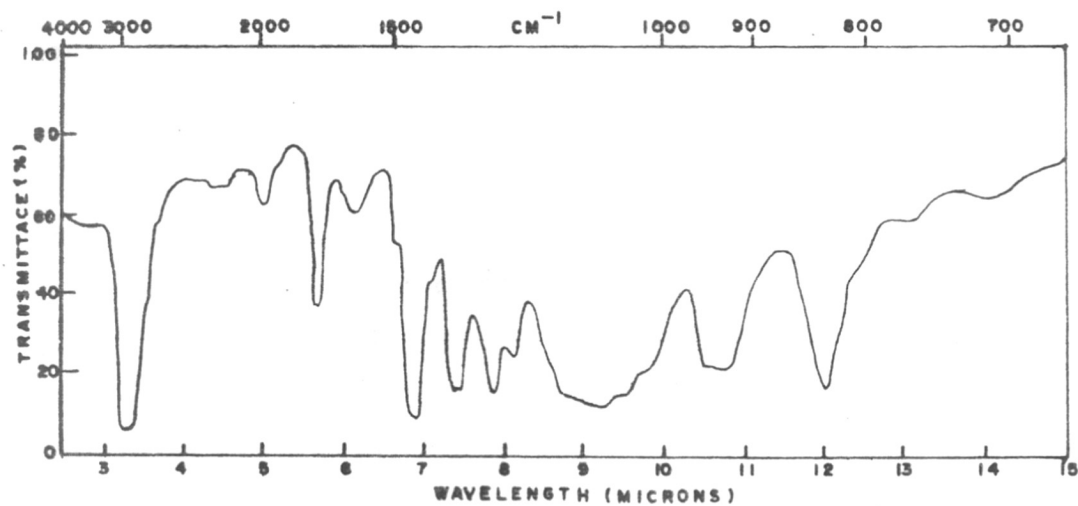


FIG. 5. IR SPECTRUM OF POLY(ETHYLENE GLYCOL)DI-MONOCHLOROACETATE,(B)

2.2.3 GAS CHROMATOGRAPHIC COLUMNS

Five columns of 5% load were prepared. They are as follows:

- 1) Poly (ethylene glycol)diacetate (Column A)
- 2) Poly (ethylene glycol)di-monochloroacetate (column B)
- 3) Poly (ethylene glycol)di-dichloroacetate (Column C)
- 4) Poly (ethylene glycol)di-trichloroacetate (Column D)
- 5) Poly (ethylene glycol) or (Carbowax-20M) (Column X)

General method of preparations and material specifications are as follows:

2.2.3.1 SOLID SUPPORT: It was a flux-calcinated diatomic support, prepared from Johns-Manville's Celite diatomaceous silica, has a medium capacity to hold liquid phases (15%), was acid washed and dimethylchlorosilane treated.

It was obtained from Analabs, Inc. (USA) as Chromosorb W (AW-DMCS) - 60-80 mesh (Cat. No.GCB-006B)

2.2.3.2 SOLVENTS: Commercial chloroform was dried over calcium chloride for 48 hr and distilled at 58-61°C.

2.2.3.3 ALUMINIUM TUBINGS: Seamless tubings with 2 mm I.D. and 4 mm O.D. and 2 meters in length were used. They were available in local market.

2.2.3.4 METHOD OF IMPREGNATION: Column material was prepared by double volume method. Volume of chromosorb W (10 g) was measured (45 ml). Carbowax-20M (1 g) was weighed accurately and dissolved in chloroform (90 ml) to get clear solution. Then chromosorb W was added to it. The mixture was gently shaken at five minutes interval four times and allowed to settle. The supernatant clear solution was decanted till it measured half the volume i.e. 45 ml and discarded. The slurry thus obtained was transferred to a flat bottom glass dish. The solvent was allowed to evaporate at 60°C on waterbath for 3 hr with gentle stirring. The free flowing material was further dried in vacuum for 1 hr till it gave constant weight. Thus 5% phase on chromosorb W was prepared.

2.2.3.5: COLUMN FILLING: Throughout the investigations, aluminium tubings from the same lot were used. Column material was filled in straight tubings which were vibrated gently to have uniform packing.

Total filling was 7.70 gms which was almost same for all columns. Both the ends of tubings were plugged with glass wool and tubing was wound to the desired shape.

2.2.3.6 COLUMN CONDITIONING: It is necessary to condition columns before use as the low boiling contaminations vaporise to disturb the baseline. For this purpose columns were

heated at oven temperature 100°C for two hours, at 150°C for two hours and further at 200°C for two hours. At the end of conditioning procedure, a steady baseline was observed with recorder. It indicated that there was no more bleeding from the columns. As a precautionary measure detector was kept at 300°C.

2.2.3.7 RECORDING OF RETENTION DATA: Retention time was recorded with the help of a stop watch. When air peak reached its maxima, the stopwatch was started and was stopped when the sample peak reached its maxima. Internal standard samples were reinjected to check their retention times at regular intervals. The data was collected by taking several concurrent readings in each case.

Throughout the investigation, equal gas flow was maintained by checking inlet and outlet gas flows. Uniform column packing and constant column temperature was also ensured in each case for consistency.

2.2.4 THE INSTRUMENT

AIMIL-NCL (India) Dual Column Gas Chromatograph, Mark II, No.70020 was used (Chapter-1, Fig. 1). Two filament thermal conductivity Detector (GOW-MAC, Part 13-004, Code W2X, Mount (333) was used.

Instrumental parameters were as follows:

- 1) Hydrogen gas flow: 6 litres/hr.

- 2) Injector temperature: (a) 120°C (b) 160°C.
- 3) Column temperature: (a) 80°C (b) 120°C
- 4) Detector temperature: 200°C
- 5) Recorder: Messgeratewerk (E. Germany),
Type EKN/T1 with variable speeds, 200 mm/hr,
600^{mm}/hr, 1200 mm/hr, 3600 mm/hr.

2.2.5 ANALYSIS OF SAMPLES: Samples were analysed on columns A, B, C, D and on X for comparison (Section 2.2.3).

Following groups of samples were analysed at the temperature mentioned therewith.

- 1) Straight chain aliphatic alcohols
(C₁ to C₇ alcohols): 80°C.
- 2) Isomeric aliphatic alcohols: 80°C
- 3) Aliphatic compounds with different
polarities: 80°C
- 4) Chloromethanes: 80°C
- 5) Chlorobenzenes: 120°C
- 6) Methylbenzenes: 120°C
- 7) Chlorotoluenes: 120°C

Details of each group are described below.

TABLE 1
STRAIGHT CHAIN ALIPHATIC ALCOHOLS

NO	NAME	STRUCTURAL FORMULA	BOILING POINT °C
1	METHANOL	CH_3OH	65
2	ETHANOL	$\text{C}_2\text{H}_5\text{OH}$	78
3	N-PROPANOL	$\text{C}_3\text{H}_7\text{OH}$	98
4	N-BUTANOL	$\text{C}_4\text{H}_9\text{OH}$	117
5	N-PENTANOL	$\text{C}_5\text{H}_{11}\text{OH}$	138
6	N-HEXANOL	$\text{C}_6\text{H}_{13}\text{OH}$	157
7	N-HEPTANOL	$\text{C}_7\text{H}_{15}\text{OH}$	177

TABLE 2
ISOMERIC ALIPHATIC ALCOHOLS

1	N-PROPANOL	$\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-OH}$	98
2	ISO-PROPANOL	$\begin{array}{c} \text{H}_3\text{C} \\ \\ \text{CH-OH} \end{array}$	82
3	N-BUTANOL	$\text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OH}$	117
4	ISO-BUTANOL	$\begin{array}{c} \text{H}_3\text{C} \\ \\ \text{CH-CH}_2\text{-OH} \end{array}$	108
5	SEC-BUTANOL	$\begin{array}{c} \text{H}_3\text{C} \quad \text{OH} \\ \quad \\ \text{CH}_3\text{-CH}_2\text{-CH-CH}_3 \end{array}$	100
6	TER-BUTANOL	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H}_3\text{C-C-OH} \\ \\ \text{CH}_3 \end{array}$	82

TABLE 3
ALIPHATIC COMPOUNDS WITH DIFFERENT POLARITIES

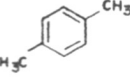
1	WATER	H_2O	100
2	ACETONE	$\text{H}_3\text{C-CO-CH}_3$	56
3	METHANOL	$\text{CH}_3\text{-OH}$	65
4	ETHANOL	$\text{C}_2\text{H}_5\text{OH}$	78
5	ETHYL ACETATE	$\text{CH}_3\text{COOC}_2\text{H}_5$	77
6	CHLOROFORM	CHCl_3	61
7	METHYL ISOBUTYL KETONE	$\begin{array}{c} \text{CH}_3\text{CO-CH}_2\text{-CH-CH}_3 \\ \\ \text{CH}_3 \end{array}$	117

CHLOROMETHANES **TABLE 4**

1	METHYLENE CHLORIDE	CH_2Cl_2	44
2	CHLOROFORM	CHCl_3	61
3	CARBON TETRACHLORIDE	CCl_4	76

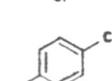
METHYL BENZENES

TABLE 5

No	NAME	STRUCTURAL FORMULA	BOILING POINT
1	BENZENE		80
2	TOLUENE		110
3	o-XYLENE		144
4	m-XYLENE		139
5	p-XYLENE		138

CHLOROBENZENES

TABLE 6

1	BENZENE		80
2	CHLOROBENZENE		132
3	o-DICHLOROBENZENE		179
4	m-DICHLOROBENZENE		172
5	p-DICHLOROBENZENE		174

CHLOROTOLUENES

TABLE-7

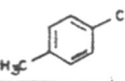
1	BENZENE		80
2	TOLUENE		110
3	CHLOROBENZENE		132
4	o-CHLOROTOLUENE		159.5
5	m-CHLOROTOLUENE		162
6	p-CHLOROTOLUENE		162

Table 8

Straight Chain Aliphatic Alcohols

Name	Retention Time					
	X	A	B	C	D	SE-30
1 Methanol	30"	30"	36"	21"	24"	10"
2 Ethanol	48"	39"	39"	25"	29"	15"
3 n-Propanol	1'35"	1'27"	1'25"	33"	1'07"	22"
4 n-Butanol	3'31"	2'55"	2'41"	1'36"	2'23"	27"
5 n-Pentanol	7'37"	7'13"	5'55"	2'43"	6'31"	1'05"
6 n-Hexanol	15'17"	12'17"	15'54"	9'04"	11'02"	2'24"
7 n-Heptanol	22'30"	25'06"	23'01"	17'22"	19'13"	6'04"

Table 9

Isomeric Aliphatic Alcohols

Name	Retention Time				
	X	A	B	C	D
1 n-Propanol	1'35"	1'27"	1'25"	33"	1'07"
2 iso-propanol	15"	33"	34"	19"	59"
3 n-Butanol	3'31"	2'55"	2'41"	1'35"	2'23"
4 iso-Butanol	2'24"	1'59"	2'11"	1'18"	1'36"
5 sec-Butanol	1'27"	1'13"	1'40"	1'05"	49"
6 tert-Butanol	38"	27"	43"	18"	35"

Table 10

Aliphatic compounds with different polarities

1 Water	49"	1'20"	1'53"	2'02"	1'31"
2 Ethanol	48"	39"	39"	25"	29"
3 Methanol	30"	30"	36"	21"	24"
4 Acetone	10"	17"	24"	13"	15"
5 4-Methyl-2-pentanone (MIBK)	32"	1'04"	1'28"	52"	1'05"
6 Chloroform	35"	1'05"	1'27"	26"	49"
7 Ethyl acetate	12"	34"	36"	23"	27"

Table 11Chloromethanes

Name	Retention Time				
	X	A	B	C	D
1 Methylene chloride	6"	35"	50"	11"	25"
2. Chloroform	35"	1'05"	1'27"	26"	49"
3 Carbon tetra-chloride	11"	29"	38"	12"	19"

Table 12Methylbenzenes

1 Benzene	5"	13"	14"	7"	10"
2 Toluene	9"	24"	23"	12"	22"
3 o-Xylene	29"	1'02"	1'31"	33"	53"
4 m-Xylene	27"	54"	1'13"	30"	48"
5 p-Xylene	28"	49"	1'05"	29"	48"

Table 13
Chlorobenzenes

Name	Retention Time				
	X	A	B	C	D
1 Benzene	5"	13"	14"	7"	10"
2 Chlorobenzene	23"	1' 11"	1'12"	38"	1'10"
3 o-Dichlorobenzene	2'34"	5' 00"	7'20"	2'55"	4'12"
4 m-Dichloro- benzene	2'13"	3'57"	5'04"	2'22"	3'25"
5 p-Dichloro- benzene	2'26"	4'02"	6'06"	2'34"	3'26"

Table 14
Chlorotoluenes

1 Benzene	5"	13"	14"	7"	10"
2 Toluene	9"	24"	23"	12"	22"
3 Chlorobenzene	23"	1'11"	1'12"	38"	1'10"
4 o-Chlorotoluene	2'06"	1'22"	2'50"	42"	1'30"
5 m-Chlorotoluene	3'12"	2'07"	3'00"	1'56"	1'35"
6 p-Chlorotoluene	3'03"	1'59"	3'07"	1'15"	1'41"

2.3 RESULTS AND DISCUSSION

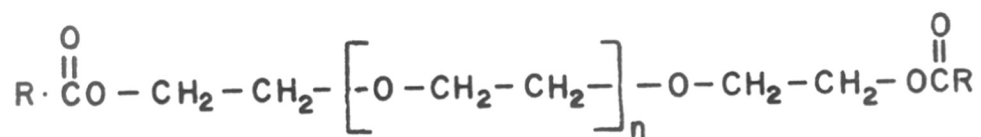
Among the variety of poly (ethylene glycol) stationary phases, carbowax-20M has been chosen as it is widely used and preferred phase^{27,28,29}. A study of McReynolds constants³ shows that carbowax-20M is capable of substituting nineteen other liquid phases⁵.

Chloroacetic acids were condensed with Carbowax-20 M as to get acetates. They are as follows (Fig. 6). Poly (ethylene glycol)di-acetate (A), Poly (ethylene glycol)di-monochloroacetate (B), Poly (ethylene glycol)di-dichloroacetate (C), Poly (ethylene glycol)di-trichloroacetate (D).

These phases were evaluated as stationary phases for gas chromatography. Under identical conditions the parent phase: Carbowax-20M (X) was also studied for comparison.

The groups of samples analysed are as follows:

1. Straight chain aliphatic alcohols (C_1-C_7)
2. Isomeric aliphatic alcohols (C_3 and C_4)
3. Aliphatic compounds with different polarities
4. Chloromethanes
5. Chlorobenzenes
6. Methylbenzenes
7. Chlorotoluenes



POLY(ETHYLENE GLYCOL) DI-ACETATES

- $\text{R} = \text{CH}_3 - , (\text{A})$
 $= \text{Cl} \cdot \text{CH}_2 - , (\text{B})$
 $= \text{Cl}_2 \cdot \text{CH} - , (\text{C})$
 $= \text{Cl}_3 \cdot \text{C} - , (\text{D})$

FIG. 6

2.3.1 ANALYSIS OF SAMPLES

Observations and interpretation of results are discussed groupwise as follows:

1) Straight Chain Aliphatic Alcohols: The retention data is represented graphically against number of carbon atoms in alcohols (C_1 to C_7) (Fig. 7). Curve obtained for carbowax-20M phase (X) is initially little deviating to axis (C_1 to C_4) but deviates sharply for higher homologues. Stationary phases (A-D) behave similarly as positions of the curves do not differ much. The retention times are found to be decreased on condensed phases for C_1 -OH to C_5 -OH (Table 2). The data clearly indicates the failure of terminal chloroacetate groups for enhanced resolution.

In another graph of boiling point against retention time, the function is non-linear (Fig. 8). The relation is linear only for C_1 -OH to C_4 -OH. It also shows identical behaviour of carbowax-20M and condensed chloroacetate phases (A-D). It has already been reported that the alcohols have identical polar nature which causes hydrogen bonding of equal strength. So they are eluted in the order of increasing boiling points³¹. Same also holds true for the condensed phases. Number of chlorine atoms in terminal acetate groups seem to be insignificant though it has been

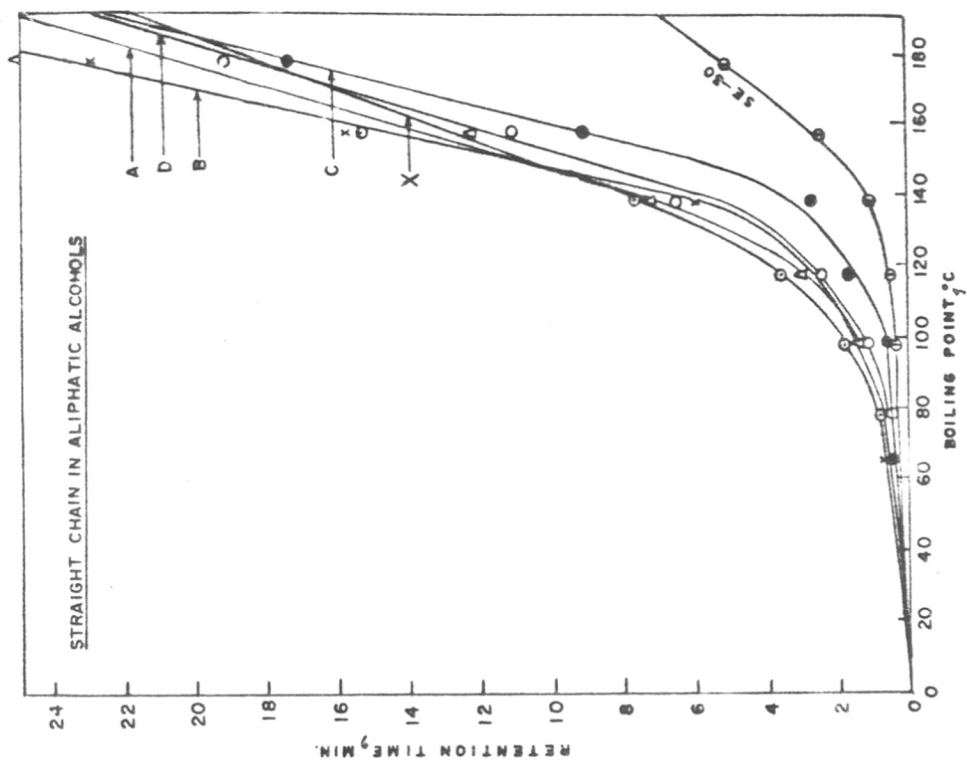


FIG. 8

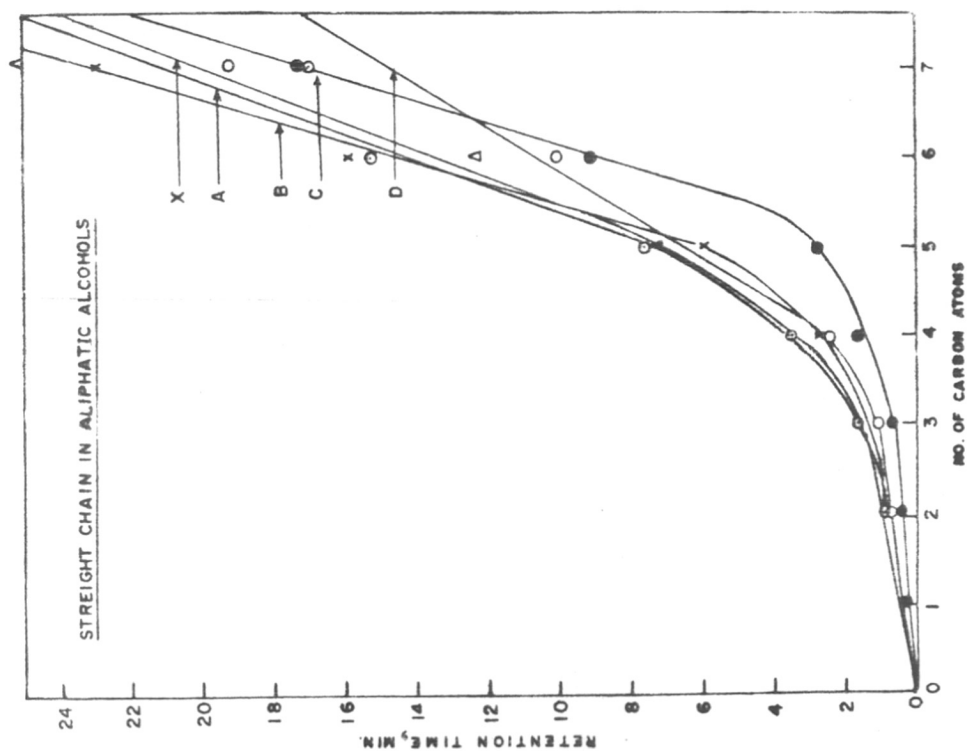


FIG. 7

reported that addition of chlorine atoms in dialkyl phthalate polyesters improves the separation³².

Figure 9 represents chromatograms of alcohols on carbowax-20M (X) and on poly (ethylene glycol)di-mono-chloroacetate (B).

2) Isomeric Aliphatic Alcohols (C_3 and C_4): Following are the retention ratios of normal alcohols to iso-alcohols.

Table 15

	X	A	B	C	D
<u>n-propanol</u> iso-propanol	6.40	2.64	2.54	1.83	2.20
<u>n-butanol</u> iso-butanol	1.46	1.44	1.23	1.25	1.40

Best resolution of isomeric alcohols was possible on the parent phase (X) but the alcohols ^{were} are very poorly separated on the new phases (A-D). It shows that polarity of the phases has been decreased substantially due to the replacement by chloroacetate groups.

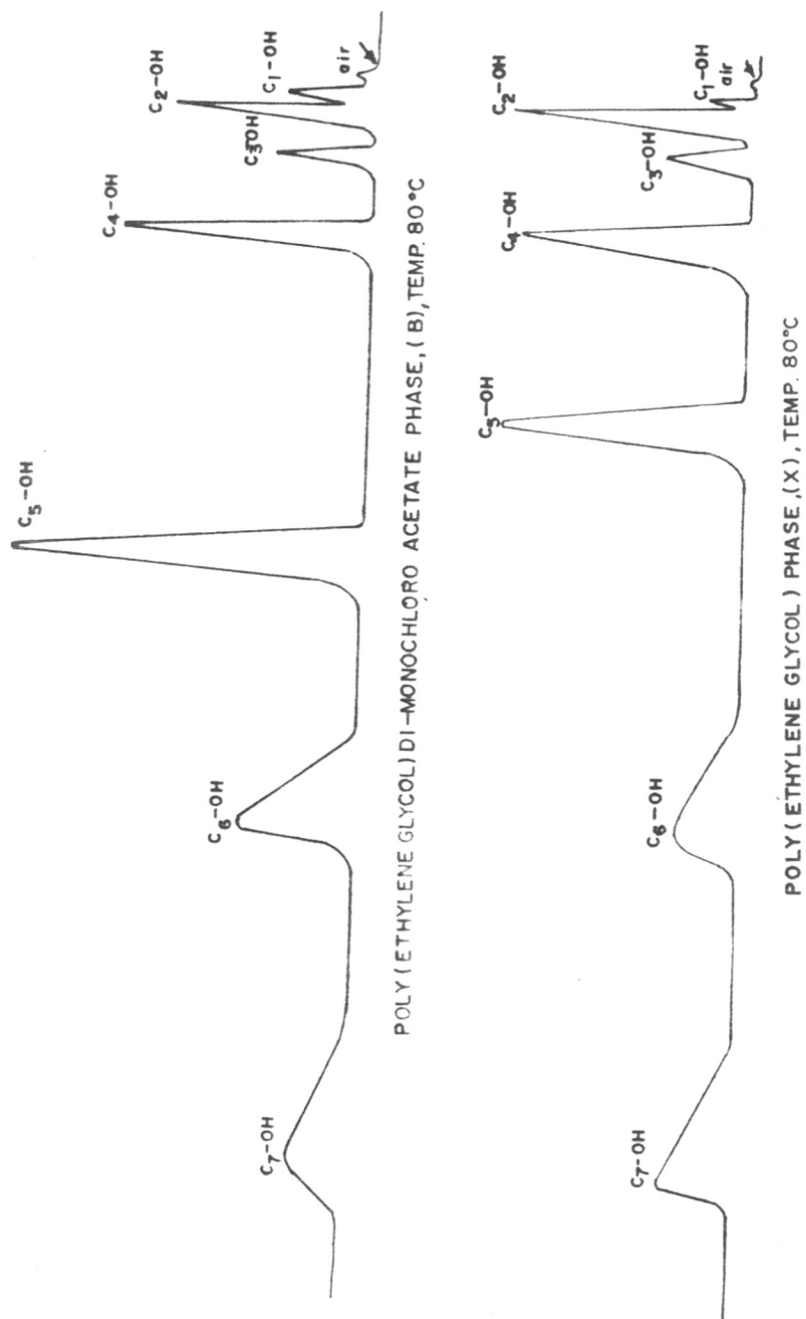


FIG. 9

3) Aliphatic Compounds with different polarities: Samples analysed were water, methanol, ethanol, acetone, ethyl acetate, 4-methyl-2-pentanone (MIBK) and chloroform. The retention data in different combinations were studied. Very interesting results were obtained in this section. They are described below:

Retention ratios of water and methanol are as follows:

Table 16

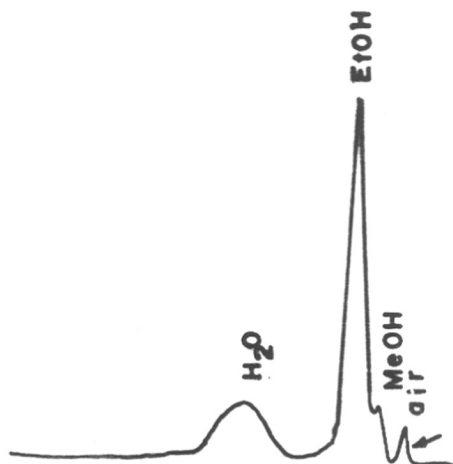
	X	A	B	C	D
<u>H₂O</u>	1.63	2.67	3.14	5.80	3.79
<u>MeOH</u>					

Improved separation was obtained on condensed phases. Best separation was achieved on poly (ethylene glycol)-di-dichloroacetate phase (C) with which the separation factor is nearly four times ^{greater} than that of the parent phase (Fig. 10).

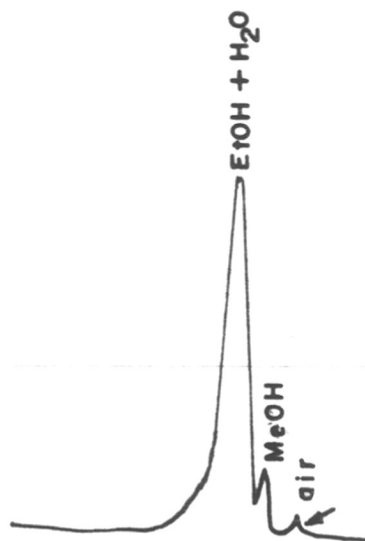
Separation factors for the pair i.e. water and ethanol are as follows:

Table 17

	X	A	B	C	D
<u>H₂O</u>	1.02	2.05	2.90	4.88	3.15
<u>EtOH</u>					



POLY(ETHYLENE GLYCOL) DI-DICHLORO
ACETATE PHASE, (C), TEMP. 80°C



POLY(ETHYLENE GLYCOL) PHASE, (X), TEMP. 80°C

FIG. 10

As compared with ethanol, water is more polar and high boiling also (b.ps. 78° and 100°C respectively) but carbowax-20 M phase (X) has not shown the separation. It may be due to higher column temperature and lesser percentage of the phase in the column. Enhanced separation for the pair has been obtained with all new acetate polymers (A-D). The phase: poly (ethylene glycol) di-dichloroacetate (C) tops the list, the separation factor being equal to five (Fig. 10).

It has already been mentioned that hydrogen bonding of equal strength occurs with alcohols³¹. Therefore on carbowax-20M phase (X); water, ethanol and methanol are eluted in the order of their boiling points. Higher resolution on condensed phases (A-D) may be attributed to the double effect i.e. boiling point difference and hydrogen bonding of varied strength. The latter is less in ethanol and methanol due to inductive effect (+I) of alkyl groups, while water is a small molecule and lone pair on oxygen atom is easily available as no steric hinderance is present. Highest resolution ^{on} poly (ethylene glycol) di-dichloroacetate may be accounted for availability of highly acidic hydrogen atom in terminal acetate molecules due to the presence of two chlorine atoms attached to the same carbon atom. This hydrogen atom may be forming strong hydrogen bonding with oxygen atom in alcohols and water.

Different types of hydrogen bonding occurring in the column are shown in Fig. 11.

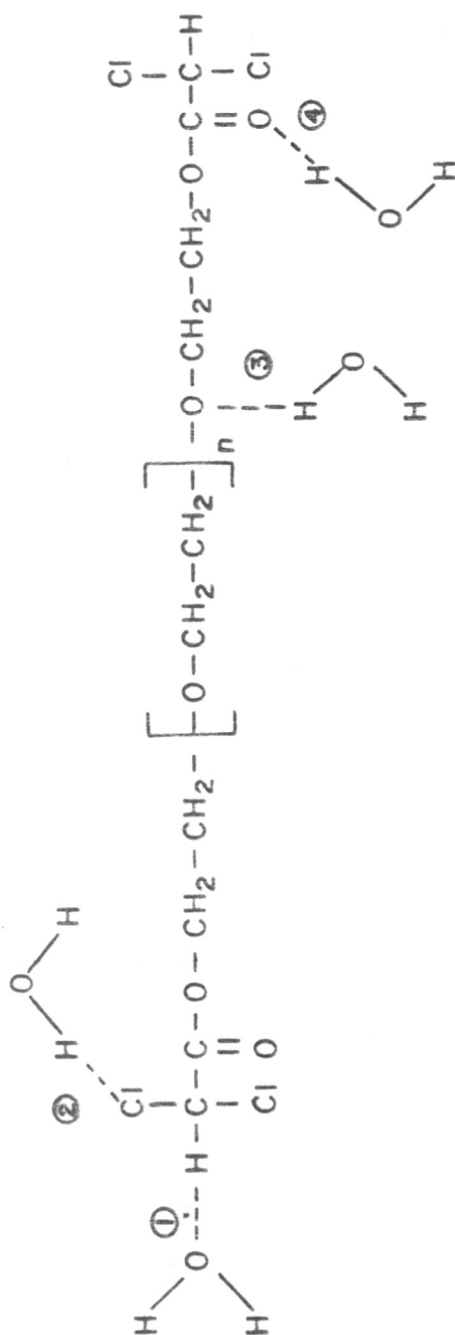
Compared with the parent phase, very poor separation of ethanol and methanol has been obtained on condensed phases.

For a pair:ethanol and ethyl acetate, separation was expected to be on the basis of polarity as boiling points are identical. Though they are separated on poly (ethylene glycol) phase (X), poor resolution has been obtained on phases (A-D). Therefore it seems that the phases have acquired less polar character than the parent phase. Some astonishing results were obtained for chloroform and 4-methyl—2-pentanone (MIBK) (Table 18).

Table 18

	X	A	B	C	D
Chloroform	35"	1'05"	1'27"	26"	49"
MIBK	32"	1'04"	1'28"	52"	1'05"

At 80°C, the phase (X) has shown little separation. Two parameters viz. vapour pressure and polarity work simultaneously. Chloroform contains highly acidic proton. Therefore it was retained, while MIBK was eluted late due to high boiling point (117°C) and not due to polarity. This



HYDROGEN BONDING WITH 1 ACIDIC PROTON

2 CHLORINE ATOM

3 ETHERIC OXYGEN

4 CARBONYL OXYGEN

POLY (ETHYLENE GLYCOL) DI-DICHLORO ACETATE PHASE, (C)

FIG. 11

two fold interaction gives overlapping retention times. The same was also observed on phase A and B but separation is enhanced on poly (ethylene glycol)di-dichloroacetate phase (C) (Fig. 12). Polarity effect is much more reduced in this case. The dependance of retention is entirely on boiling points (61°C and 117°C). It was confirmed when the pair was analysed on SE-30 column which separates the components according to their boiling points. Therefore the behaviour of the phases (A-D) seems to be tending towards the non-polar character in this case also.

4) Chloromethanes: Carbon tetrachloride, chloroform and methylene chloride were analysed in this group.

Chlorine atom has negative inductive effect (-I). Therefore both the hydrogen atoms in methylene chloride become acidic and readily available for hydrogen bonding. The hydrogen atom in chloroform is also highly acidic due to three chlorine^{atoms} attached to the same carbon atom. It increases polarity of the proton for a stronger hydrogen bonding. As carbon tetrachloride is non-polar, hydrogen bonding does not occur. Therefore according to the strength of the hydrogen bonding that the compounds may be experiencing, the order of elution is carbon tetrachloride, methylene chloride and chloroform. All condensed phases have shown similar type of behaviour. Eventhough methylene

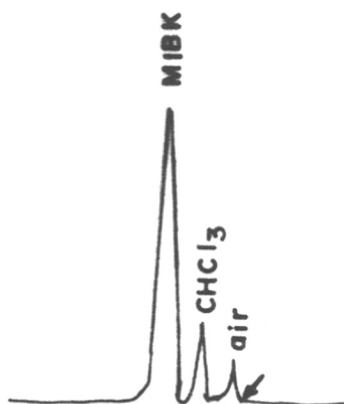
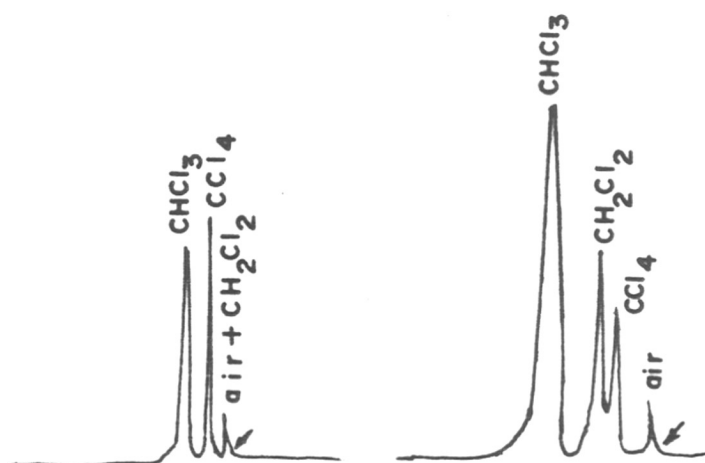


FIG. 12. POLY(ETHYLENE GLYCOL) DI-DICHLORO ACETATE
PHASE, (C), TEMP. 80°C



POLY(ETHYLENE GLYCOL)
PHASE, (X), TEMP. 80°C

POLY(ETHYLENE GLYCOL) DI-
- MONOCHLORO ACETATE
PHASE, (B), TEMP. 80°C

FIG. 13

chloride and carbon tetrachloride differ in polarities, the resolution is comparatively poor. It may be attributed to the principle of double effect i.e. due to boiling point and polarity. Carbon tetrachloride though non-polar, was retained due to higher vapour pressure and higher molecular weight. Therefore resolution that occurs due to hydrogen bonding is partially nullified. The separation factors are as follows.

Table 19

Phase	X	A	B	C	D
$\frac{\text{CCl}_4}{\text{CH}_2\text{Cl}_2}$	0.5	1.30	1.30	1.10	1.30
$\frac{\text{CHCl}_3}{\text{CH}_2\text{Cl}_2}$	6.0	1.83	1.74	2.37	2.00

Separation factors for the second pair are in the range of 1.8 - 2.3 on the acetate phases and the boiling point ratio is 1.5 (61°C and 42°C respectively). Therefore the retention is mostly on the basis of boiling points. Hydrogen bonding of equal strength may be occurring in this case so that its role in resolution is negligible. This behaviour again indicates less polar character of the phases.

The resolution is enhanced on phase X because of stronger hydrogen bonding (Fig. 13).

Except poly (ethylene glycol)di-dichloroacetate phase (C) all other phases have shown increase in retention time for chloromethanes with almost similar resolution which is better than on parent phase (X), (Fig. 13).

5) Methylbenzenes: Benzene, toluene and xylene isomers were analysed. Eventhough no characteristic observations have been made, some are worth noting. All the phases, including carbowax-20M (X) record double the retention time for toluene than benzene.

Orthoxylene, the highest boiling dimethyl benzene (b.p. 144°C) had highest retention time while meta and para-isomers with very close boiling points (139° and 138.5°C) had little or no separation on these phases. Consequently the elution seems to be dependent upon the boiling points. Therefore they were compared on SE-30 phase. It showed almost linear relation while the phases under consideration are represented by curves (Fig. 14), sharply deviating in the region $120-140^{\circ}\text{C}$. It suggests that there is some interaction other than that related to vapour pressure. There occur inductive forces between the phase and xylenes³⁹. The same also may be accounted due to the difference in molecular volumes (benzene < toluene < xylenes) which may be affecting partition

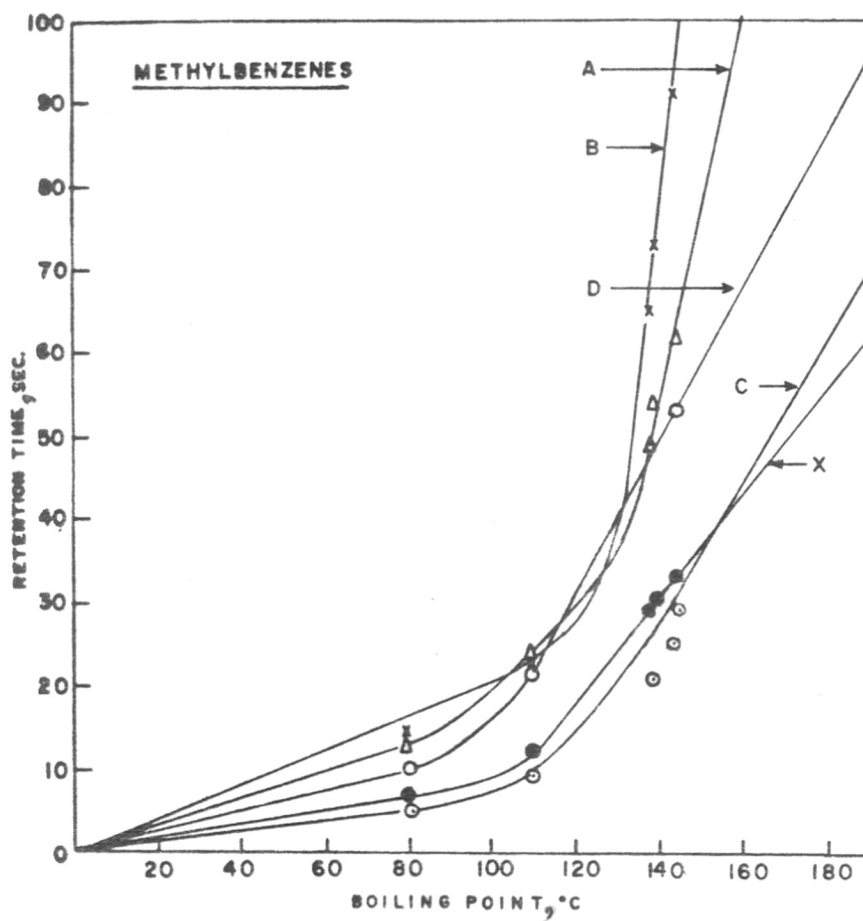
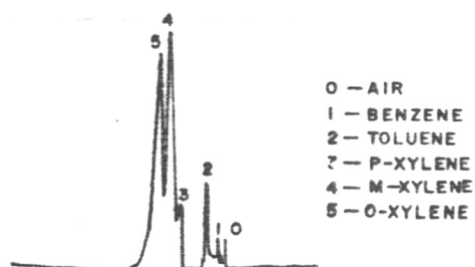


FIG. 14

FIG. 15 POLY(ETHYL GLYCOL)DI-MONOCHLORO ACETATE
PHASE, (B), TEMP. 120°C

co-efficients³³. Highest retention has been observed on poly(ethylene glycol)di-monochloroacetate phase (B). The chromatogram is shown in Fig. 15.

6) Chlorobenzenes: Benzene, monochlorobenzene and dichlorobenzene isomers were analysed. The order of elution is benzene < chlorobenzene < meta-dichlorobenzene < para-dichlorobenzene < orthodichlorobenzene which is in the order of their boiling points also (80°, 132°, 172°, 174°, 179° respectively). Eventhough the graph is a curve (Fig. 16), the isomers lie on a segment which is a straight line representing a linear function over a range of boiling points of isomers. As the boiling points are close, marginal resolution obtained may be due to the electrostatic properties. The relation therefore can be represented by an equation.

$$Z = ax + by \text{ where } a \text{ and } b \text{ are constants}$$

x = structural factor

y = boiling point

Thus whatever marginal separation occurred due to difference in boiling points, is enhanced by electrostatic properties. Combined forces: inductive effect (I) and mesomeric effect (+M) which impart partial double bond character to C-X linkage^{6,11} may be responsible for the same.

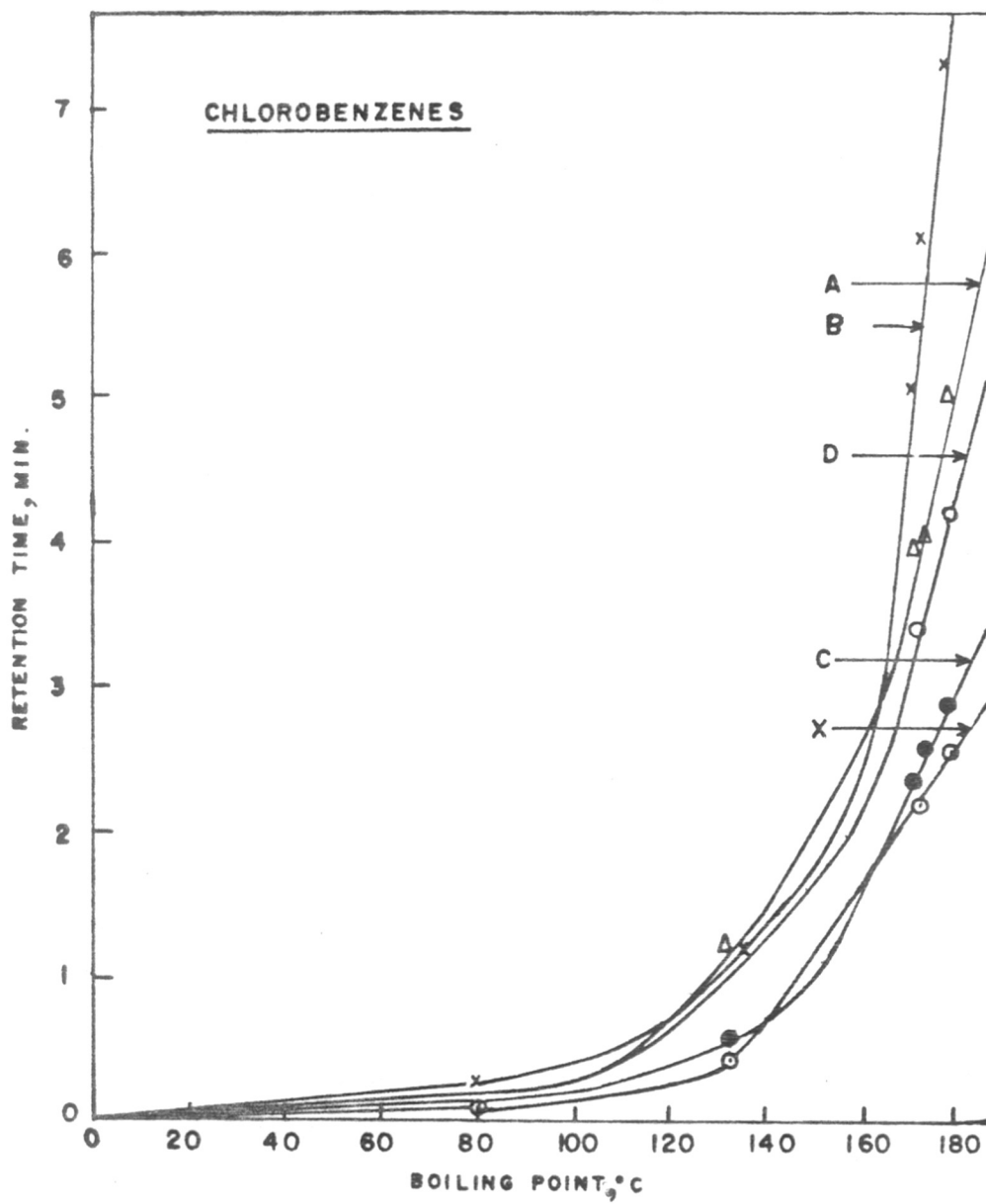


FIG 16

The separation factors on various phases are as follows:

Table 20

	X	A	B	C	D
meta	1.00	1.00	1.00	1.00	1.00
para	1.10	1.02	1.20	1.09	1.00
ortho	1.16	1.26	1.45	1.23	1.23

Optimum resolution was obtained ^{with} poly(ethylene glycol)-di-monochloroacetate phase (B). The phase is selective for dichlorobenzenes (Fig. 17).

7) Chlorotoluenes: Benzene, chlorobenzene, toluene and chlorotoluene isomers were studied in this group. Combined effect of methyl and chloro groups at various aromatic positions is discussed. Except the isomers, elution of the compounds is in the order of increasing boiling points. (Benzene < toluene < chlorobenzene < chlorotoluene) (Table 7). The retention data (Table 14) clearly indicates the independance of elution of the isomers over vapour pressure which is mainly responsible for the elution of xylenes and dichlorobenzenes¹⁰.

Electronegativity of chlorine atom in ortho-chloro-toluene is maximum due to inductive effect (+I) of the methyl group, but the isomer is not retained much. So the electrostatic factor may be neglected. In metachloro-toluene, hydrogen atom ortho to chloro group is highly electron deficient due to mesomeric and inductive effects which act in opposite directions. Therefore it may be having strong hydrogen bonding with the phase, resulting in more retention than other isomers. Same behaviour has been observed on the phases X, A and C but the phases B and D do not show good resolution. Highest retention has been observed on poly(ethylene glycol)di-monochloroacetate phase (B). The separation factors are as follows:

Table 21

	X	A	B	C	D
ortho	1.00	1.00	1.00	1.00	1.00
meta	1.53	1.50	1.07	1.27	1.04
para	1.45	1.45	1.11	1.80	1.08

Satisfactory resolution has been obtained on poly (ethylene glycol)^{di}chloroacetate (c). Therefore it is selective for chlorotoluenes (Fig. 18).

2.3.2 NATURE OF CONDENSED PHASES

Attempts were made to find relation between structure of the phases and retention data. The function of number of chlorine atoms in the phases is not linear because poly (ethylene glycol)di-dichloroacetate phase (C) records lowest retention time for all solutes. The difference increases substantially for high boiling samples.

The function of melting points (A=35°C, B=52°C, C= - 3°C, D = 31°C) is also nonlinear. In this case also the phase 'C' shows abnormal behaviour. Therefore no uniform behaviour has been observed for poly (ethylene glycol)di-chloroacetate phases.

Poly (ethylene glycol)di-monochloroacetate phase (B) possesses highest melting point and also shows maximum retention of solute samples while the phase: poly (ethylene glycol)di-dichloroacetate (C) records lowest retention for all samples analysed in these studies.

Selectivity of the phases towards efficient resolution of samples has been tabulised below.

Table 22

No.	Samples	Phase
1.	Straight chain aliphatic alcohols (C ₁ -C ₇)	B
2.	Methanol-water and ethanol-water	C
3.	Methylisobutylketone-chloroform	C
4.	Chloromethanes	C
5.	Xylene isomers	B
6.	Dichlorobenzenes	B
7.	Chlorotoluenes	C

Behaviour of each group of samples has already been discussed in previous sections. It has been pointed out that elution of alcohols, xylenes and dichlorobenzenes depends upon their boiling points and whatever interactions occur are not significant enough to affect the order of elution. It indicates that the phases have acquired non-polar character like squalene and SE-30 which separate the components according to their capacity of dispersion³⁴. Eventhough the dichloro phase (C) records lowest retention time, it is highly selective for the resolution of samples in which polarity factor predominates over vapour pressure. They are water-alcohol, chloromethanes, chlorotoluenes, etc. The behaviour should be essentially accounted for the highly acidic protons in terminal acetate groups of the phase. It forms significant hydrogen bonding with electro-negative atoms in solute molecules, resulting in enhanced separation of components. Additionally the dichloroacetate phase (C) has highest percentage of chlorine contents (Section 2.2.2). The observations are non-agreeable with work reported by Igasen et al.³⁵ who have mentioned that the improved retention in chlorinated poly (ethylene glycol) was due to increase of molecular weight and partially due to positions of chlorine atoms.

Studies of hydrolysis estimation of the acetate polymers revealed that Carbowax-20 M molecule has been

broken upto reduce the chain length during the condensation process³⁶. Intense absorptions in IR spectrum of the phases (section 2.2.2) support this. Bond fission phenomenon in poly (ethylene glycol)di-dichloroacetate (C) seems to substantial to add up more number of dichloroacetic acid molecules.

It has been reported that at temperature below the melting point, Carbowax-20M also works satisfactory³⁷. As the condensed phases (A-D) have lower melting points ~~depends~~ than that of Carbowax-20M, the lowest limit of operational temperature has been extended upto 0°C. Thus the derivatives are preferred in this case also. They were also examined for high temperature (200°C) analysis and found to give satisfactory performance³⁶. Among them, the phase 'C' is preferred due to lowest melting point and highest resolution power. It also has low retention volume. Thus it is the best substitute for Carbowax-20M phase.

2.4 APPLICATIONS

Glycerol has been used to remove water contents from liquid samples³⁸. On this basis, Adlrod³⁹ has analysed alcohols for moisture contents on poly (ethylene glycol)-600. He has reported the resolution of alcohols (upto C_3) from water. Usually Carbowax-20 M is employed for these types of analyses. Eventhough better separation can be achieved on it, higher percentage of the phase is required and column temperature also is to be maintained critically at about 70°C . Actually elevation in column temperature has been observed due to poor insulation of oven from injector and detector systems. This results in variable retention time and poorer separation.

Better resolution of alcohol (C_1 and C_2) and water has been obtained on the chloroacetate phases (A-D) than on the parent phase in present investigations. The phase poly (ethylene glycol)di-dichloroacetate (C), m.p. -3°C has been found highly selective for the pairs. It has a wide range of operational temperature, 0°C being lowest limit. Therefore the phase can be used safely even at room temperature (25°C). So the phase has been employed for analysis of samples from the pesticide processes.

National Chemical Laboratory, Poona has taken up pesticide projects as a part of process development of pest control agents. The analytical group is playing a key role in developing efficient gas chromatographic methods for the same.

Analysis of aqueous effluent solutions from the processes is essential. Alcohol contents in aqueous layers are determined for material balance. Their presence in aqueous solutions increases solubility of hazardous organophosphorus compounds such as dialkyl phosphorodithioic acids, intermediate products and the pesticides. This causes serious concern over contamination of the pesticides in effluent waters. Therefore the estimation is a vital need to control water pollution.

Analysis of aqueous portions from dimethoate and ethion processes has been described below.

2.4.1 DIMETHOATE PROCESS⁴⁰ (Fig.12)

A) Phosphorus pentasulphide and methanol in benzene were warmed to get O,O-dimethyl phosphorodithioic acid (I). The scrubber solution distillate contains methanol dissolved in water in trace quantities.

B) Monochloroacetic acid was esterified using methanol to get methyl chloroacetate (II). Excess of methanol remains in aqueous layer which was to be rejected (B-1). Trace quantities of methanol and water remains dissolved in methyl chloroacetate (B-2).

C) Sodium salt of O,O-dimethyl phosphorodithioic acid (I') was condensed with methyl chloroacetate (II) to get

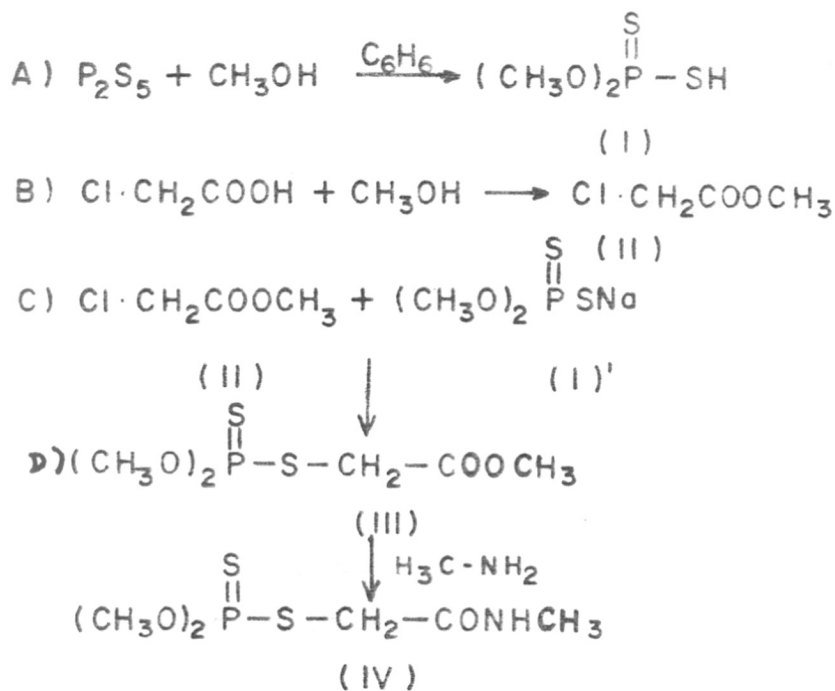


FIG. 19

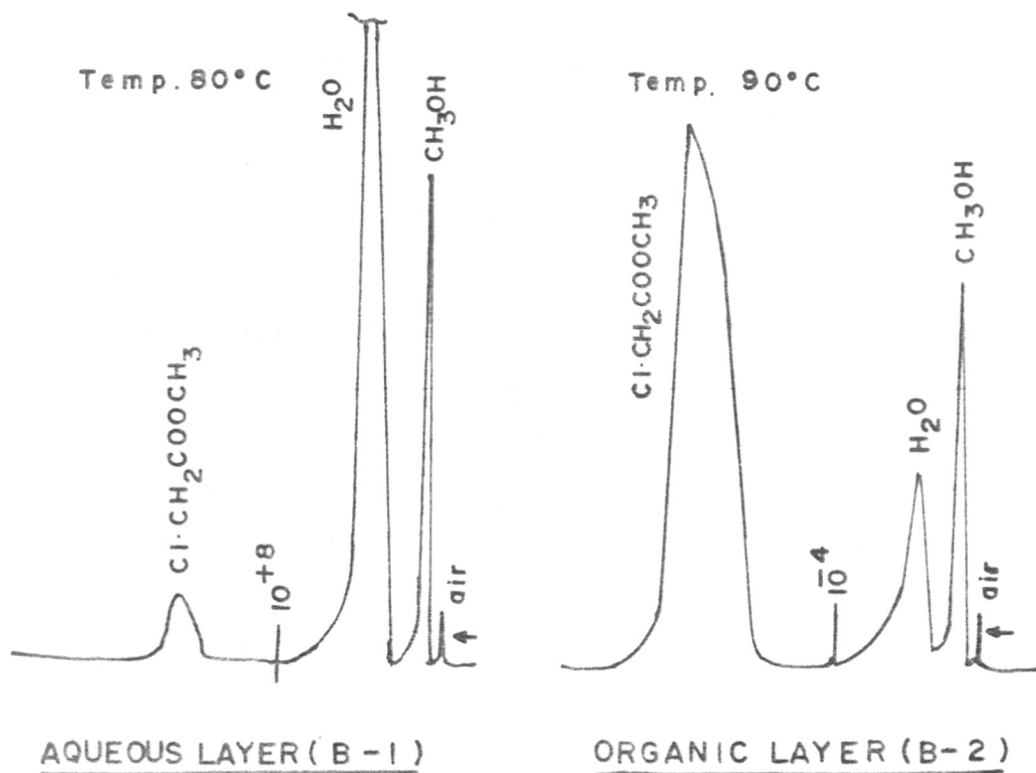


FIG. 20.

O,O-dimethyl S-(methoxycarbonylmethyl) phosphorothiolothionate (III). The organic layer of the acid (I) also contains some methanol in dissolved state. When sodium salt was prepared, it went in aqueous layer. After the condensation, the aqueous layer was separated.

D) The ester (III) was allowed to react with methyl amine to yield the pesticide:dimethoate (IV).

Above mentioned aqueous solutions (A,B-1 & B-2) were analysed on the phase 'C' (Fig. 20). Their percentages were determined by triangulation method⁴¹. The results are summarised in Table 23.

2.4.2 ETHION PROCESS³⁰ (Fig. 21)

A) Phosphorus pentasulphide and ethanol in benzene were warmed to get O,O-diethyl phosphorodithioic acid (I). The scrubber solution distillate contains ethanol in aqueous solution in trace quantities.

B) Sodium salt of O,O-diethyl phosphorodithioic acid (I') was allowed to react with methylene dibromide. Ethanol was added to make the reaction mixture homogeneous. After the reaction was over, major quantity of low boiling portion was distilled out (B-1). Aqueous layer was separated from the residue to get the pesticide:ethion (II) (B-2).

Table 23

No.	Step	Contents	Percentage
1	A	methanol	0.30
		water	99.70
2	B-1	methanol	14.25
		water	86.45
		methyl chloro- acetate	1.30
3	B-2	methanol	2.33
		water	3.17
		methyl chloro- acetate	94.51

Table 24

No.	Step	Contents	Percentage
1	A	ethanol	0.14
		water	99.86
2	B-1	ethanol	52.70
		water	11.10
		benzene	27.60
		methylene- dibromide	8.60
3	B-2	ethanol	5.04
		water	94.96
		benzene	0.00
		methylene- dibromide	0.00

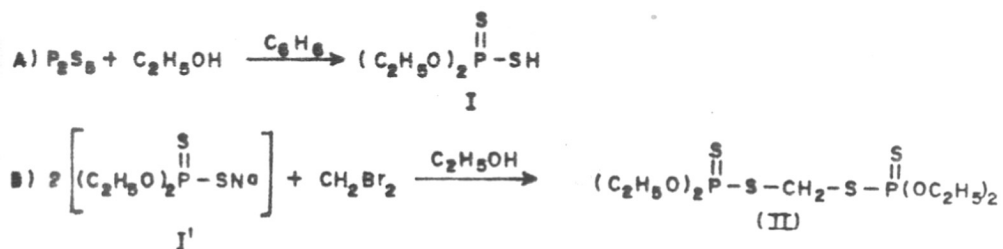


FIG 21

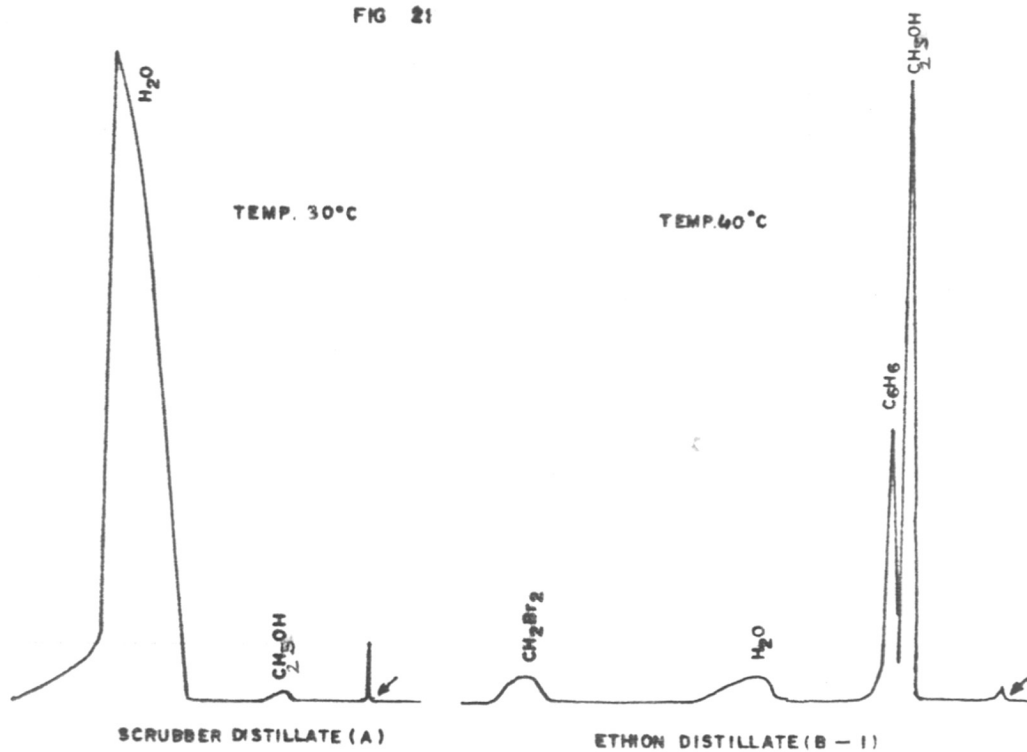


FIG. 22

The aqueous solutions from A, B-1 and B-2 were analysed on the phase 'C' (Fig. 22). Their percentages were calculated by triangulation method⁴¹. The details are mentioned in Table 24.

REFERENCES

1. Evans, M.B. and Smith, J.P., J.Chromatogr., 36, 489 (1968).
2. Perry, S. and Hibbert, H., J.Am.Chem.Soc., 62, 2599 (1940).
3. McReynolds, W.O., J.Chromat. Sci., 8, 685 (1970).
4. Adlond, E.R., Symposium on VPC, Institute of London, May (1956).
5. Castello, G. and Damato, G., J. Chromatogr., 90, 291 (1974).
6. Castello, G., *ibid.*, 66, 213 (1972).
7. Smith, B., Acta Chem. Scand., 13(3), 480 (1959).
8. Durrett, L.R., Anal. Chem., 32, 1393 (1960).
9. Aue, W.A., Hastings, C.R. and Kapila, S., J.Chromatogr., 77, 299 (1973).
10. Bighi, C., Bett, A., Saglietto, G. and Dondi, F., *ibid.*, 35, 309 (1968).
11. McReynolds, W.O., Gas Chromatographic Data, Preston, Technical Abstracts Co., Evanston, III (1966).
12. Kirland, J.J., Pittsburgh Conf. Anal.Chem. and App. Spectroscopy, Pittsburgh (1963).
13. Roupp, G., Z. Anal.Chem., 164, 135 (1958).
14. Cronin, D.A., J.Chromatogr., 97, 263 (1974).

15. Verachtert, H., Van Oevelen, D. and Bevers, J., *ibid.*,
117, 295 (1976).
16. Fox, J.E., *Proc.Soc.Expt.Biol.Med.*, 97, 236 (1958).
17. Singer, D.D., *Analyst*, 91, 127 (1966).
18. Bonnichsen, R. and Linturi, M., *Acta.Chem.Scand.*,
16, 1289 (1962).
19. Brealey, L., Elvidge, D.A. and Proctor, K.A., *Analyst*,
84, 221 (1959).
20. Schroeter, S. and Eliel, E.L., *J.Org.Chem.*, 30,
1 (1965).
21. Grob, K., *Beitr. Tobakforsch.*, 9, 315 (1962).
22. Grob, K., *J.Gas Chromatogr.*, 2(2), 80 (1964).
23. Hrivnak, J., Sojak, L., Baska, E. and Janak, J.,
J.Chromatogr., 68, 55 (1972).
24. Byare, B. and Jordan, G., *J. Gas Chromatogr.*,
2, 304 (1964).
25. Grob, K., *ibid.* 3(2), 52 (1965).
26. Assmann, K., Geppert, G., Struppe, H.G. and Serfas, O.,
Gas Chromatographie, Akademik-Verlag, Berlin, 33 (1968).
27. Persinger, H.E. and Shank, J.T., *J.Chromat.Sci.*,
11, 190 (1973).
28. Leary, J.J., Justice, J.B. Tsuge, S., Lowry, S.R.
and Isenhour, T.L., *ibid.*, 11, 201 (1973).
29. Mann, J.R. and Preston, S.T.^{Jr.}, *ibid.*, 11, 216 (1973).

30. Taussant, A., French Patent 1,231,107 (1960).
31. Baiulescu, G.E. and Ilie, V.A., Stationary phases for Gas Chromatography, Pergamon Press, 178 (1975).
32. Langer, S.H., Anal.Chem., 44, 1915 (1972).
33. Littlewood, A.B., J.Gas Chromatogr., 1, 6 (1963).
34. Nogare, S.D. Juvet, R.S. Jr., Gas Chromatography, Interscience, 112 (1962).
35. Igansen, A.V. and Semina, G.N., Grozov Khromatogr., No.4, 17 (1966).
36. Kadam, A.N. and Ghatge, B.B. unpublished results (1979).
37. Fiddler, W. and Doerr, R.C., J.Chromatogr., 21, 481 (1966).
38. Harvey, D. and Chalkley, D.E., Fuel Land. 34, 191 (1955).
39. Adlond, E.R., Vapour Phase Chromatography - Desty, D.H., Ed., Butterworths, London, 108 (1957).
40. Italian Patent 661, 487 (1964).
41. Condal-Busch., J.Chem.Ed., 41, A235 (1964).

●

CHAPTER THREE

THERMAL DECOMPOSITION
OF
DIMETHOATE

●

3·1 INTRODUCTION

3·2 EXPERIMENTAL

3·3 RESULTS AND DISCUSSION

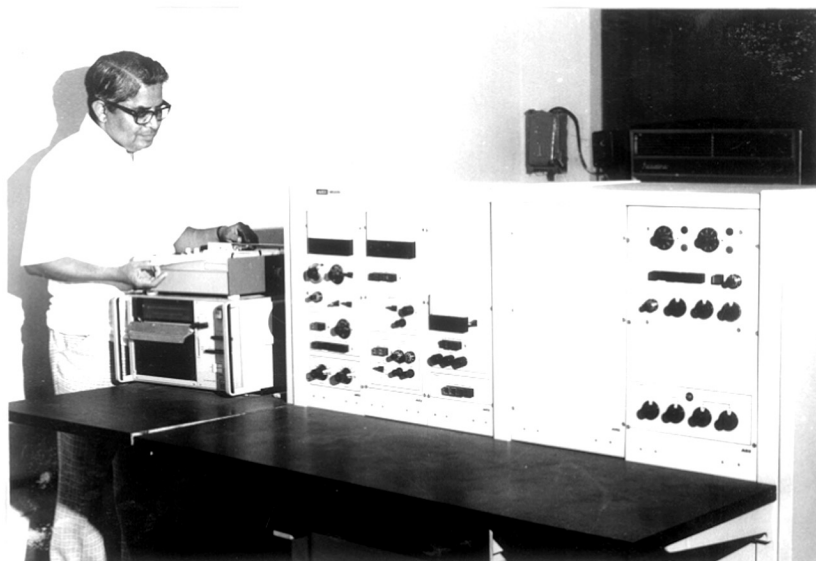
●

3.1 I N T R O D U C T I O N

3.1.1 COUPLED GAS CHROMATOGRAPHY / MASS SPECTROMETRY (GCMS)

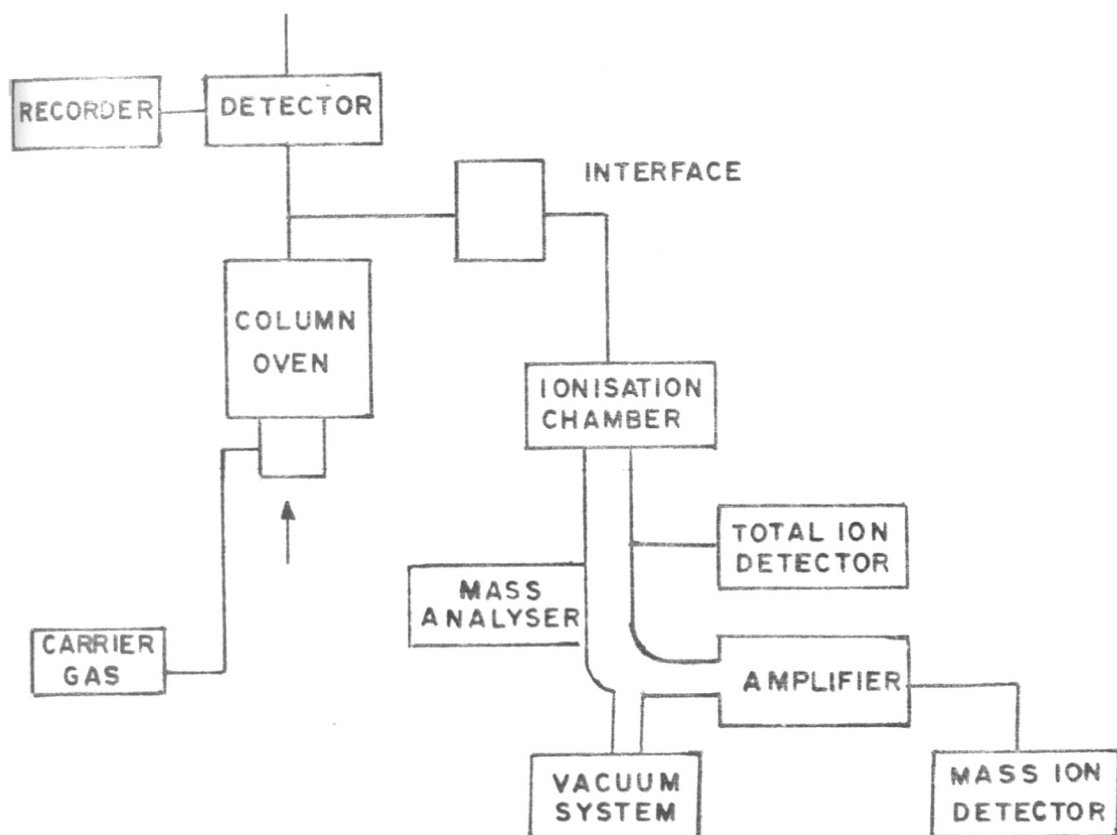
3.1.1.1 THE INSTRUMENT (Fig. 1): In a mass spectrometer, a compound under investigation is bombarded with high energy electron beam. The molecule breaks due to bond fission and rearrangement of atoms. Positively charged molecular ions are formed and recorded by a suitable device. Mass fragmentation pattern thus obtained, provides information about structure of a compound.

Advantages of mass spectroscopy become multifold by coupling it with gas chromatograph. The development is a great achievement in analytical chemistry. A brief account is as follows. James and Martin¹ first discovered gas chromatographic technique in 1952. Due to the great potential of the analytical method, it was acknowledged soon. Though the method has marked advantages, due to the principle of relative determination, its applicability is restricted. Many attempts were made to improve the utility by coupling the instrument with another analytical systems^{2,3}. Among them, mass spectrometry is most effective ancillary device^{4,5}. It was first reported by Homes and Morrell⁶ in 1957. First practical GCMS on-line combination was described by Gohlke⁷ in 1959.



COUPLED GAS CHROMATOGRAPHY / MASS SPECTROMETRY INSTRUMENT
(PHOTOGRAPH)

FIG. 1



COUPLED GAS CHROMATOGRAPHY / MASS SPECTROMETRY INSTRUMENT
(SCHEMATIC VIEW)

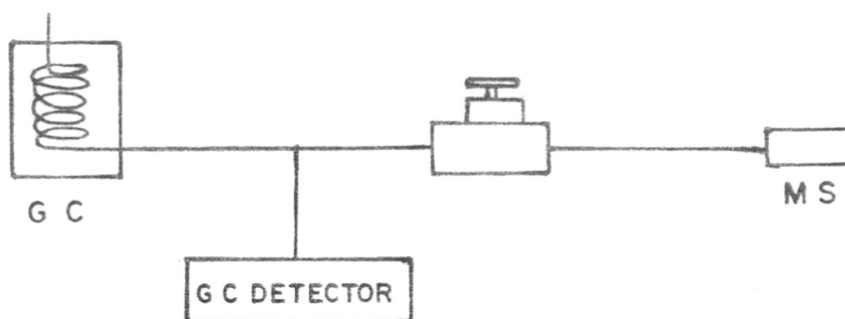
FIG. 2

Basic components in GCMS apparatus are shown in Figure 2. (Please refer previous chapter for gas chromatographic part.) The coupling of gas chromatograph and mass spectrometer (Interface) is most important component of the instrument. The spectroscopic portion has following components. (1) Interface, (2) Ionisation Chamber, (3) Mass Analyser, (4) Vacuum system, (5) Amplifier and (6) Recorder. They are described in detail as follows:

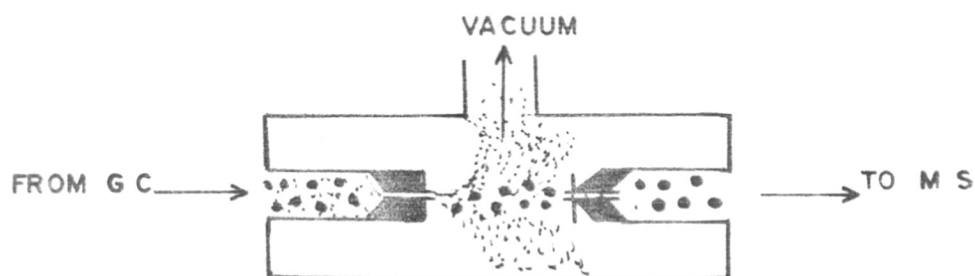
(1) Interface: It is a linkage between two different types of system and a most critical part of combined operation.

At the beginning, a simple needle valve (Fig. 3) was used. It splits the carrier gas stream at the exit of column and accepts only the small amount of the gas directly entering into the ion chamber⁶.

For more sophisticated analysis, enrichment of sample vapours in effluent carrier gas is essential. The fractionation is achieved by molecular separator device. First of this type includes the enrichment by effusion through fine pores or a narrow slit. Glass frit separator⁸ is also popular. A sintered glass tube having a ultrafine porosity is enclosed in a vacuum envelop with glass capillaries which cause a drop in pressure. The effluent gas effuses through the porous tube and only some quantity of enriched fraction leads to mass spectrometer. Silver, stainless steel and ceramic porous



NEEDLE VALVE
FIG 3



RYHAGE JET SEPARATOR

FIG 4

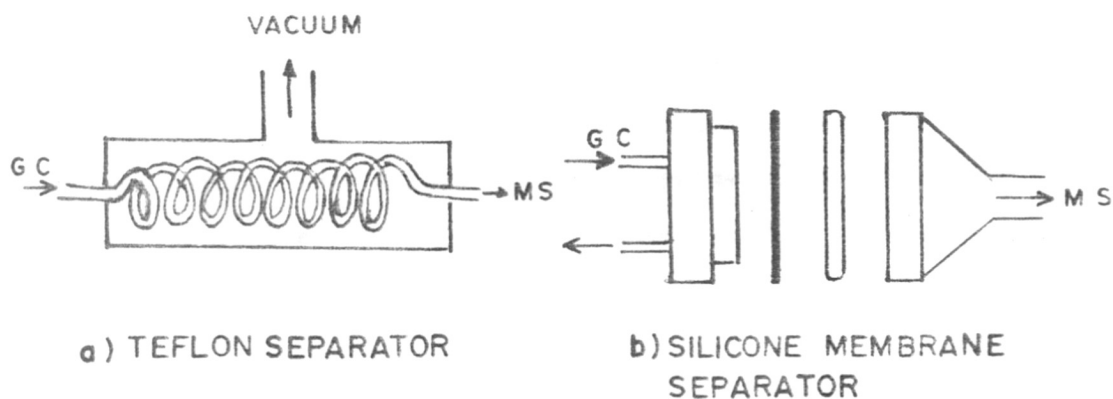


FIG 5

separators have also been proposed^{9,10}. Metal sinters are more convenient due to uniform pore size but they have a major drawback that they are optimised only at one set of flow conduction. The variable conductance separators can be used with different flow rates¹¹. The effusion occurs through a small adjustable slit formed between a flat cover-plate and the edges of two annular rings, 2 cm in diameter.

In another type called as jet separator¹², effluent jet from chromatograph expands rapidly in the evacuated area resulting in enrichment of heavier molecules of solute in the core which only enters the mass spectrometer (Fig. 4).

Semipermeable membrane separators are most widely accepted. These membranes eliminate molecules having low molecular weights. So carrier gas as well as solvent molecules are rejected and highly enriched solute molecules only enter in ionisation chamber. The Teflon separator which contains thin Teflon film is described by Lipskey et al.¹³. It consists of 200 cm long, thin walled 0.01 cm Teflon capillary tubing housed in a glass jacket which is attached to a rotary pump (Fig. 5a). Permeability and durability of these separators collapse at higher temperature ($>250^{\circ}\text{C}$). Palladium-silver alloys are highly permeable to hydrogen. Therefore they are used to eliminate hydrogen molecules from

the carrier gas¹⁴. It gives 100% enrichment as carrier gas molecules are totally rejected. Due to the possibility of catalytic reduction of sensitive compounds and corrosivity of sulfur and iodine compounds, its use is very limited.

Silicone membrane separator consists of a thin polymer barrier which preferentially allows organic vapours. In a small chamber, thin silicon rubber membrane is supported on a fine metal or glass sinter (Fig. 5b). Inorganic carrier gas is totally eliminated and passes through the exit while only organic molecules are permitted to mass spectrometer. It was first proposed by Llewellyn and Littlejohn¹⁵. Lot of research, mathematical derivations and number of modifications are carried out on this type of membrane¹⁶.

2) Ionisation Chamber: Positively charged ions are produced in this part. Electron impact process is most widely used for organic compounds. Electrons are emitted from a hot filament and are accelerated towards anode. Interaction between the electron beam and organic molecules produce ions. This method is very popular because of stability, high efficiency, precise control and simplicity of construction. Sometimes a signal of molecular ion is very weak. To overcome this drawback, chemical ionisation¹⁷ is very useful. It occurs between sample gas and reactant gas at differential pressures in an ion chamber. The process yields mostly

molecular ion due to low energy transfer. Simple hydrocarbons like methane, isobutane are common reactant gases. In field ionisation process, sample ionization is at low energy level, resulting in increase in abundance of parent ion. Its use in GCMS is found to be valuable¹⁸.

(3) Mass Analyser: It disperses mass ions. A simplest type, single focussing mass spectrometer is commonly used. While passing through an homogeneous magnetic field, the ions get dispersed according to their masses and follow a curved path. In double focussing mass spectrometer, the ions are passed through electric field followed by a magnetic field. In former, ions are dispersed according to differential energy; while in the latter, they are separated according to their momentum. So ions get separated individually for accurate mass determination. The double beam technique is very useful in high resolution applications for small amount of samples. In time-of-flight mass spectrometer, a repeller voltage pulse of a few nanoseconds duration is applied to eject out ^a bundle of ions, which is accelerated by multi-thousand volts. The ions then enter into the field-free drift region at a constant velocity. During the flight, ions with different masses are separated according to the time of the original voltage pulse. Its advantages are; higher abundance of molecular ions (M^+) and fast response which is

particularly required for GCMS. More recently quadrupole mass filters¹⁹ are very popular. It consists of a quadrant of parallel hyperbolic or circular rods which provides radio frequency field. The oscillating field causes oscillations of ions with increased amplitude and ions are scanned according to their masses.

4) Amplifier: There are two types of amplifiers; electrometer amplifier and electron amplifier. In the former, current is amplified to give high precision and accuracy of measurement of ion beam intensity. The electron multiplier²⁰ is universally used as it amplifies the current. As it lacks stability and sensitivity, it is coupled with electrometer amplifier to facilitate fast recording. Sixteen stage copper Beryllium multipliers or dynodes containing two parallel glass plates are in use.

5) Recorder: There are several types of recorders. In modern oscillographic recorder, several small galvanometer assemblies with its own input terminal are inserted into magnetic block. Current passing through small coil causes deflection of mirror which in turn reflects light beam from mercury arc lamp on UV sensitive paper. These recorders are relatively insensitive. Magnetic tape recorder²¹ is mostly useful to record very narrow peaks' width for high resolution

and to reduce the volume of data which can be computer-processed. Photoplate recording has an advantage that scanning of mass spectrum is not required but due to various drawbacks with the system, it is recommended to be avoided. Old set of differential potentiometric recorders are still in use for low molecular weight compounds.

Vacuum system is not described here as it mostly involves mechanical engineering.

3.1.1.2 APPLICATIONS IN PESTICIDES: Now-a-days GCMS techniques has been used in almost all fields of investigations^{22,23}. The use of GCMS in pesticide chemistry is relatively a recent development. Therefore its usefulness in this field is still to be exploited fully. Recent reports on pollution studies have emphasised the need of confirming pesticide residues from environment. Among the spectroscopic and chemical methods available for the same, GCMS is outstanding technique due to rapid analysis, elimination of laborious job of isolating pure compounds and greater certainty of identification.

Earlier studies were mostly concentrated on chlorinated pesticides where confirmation of residue, characterisation of metabolites and photodecomposition products are facilitated due to larger accumulation of mass spectroscopic data of related compounds and also due to immediate need of determining long persistent pollutants.

Barthel²⁴ has analysed p-chlorophenol in human blood and urine using diagnostic peaks. In the same year, Bellman and Barry²⁵ have reviewed utility of GCMS by using molecular separator for organochlorine pesticides. Biros and Sullivan²⁶ determined 1-naphthol, a major metabolite of carbaryl in urine. They developed a method of hydrolysis of urine, benzene extraction, derivatization and column chromatography. In case of 2,4-D and 2,4,5-T; the technique was used to confirm intact residues, known metabolites and also to characterise unknown metabolites²⁷. Confirmatory evidence for the presence of polychlorinated biphenyls (PCB) in fish, seabird and human fat was collected by Widmark²⁸. Aniline derivatives of azobenzenes in soil were characterised using the same technique by Kearney²⁹. A long persistent pesticides and most threatening pollution hazard: DDE and DDT were studied by Plimmer³⁰ by characterising several photolysis products from them. We have also reported studies of chlordane mixture using the same technique³¹.

Determination of organophosphorus pesticide residues is more complicated due to insufficient mass spectral data of degraded products. Parathion metabolites such as diethyl phosphoric and diethyl thiophosphoric acids in urine were examined by Shafik³². Birds and Walker³³ have reported detailed application of GCMS technique to pesticide residue in human tissue. Structure elucidation of ethyl parathion,

a residue from green tea was done by Yugi³⁴, while Bonelli et al.³⁵ have evaluated the technique to determine metabolic pathways of O^{18} organophosphate pesticides. They also introduced computerised methods³⁶. Variety of compounds which are relevant with trialkyl phosphate esters were studied by Biros et al.³⁷.

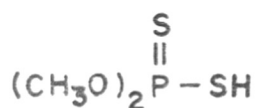
3.1.2 THE PESTICIDE: DIMETHOATE

O,O-Dimethyl S-(N-methylcarbomoylmethyl) phosphorothiolothionate or Dimethoate³⁸ (Fig. 6, III) belongs to a class of thiolothionates. It was first introduced by American Cyanamide Co. (USA).

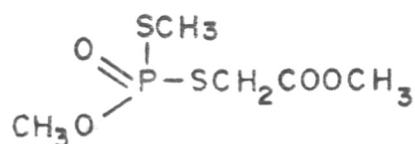
Its method of preparation³⁹ is as follows (Fig. 6). Heterogeneous reaction mixture of phosphorus pentasulphide and methanol in benzene is refluxed for 2 hr to get a solution of O,O-dimethyl phosphorodithioic acid (I). Its sodium salt in aqueous solution is then allowed to react with methyl chloroacetate to get O,O-dimethyl S-(methoxycarbonylmethyl) phosphorothiolothionate (II) in 90% yield. Aminolysation using methyl amine gives the pesticide: Dimethoate (III) in 87% yield. It is a white crystalline compound (m.p. 52°C). Technical product is about 85-90% pure. The nature of impurities vary according to the mode



PREPARATION OF DIMETHOATE, (III)



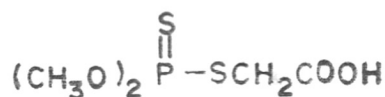
(I)



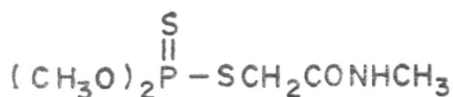
(V)



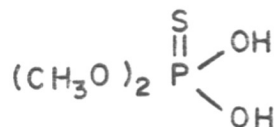
(II)



(VI)



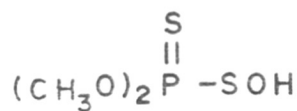
(III)



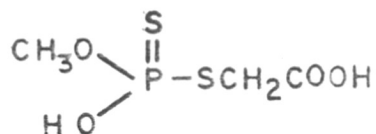
(VII)



(IV)



(VIII)



(IX)

FIG. 6

of preparation. Alongwith O,O -^S-trimethyl phosphorothiolothionate, the oxo analogue: Dimethoxon (Fig. 6, IV) is also present in trace quantities but causes toxicity to mammals. Dimethoate prepared by above method contains II-6.9%, V-5.0% (Fig. 6).

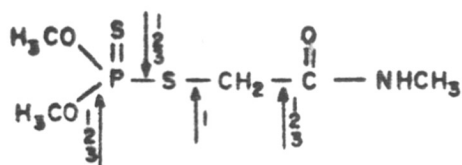
It is a broad spectrum systemic pesticide which is particularly effective against sucking insects such as Diptera and susceptible spider mites.

Dimethoate is one of the most studied pesticide. Its metabolism has different routes depending upon the species⁴⁰. The degradation in rat and mouse liver yields compounds such as dimethoate acid (Fig. 6, VI) and O,O -dimethyl phosphorodithioic acid (I). From sheep liver, only VI has been isolated. In many insects, phosphatase reaction is predominant over amidase reaction⁴¹. In boll weevil, there is a hydrolytic cleavage at C-N and C-S bonds producing I and VI⁴². IV is also produced but rapidly hydrolyses to other non-toxic forms such as VI, dimethyl^{thio}phosphate (Fig. 6, VII), dimethyl dithiophosphate (Fig. 6, VIII) and desmethyl dimethoate acid (Fig. 6, IX). In olive fruit metabolism⁴³, end products are inorganic phosphates. The degradation in rat mainly depends upon concentration of the pesticide⁴⁰. At higher concentration (10^{-3} mole), there occurs C-N fission while at lower

concentration, C-S fission takes place. The effect of amidase action depends upon species and sex. Comparative studies of mode of metabolism in plants, insects and rat has been reported by Morikowa and Saito⁴⁴ (Fig. 7). Its main features are as follows. O-Dealkylation and carboxylation occur in plants while in rat, S-C bond fission is predominant. General metabolic pathways can be summarised as shown in Fig. 8.

Hydrolysis of Dimethoate readily occurs in alkaline medium (at 70°C and 9 pH, half life 0.8 hr) but in acidic medium it is very stable (at 70°C and 2 pH, half life 21 days).

Numerous reports are available on decomposition of Dimethoate due to environmental effects. Reports pertaining to temperature are mentioned here. Dimethoate is highly photosensitive⁴⁵. It degrades fast when exposed to sunlight⁴⁶. On storage at room temperature, it degrades slowly into methyl amine and mercapto acetic acid⁴⁷. Humidity was considered as vital factor in the decomposition rather than light and temperature. Semenova and Siforova⁴⁸ have studied detoxication of Dimethoate at elevated temperature. Acetone solution of Dimethoate was coated on glass plates and kept at different temperatures. They discovered that toxicity reduced to one half by keeping it at room temperature for 50 days, but its half life is only



1. rat metabolism
2. insect metabolism
3. plant metabolism

FIG. 7

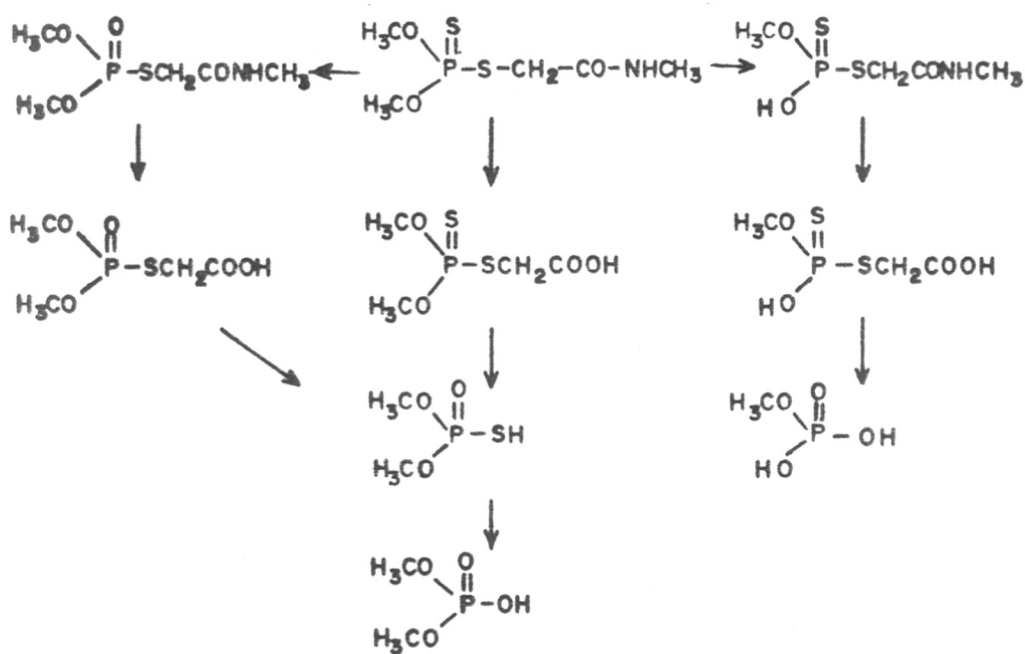


FIG. 8

4 hr at 55°C.

Temperature seems to play a key role in decreasing toxicity of Dimethoate in soil. When disappearance curves in semi-logarithmic co-ordinate systems were plotted, it was shown that rate of decomposition is proportional to the temperature of soil and moisture contents⁴⁹. In another experiment using irrigated and autoclaved soil, it was reported that decomposition was mainly due to heat⁵⁰. Mishra et al.⁵¹ have reported that Dimethoate does not persist in soil for very long time.

Spynu et al.⁵² developed a mathematical model to forecast kinetics of Dimethoate in plants. They reported that elapsed time and temperature are major factors. They further evaluated the model to predict Dimethoate residues in plants, vegetables and fruits⁵³. The level and duration of residue shown to be dependent on climatic conditions, particularly temperature. Eventhough Dimethoate hydrolyses fast in water^{49,54} at elevated temperature of water, the toxicity increases multifold⁵⁵ presumably due to oxo analogue (IV).

The effect of temperature on rate of hydrolysis is also very important as the rate increases four times by the temperature rise of each 10°C ⁵⁶. Thus temperature is the vital factor which is responsible for environmental decomposition of the pesticide.

3.1.3 PRESENT INVESTIGATIONS

Effect of temperature on decomposition of dimethoate has been discussed in 3.1.2 . Some laboratory observations pertaining to the same are worth noted. When technical product was gas chromatographed at 180°C on SE-30 (5%) phase, packed in aluminium columns, it has been observed that the chromatogram possesses a disordered pattern of number of peaks ranging from six to ten⁵⁷. As injector port, oven and detector were at 150°-250°C temperature range, temperature may be mainly responsible for the decomposition alongwith other catalytic reactions. In another experiment⁵⁷, attempts were made to remove low boiling components in technical Dimethoate by distillation under reduced pressure and it was observed that it instantly decomposed into blackish solid and dark brownish liquid collected in receiver.

In view of above mentioned observations, it was a prime importance to explore exact nature of role played by temperature in these cases. The explosion at elevated temperature is also an unique and unidentified feature of Dimethoate. Additionally, there is a probability that ultimate fate of the pesticide in environment, particularly on storage may be the same as occurring in explosion phenomenon. Therefore the investigations may be helpful

in identifying unknown hazardous residue and to confirm presence of known decomposition products which in turn may be useful to solve the pesticide residue problems.

Due to the great potential of GCMS applications which is still to be exploited fully in such cases, it was thought to investigate in thermal decomposition of Dimethoate using GCMS technique.

3.2 EXPERIMENTAL

3.2.1 PREPARATION OF SAMPLES

1) O,O-Dimethyl S-(methoxycarbonylmethyl) phosphorothiolothionate^{39,58} (Fig. 6, II, X): Powdered phosphorus pentasulfide (10 g) was suspended in distilled benzene (10 ml) and the mixture was heated at 64°C with stirring. Methanol (8 ml) was added dropwise over a period of 1 hr with enough heating for reflux. H₂S evolved was allowed through an outlet tube. The reaction mixture was further heated for 2 hr. Initial yellow coloured mixture turned into dark brown liquid. The cooled reaction mixture was filtered to get clear benzene solution of O,O-dimethyl phosphorodithioic acid (Fig. 6, I). It was dissolved in aqueous solution of NaOH (10%, 20 ml). The organic layer was removed. The aqueous layer was acidified and saturated with sodium chloride to get colourless layer of the acid. It was dried over anhydrous Na₂SO₄. I (1.74 g, 0.011 M) was added to aqueous NaOH solution (0.44 g, in 10 ml). The clear aqueous layer was mixed with methyl chloroacetate (1.09 g, 0.01 M) in a 100 ml round bottom flask equipped with a water condenser and a stirrer. The mixture was heated at 60°C for 4 hr with vigorous stirring. It was then cooled and organic layer was removed. The layer was washed with three

parts of Na_2CO_3 solution (0.5 g in 15 ml H_2O) and then with water till washings were neutral to litmus. It was dried over anhydrous Na_2SO_4 . Yield 2.07 g (90%).

2) O,O-Dimethyl S-(N-methyl carbomoyl methyl) phosphorothiolothionate³⁸ (Fig. 6, III): Technical grade sample was supplied by Bharat Pulverising Mills, Bombay (India), m.p. 49°C.

It was recrystallized from benzene, m.p. 52°C.

$\text{ClCH}_2\text{COOCH}_3$, 1 and 2 were analysed by TLC.

TLC : Pet. ether: Acetone (3:1) solvent system and silica gel, single spot for each compound, R_f values $\text{ClCH}_2\text{COOCH}_3$ -1.00, 1-0.50, 2-0.40.

3.2.2 THERMAL DECOMPOSITION (Fig. 9)

In a 500 ml round bottom flask with an air distillation condenser having a reflux column (30 cm) and a thermometer, Dimethoate (2, 6.546 g) was taken. The flask was slowly heated on a sand bath with a Bunsen burner.

At bath temperature of 148°C, the colourless syrupy liquid instantly decomposed into blackish deformed, solid mass and a dark brown liquid which was collected in the receiver. Evolution of white fumes was also observed.

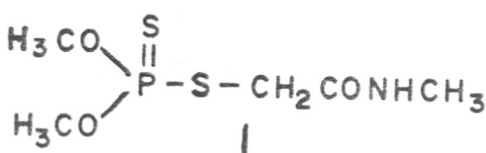


FIG. 9

The solid mass in the reaction flask was extracted with ether, the ethereal layer dried over anhydrous Na_2SO_4 and the solvent was evaporated at room temperature. The residue thus obtained was combined with liquid portion from the receiver and was named as 'organic part' (1.30 g, 19.9%). Ether insoluble portion was dried in vacuum at room temperature for 2 hr. It was named as inorganic part (5.66 g, 77.4%).

Quantitative determination of various components from decomposed dimethoate is summarised in Table 1.

3.2.2.1 INORGANIC PART : The highly hygroscopic material was dissolved in water (50 ml). The solution was filtered. The black residue was washed with water (50 ml) and dried at 120°C for 2 hr (0.0301 g, 4.6%). The acidic filtrate when titrated against NaOH solution, found to be of 0.182 N. A small portion of the filtrate (10 ml) was heated ($95^\circ\text{C}/50$ mm) to remove water completely to recover back the solid material. Residue and filtrate were analysed for C, H, N, P & S. The results are mentioned in Table 2. Water insoluble part was therefore identified as pure charcoal, while water soluble part as oxides of phosphorus.

Table 1

No.	Details	Percentage*
1.	Total organic part	19.9
2.	Total inorganic part	77.4
3.	Water soluble inorganic part	72.8
4.	Water insoluble inorganic part	4.6
5.	Loss in weight	3.7

* Percentages were reported with respect to the initial weight of Dimethoate

Table 2

No.	Details	Elements present (C, H, N, P and S)	Percentage
1.	Water insoluble part	Carbon only	100
2.	Water soluble part	phosphorus only	18

3.2.2.2 ORGANIC PART: It was a dark brown, thick syrupy liquid with a very offensive odour. Elemental analysis is as follows.

C-20.93%, H-6.63%, N-4.68%, P-8.0%

This part was further studied for quantitative determination of the components.

3.2.3 ANALYTICAL METHODS

The organic part was first studied by gas chromatography for percentage determination of the components and then by GCMS for identification of components.

3.2.3.1 GAS CHROMATOGRAPHY: The details of instrumental parameters are as follows:

Unit	: AIMIL-NCL Dual Column chromatograph, Mark II, No.70020.
Columns	: Aluminium, 930 mm x 2 mm (ID)
Phase	: 5% Carbowax-20 M (Varian aero GR, No.82-1115) on 60-80 mesh chromosorb W. (Analabs, Inc-USA, AW-DMCS, Cat. No.-GCB-0068).
Detector	: Two filament thermal conductivity detector (GOW-MAC, Part 13-004, Code W2X).
Carrier Gas	: Hydrogen, 20 ml/min.
Temperature	: Oven 200°C : Injector 270°C : Detector 270°C

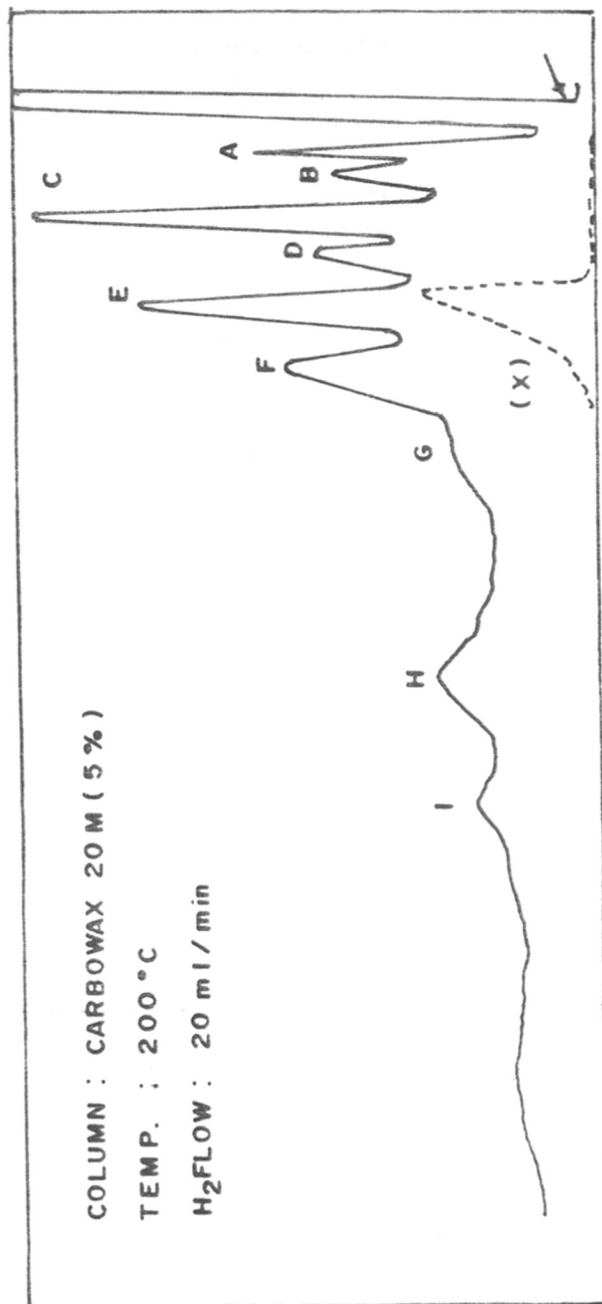
The chromatogram obtained is a mixture of nine components (Fig. 10). Percentage of the components were determined using standard solution technique⁵⁹. Pure O,O-dimethyl S-(methoxycarbonylmethyl) phosphorothiothionate⁵⁸ ^(X) was taken as standard.

Organic part (1.00 g) and X (0.102 g) weighed accurately, and diluted to 10 cc in separate volumetric flasks using distilled acetone (treated with KMnO_4). These two standard solutions were used for quantitative estimation of the components. Under identical conditions as mentioned above, equal amount of both solutions were injected to get good and consistent chromatograms (Fig. 10). Percentage of the components was calculated by triangulation method⁶⁰ in which area under triangular curves was compared with the area of peak obtained from standard solution of (X). The percentage and retention times have been summarised in Table 3.

3.2.3.2 COUPLED GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GCMS):

The instrumental parameters are as follows:

Unit	: ARI-30
Columns	: Glass, 930 mm x 2 mm (I.D.)
Phase	: 5% Carbowax-20 M on 60-80 mesh chromosorb-W (temperature 200°C)
Carrier Gas	: Helium, 20 ml/min.
Interface	: Silicon membrane.
Detector	: Total ion detector



GAS CHROMATOGRAM OF ORGANIC PART

FIG 10

Table 3 (Fig. 10)

Peak No.	<u>Retention Time</u>		Percentage*
	min.	sec.	
X	2	46	10.290
A	0	31	0.683
B	0	48	0.653
C	1	25	3.205
D	2	01	1.053
E	2	42	2.727
F	3	35	2.434
G	5	12	0.321
H	8	36	1.683
I	10	49	0.725

*Percentages were reported with respect to the initial weight of Dimethoate

Table 4 (Fig. 11)

Peak No.	Base Peak	Mole.Wt.
A	126	158
B	61	140
C	93	172
D	125	172
E	110	156
F	129	168
G	73	168
H	58	188
I	141	188

The vapourised samples were fed to mass spectrometer through silicon membrane and at peak maxima they were scanned. Complete mass spectrum of each component was obtained. Fragmentation patterns of these components were examined for identification purposes. Mass ions of fragments are mentioned in Table 4 (Fig. 11).

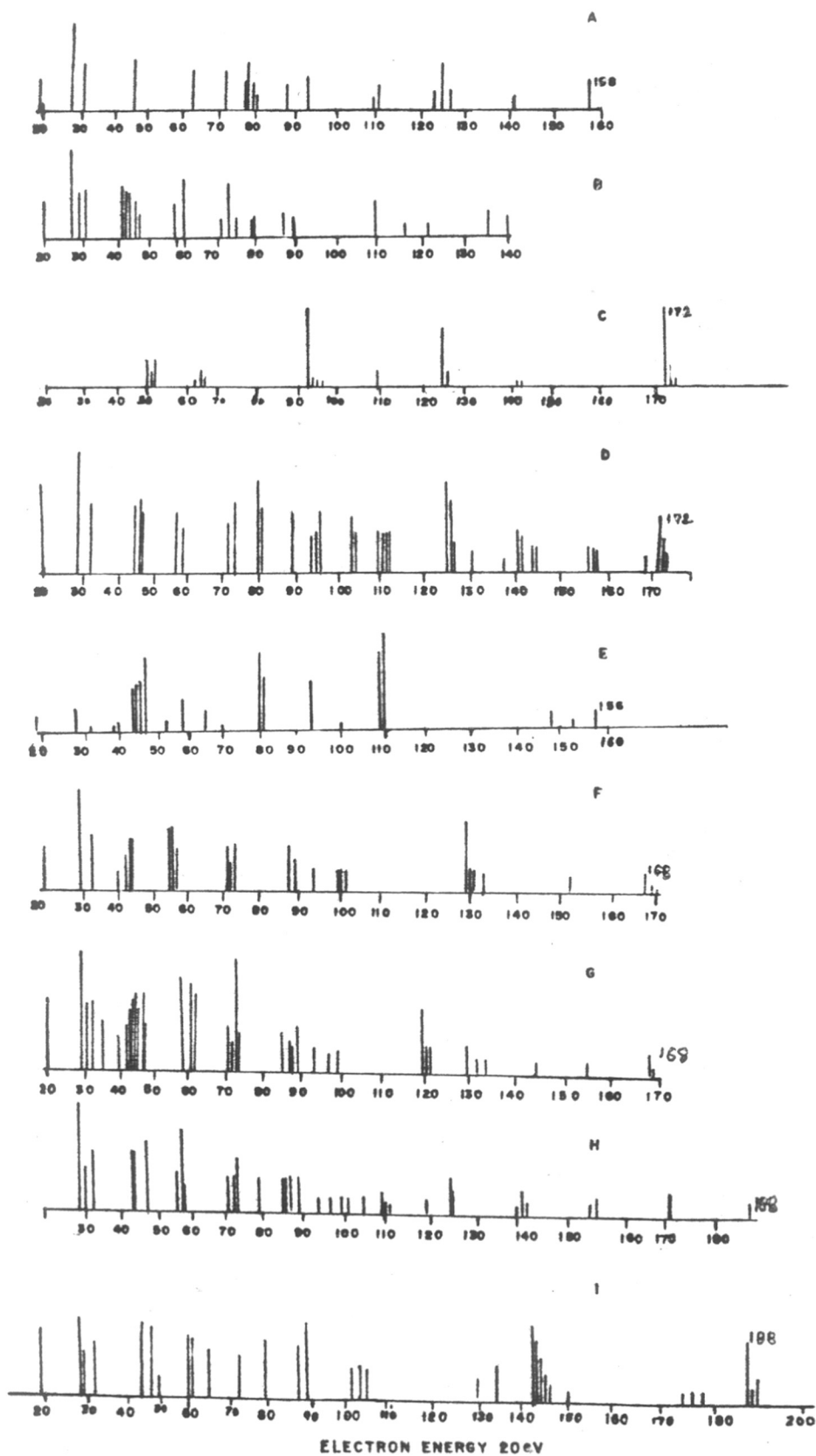


FIG. 11.

3.3. RESULTS AND DISCUSSION

Present studies were taken up to examine the role of temperature in decomposition of Dimethoate and to co-relate the products with the compounds obtained in environmental degradation. It may also provide more information about the cause of explosion phenomenon occurring exclusively with Dimethoate.

Dimethoate was heated upto explosion temperature (148°C) and the products were separated into inorganic and organic parts. They are described in detail as given below.

3.3.1 INORGANIC PART

It is a black, hygroscopic and deformed solid mass. It was dissolved in water and separated into filtrate and residue.

3.3.1.1 FILTRATE: It was blackish solution which became yellowish on dilution. When the material was analysed for the presence of carbon, hydrogen, nitrogen, phosphorus and sulfur; only phosphorus (18%) found to be present in it. The solid was therefore identified as phosphorus oxides of which acidic nature has been confirmed by titrating it against standard NaOH solution. Eventhough phosphorus is 13.5% in Dimethoate, phosphorus derivatives aggregate

about 72.8%. It may be due to formation of higher oxidation products of phosphorus in Dimethoate.

Many workers have reported that inorganic phosphates are the ultimate end products of Dimethoate in enzymatic as well as nonenzymatic transformations^{42,43}. They have been undoubtedly formed due to the replacement of sulfur atoms by oxygen and also by desmethylation of $P-OCH_3$ linkages⁴². Incidentally desmethylation is also observed in the formation of phosphorus oxides in present investigations. This identity serves to unfold the nature of decomposition of Dimethoate during storage and in soil i.e. reactions occurring in absence of enzymes. Thus temperature is the major factor alongwith the moisture for desmethyl reactions. Eventhough the decomposition is rather slow at room temperature which is far below the explosion temperature, final residue is essentially the same. Noting these facts, present studies seem to be very useful in explaining formation of the inorganic residue.

3.3.1.2 RESIDUE: It was a black coarse mass having nonadhering character. When it was analysed for elemental presence, it has been found to be pure carbon in the form of charcoal. Eventhough Dimethoate molecule contains 26.2% carbon, only 4.6% was found in the isolated form. At the beginning, it was assumed that some carbon might have escaped in the form of CO_2 but the explosion temperature does

not seem to be high enough to cause the conversion. Carbon contents could not be accounted fully. Carbon as one of the decomposition product has been reported for the first time. In thermal decomposition; it seems that at the explosion temperature, the molecule becomes highly unstable and the heat energy at 148°C is sufficient to break strong sigma bonds between carbon and hydrogen atoms. The low energy requirement may be due to polarisation of bonds in the molecule. Therefore presence of carbon in free form may be possible. Eventhough formation of CO_2 does not sound probable, it is one of the possibilities. The thermal decomposition phenomenon has similarities with the proceeding reactions occurring in earthquake i.e. Biological material which gets buried is converted into coal, natural gas and petroleum. Eventhough basic difference of high temperature and availability of oxygen remains, the phenomenon seems to be identical as it involves charcoal, oxides as residues, fragmented organic molecules and gases in the form of fumes.

3.3.1.3 WHITE FUMES: The fumes could not be trapped at the temperature of ice cold water using 40 cm long distillation column. The discrepancy in accounting percentages of elements in the decomposed products suggests that the gases may be containing sulfur dioxide, nitrogen oxides and

carbon dioxide along with ammonia and methyl amine. Any possibility of methyl amine was rejected due to obvious reasons. As the fumes did not affect moist turmeric paper, ammonia is also not present in it. Formation of gases is supposed to be due to combination of elements in polarised form. Eventhough it has been reported that $\text{CO}_2, \text{CH}_3.\text{NH}_2$ evolved in the degradation of Dimethoate⁶¹; the explosion temperature is not enough for these conversions to take place. Therefore presence of polarised forms seems to be more logical. As the environmental conditions are comparatively milder, this phenomenon may not occur but methyl amine, as one of the metabolites has been reported⁶². The observations are not confirmed. Therefore the conclusions are mentioned in brief.

3.3.2 ORGANIC PART

It was a dark brown thick liquid only 13.5% of total Dimethoate. This part when analysed by gas chromatography, resolved into nine components (Fig. 10). Their percentages were determined using standard solution technique⁵⁹ in which pure O,O-dimethyl S-(methoxycarbonylmethyl)phosphorodithiolothionate⁵⁸ (Fig. 6, II, X) was used as standard (Table 3). Total volatile portion in the organic part was found to be 68%. Remaining nonvolatile residue may be a

complex mixture of the compounds with high molecular weights.

3.3.2.1 APPLICATION OF GCMS: When distillation of the mixture was tried at atmospheric as well as at reduced pressure, it was difficult to get a distillate.

Additionally, the gas chromatographic pattern changed; probably due to the intermolecular conversions. Column chromatography using silica gel in the ratio 1:50 was also tried. When the chromatographic fractions and the total mixture was gas chromatographed, many irrelevant peaks appeared in chromatograms. They were presumably due to further decomposition, enrichment of components in trace amounts or some catalytic reactions. These failures hamper isolation of pure components in the mixture.

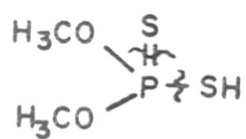
Here gas chromatography/mass spectrometry (GCMS) which can overcome these difficulties comes in picture. It has many advantages over other conventional analytical methods^{4,5}. It eliminates the task of isolating small quantities of pure components, particularly by tedious procedure of column chromatography. Therefore GCMS is the ultimate technique found to be applicable to study the volatile organic part.

Eventhough the components are in lesser percentages, complete mass spectrum is obtainable with GCMS. It in turn may provide valuable information for structure elucidation.

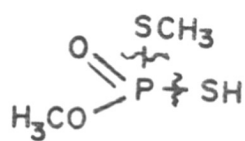
The GCMS analysis was carried on AEI-30 model where silicon membrane separator was used as interface. Studies in mass spectrometry and derivation of possible structures are discussed below.

3.3.2.2 INTERPRETATION OF SPECTRA (Fig. 12): On the basis of the fragmentation pattern of peak A, three structures were proposed (Ia-c). As m/e 125 and 93 are prominent in Ia⁶³ but absent in the spectrum, the structure was ruled out. I.b was not the possible structure as it produces mass fragments: m/e 125 and 78 by the loss of SH and SCH₃ respectively. Eventhough loss of sulfur atom from I.a is possible to produce mass ion at m/e 126, -SH is more labile to get a fragment at m/e 125⁶³. On the other hand, I.c explains all major mass fragments: m/e 126, 111, 94, 72, 64 and 47. Therefore it has been assigned the structure: O,S-dimethyl dithiophosphate (I9).

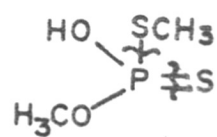
Peaks C,D and E have been assigned structures: O,O,S-trimethyl phosphorothiothionate (II); O,S,S-trimethyl phosphorodithiolate (III) and O,O,S-trimethyl phosphorothiolate (IV) respectively because their patterns were identical with that reported by Santoro⁶⁴ for the same.



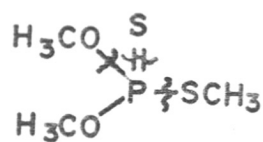
(I-a)



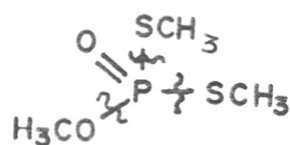
(I-b)



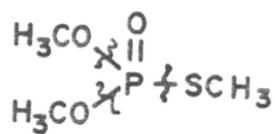
(I-c)



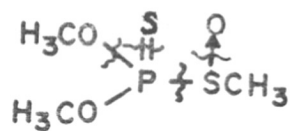
(II)



(III)



(IV)



(V)

FIG. 12

There occurs normal α cleavages of SCH_3 , S, OCH_3 , etc.⁶⁴

Major fragments of peak H: m/e 188, 172, 157, 125 and 93 explains elimination of labile oxygen atom attached to thiole sulfur and α cleavages from P-S bond⁶³. Therefore the structure: sulfoxide derivative of O,O,S-trimethyl phosphorothiolothionate (V) has been assigned to it.

Peak B (mole. wt. 140), F and G (mole.wt. 168) and I (mole.wt. 188) possess unrelevant pattern. So they were not interpreted. Proton migration theory which was proposed by Damico⁶⁵ has not been observed probably due to short chain length.

3.3.2.3 GENESIS (Fig. 13): It is notable that the organic part^{gave}/reproducible gas chromatogram. Therefore it was assumed that they have acquired stable configuration. Pistchimuka isomerisation or $\text{OCH}_3 \rightarrow \text{SCH}_3$ rearrangement (a) is the initial transformation occurring in Dimethoate molecule⁶⁶. The amido sidechain may form a cyclic intermediate with phosphoryl bond, resulting in formation of P-OH bond by proton transfer (b)⁶⁷. This electron rearrangement breaks S-C bond to form O,S-dimethyl dithiophosphate (I)⁶⁸. This type of migration has been reported by Damico⁶⁵ in interpreting mass spectra of organophosphorus compounds such as Dimethoxon (Fig. 6, IV).

Migration of terminal methyl group in side chain to thiono sulfur is also possible in the cyclic intermediate (c)⁶⁷. It cleaves S-C bond yielding in O,O,S-trimethyl phosphorothiolothionate (II)⁶⁹. Its oxidation (d) takes place at elevated temperature⁷⁰ to get O,O,S-trimethyl phosphorothiolate (IV)⁶⁹ and sulfoxide (V). The product (III) is the isomer of II which is formed due to pistchimuka isomerisation⁶⁶. However, presence of I and V was not confirmed.

Thus the thermal impact is $\text{O}-\text{CH}_3 \rightarrow \text{SCH}_3$ rearrangement, migration of H and CH_3 from the sidechain to skeleton sulfur and oxygen, predominant cleavage of S-C linkage and promotion of auto-oxidation. The migration seems to be mainly responsible to create labile centres in the side-chain, causing decomposition of the molecule.

3.3.2.4 DEGRADATION STUDIES: Eventhough O,O-dimethyl phosphorodithioic acid as a major degradation product has been reported^{40,42,44}, its isomer (I) has not been reported so far. Kubel and Ackermann⁷¹ have reported occurrence of trimethyl phosphorothiolothionates as metabolic products but their formation was not clarified. O,O-Dimethyl S-(methoxycarbonylmethyl) phosphorothiolothionate (Fig.6, II) and O,O,S-trimethyl phosphorothiolothionate (II) simultaneously occur as impurities

in technical grade sample. In addition to possibilities discussed earlier for esters' formation, II may be formed by decarboxylation of the former. Both are likely to be carried in formulation also but they are rarely detected probably due to hydrolysis.

Eventhough the products have not much relevance with the degradation studies, they explain impurity of II in stored products and other possible transformations which may occur, particularly in the technical product and intermediates such as O,O-dimethyl phosphorodithioic acid ^(I) and O,O-dimethyl S-(methoxycarbonylmethyl)phosphorothiolothionate (II) (Fig 6).

R E F E R E N C E S

1. James, A.T. and Martin, A.J.P., *Biochem.J.*, 50, 679 (1952).
2. Ettre, L.S. and McFadden, W.H., Eds., *Ancillary techniques of Gas Chromatography*, Wiley-Interscience, New York (1969).
3. Leathard, D.A. and Shurlock, B.C., *Identification Techniques in Gas Chromatography*, Wiley-Interscience, London (1970).
4. Harrington, D.B., *5th Annual Conf. on Mass Spectrom and Allied Topics*, New York (1957).
5. Donner, W., Johns, T. and Gallaway, W., *ibid.*
6. Homes, J.C. and Morrell, F.A., *Appl.Spec.*, 11, 86 (1957).
7. Gohlke, R.S., *Anal.Chem.*, 31, 535 (1959).
8. McLafferty, F.W., *Science*, 151, 641 (1966).
9. Blumer, M., *Anal.Chem.*, 40, 1590 (1968).
10. Grayson, M.A. and Levy, R.L., *J.Chromatogr.Sci.*, 9, 687 (1971).
11. Brunnee, C., Bultemann, H.J. and Kappus, G., *17th Annual Conf. on Mass Spectrom.and Allied Topics*, Dallas, Paper No.46 (1969).
12. Ryhage, R., *Anal.Chem.*, 36, 759 (1964).

13. Lipsky, S.R., Horvath, C.G. and McMurray, W.J.,
Anal.Chem., 38, 1585 (1966).
14. Lucero, D.P. and Haley, F.C., J. Gas Chromatogr.,
6, 477 (1968).
15. Hewellyn, P.M. and Littlejohn, D.P., Pittsburgh
Conf. on Anal.Chem. and Appl.Spec., Feb. (1966).
16. McFadden, W., Technique of Combined Gas Chromatography
Mass Spectrometry, Wiley-Interscience, New York
191 (1973).
17. Fales, H.M., Milne, G.W.A. and Vestal, M.L., J.Am.
Chem.Soc., 91, 3682 (1969).
18. Damico, J.N. and Barron, R.P., Anal.Chem., 43,
17 (1971).
19. Paul, W. and Steinwedel, H. Z. Naturforsch., 8a,
448 (1953).
20. Allen, J.S., Phys.Rev., 55, 966 (1939).
21. Merrit, C., Issenberg, P.Jr., Bazinet, M.L., Green,
B.N., Merren, T.O. and Murray, J.G., Anal.Chem.,
37, 1037 (1965).
22. Junk, G.A., Int.Mass Spectrom.Ion Phys., 8, 1 (1971).
23. Burlingame, A.L. and Johanson, G.A., Anal.Chem.
Annual Rev., 44, 377R (1973).
24. Barthel, W.F., Curley, A., Thrasher, C.L., Sedlak,
V.A. and Armstrong, R., J.Assoc.Offic.Anal.Chemists,
52, 294 (1969).

25. Bellman, S.W. and Barry, T.L., Private Communication Food and Drug Admin., U.S. Dept. of Health Ed. and Welfare (1970).
26. Biros, F.J. and Sullivan, H., Unpublished observations (1970).
27. Shafik, M.T. and Sullivan, H., 7th Inter.Am. Conf. on Toxic and occupal.Med., Miami, Aug. (1970).
28. Widmark, G., J. Assoc. Offic.Anal.Chemists, 50, 1069 (1967).
29. Kearney, P.C., Plimmer, J.R. and Guardia, F.B., J.Agric.Food Chem., 17, 1418 (1969).
30. Plimmer, J.R. Klingebiel, U.I. and Hummer, B.E., Science, 167, 67 (1971).
31. Kadam, A.N., Bendre, S.B. and Ghatge, B.B., Pesticides, XII(3), 13 (1978).
32. Shafik, M.T. and Braelway, D., Am.Chem.Soc.Inst., Canada Internat.Meeting, Toronto, May (1970).
33. Biros, F.J. and Walker, A.C., J.Agric.Food Chem., 18, 425 (1970).
34. Yugi, T., Takahashi, S. and Murata, T., Shimadzu Hyron, 22, 84 (1971).
35. Bonelli, E.J. and Cornelius, J., Application Tips, No.19, Finnigan Corpn., Sunnyvale Cal. (1970).
36. Bonelli, E.J. and Cornelius, J., ibid. No.15(1970).

37. Biros, F.J. and Shafik, M.T., 163rd National Meeting Am.Chem.Soc., Boston (1972).
38. Cassaday, J.T., Hoegberg, E.I. and Gleissener, B.D., U.S. Patent, 2, 494, 283 (1948).
39. Italian Patent 661, 487 (1964).
40. Uchida, T., Dautermann, W.C. and Obrien, R.D., J.Agric.Food Chem., 12, 49 (1964).
41. Uchida, T., Rahmati, H.S. and Obrien, R.D., J.Econ. Entomol., 58, 831 (1965).
42. Bull, D.C. and Lindquist, D.A. and HacsKaylo, J., *ibid.*, 56(2), 129 (1963).
43. Santi, R. and Giacomelli, R., J.Agric.Food Chem., 10, 257 (1962).
44. Morikowa, O. and Saito, T., *Batyu-Kagaku*, 31, 130 (1966).
45. Ivie Glen, W. and Casida, J.E., J.Agric.Food Chem. 19(3), 405 (1971).
46. Ivie Glen, W. and Casida, J.E., *ibid.*, 410 (1971).
47. Fontanelli, R. and Nanforti, G.F., *Rend, Ist.Super Sanila*, 22, 32 (1959).
48. Semenova, S.A. and Siforova, T.A., *Kim,Sel.Khoz.*, 9(4), 258 (1971).
49. Bro-Sasmussen, Finn, Noeddegaard, E., *Voldumclauseuk, Pesticide Sci.*, 1(5), 179 (1970).

50. Getzin, L.W., Rosefield, Irving, J. Agric. Food Chem., 10(4), 598 (1968).
51. Mishra, R.C. and Gupta, D.S., Pesticides, 6(11), 28 (1972).
52. Spynu, E.I., Sova, R.E., Stefanskii, K.S. and Akoronko, J.L., Gig Sanit, 2, 70 (1974).
53. Spynu, E.I. and Ivonova, L.N., Zashch Rast, 9, 24 (1974).
54. Ordzhonikidze, E.K., Soobshch Akad Nauk Gruz SSR, 73(3), 705 (1974).
55. Yasuhiro, N., Suisan Zoshoku, 24(4), 140 (1977) (Japan)
56. Muhlmann, R. and Schrader, G., Z. Naturforsch., 12b, 196 (1957).
57. Kadam, A.N. and Ghatge, B.B., Unpublished observations (1979).
58. Perni, M. and Speroni, G.A., U.S. Patent 3,047,459(1955).
59. Emergy, E.M., J. Gas Chromatogr., 5, 596 (1967).
60. Condal Busch, J. Chem. Ed., 41, A235 (1964).
61. Hasan, A., Zayed, S.M.A.D. and Bahig, M.R.E., Biochem. Pharmacol., 18(10), 2429 (1969).
62. Haeskaylo, J. and Bull, D.L., J. Agric. Food Chem., 11, 464 (1963).
63. Jorg, J., Houriet, R. and Spiteller, G., Monatsh. Chem., 97, 1064 (1966).
64. Santoro, E., Org. Mass Spectrom., 7(5), 589 (1973).

65. Damico, J.N., J. Offic. Anal. Chemists, 49, 1027 (1966).
66. Pistchimuka, P., Ber., 41, 3854 (1908).
67. Eto, M., Organophosphorus Pesticides: Organic and Biological Chemistry, CRC Press, Inc., 100 (1977).
68. Teichmann, H. and Phitrong Lam, Z.Chem., 9(8), 310 (1969).
69. B., I, 286.
70. Van Wazer, J.R., Phosphorus and Its Compounds, Interscience, New York, I (1958), II (1961).
71. Kubel, D., Akermann, H. and Engst, R., Nahrung, 10(7), 611 (1966).

●

CHAPTER FOUR

DEGRADATION OF IMIDAN

●

4·1 THE PESTICIDE:IMIDAN

4·2 THIN LAYER CHROMATOGRAPHY

4·3 HYDROLYSIS STUDIES

4·4 EFFLUENT TREATMENT

●

Actually research in organophosphorus pesticides had gained pace in late fifties eventhough it had been initiated during IInd World War. By that time, long persistent organochlorine pesticides had created serious problems due to their hazardous residues. Therefore the former has been approached very cautiously and development of efficient methods to analyse technical grade products, formulations and environmental residues have been started from the beginning. More attention has also been given to detoxicate the effluent from pesticide process industries as it was observed that it has adverse effects on aquatic life. Thus the field as a whole consists of synthesis, analytical techniques, fundamental chemistry of transformations and industry oriented effluent treatment procedures.

Eventhough extensive research has been carried upto 1970, it has been slowed down as their use has been reduced substantially in western countries. But as they are recently introduced in India, they require a fresh look.

Keeping these combined aspects ⁱⁿ mind, investigations on Imidan were taken up.

4.1 THE PESTICIDE: IMIDAN

Among the numerous pesticides known, O,O-dimethyl S-phthalimidomethyl phosphorothiolothionate or Imidan¹ (Fig. 1) is important in the class of phosphorothiolothionates. Other trade names for the same are Prolate, Phosmate and Phthalophos. It was first synthesised by Stauffer Chemical Co. (USA). The pesticide can be prepared by treating sodium dimethyl phosphorothiolothionate with N-chloromethyl phthalimide (Fig. 1). The process is described in detail in proceeding section.

Imidan is a white crystalline solid, m.p.72°C. It is practically insoluble in water (25 ppm at 25°C) but soluble in most of the organic solvents except aliphatic hydrocarbons³. The broad spectrum and non-systemic insecticide is effective against insects and mite pests which attack fruit, field crops, pasture and livestock. It is found active in controlling Codling moth, Plum Curculio, Boll weevil, Pear psylla, Rice Stem borer, Rice Paddy borer, Northern Cattle grub and common cattle grub.

The metabolism of Imidan is more or less same in plants, animals and soil. The C¹⁴ labelled pesticide when administered orally in rats, rapidly degraded and excreted; 19% of the dose was eliminated via feces and 79% of the dose

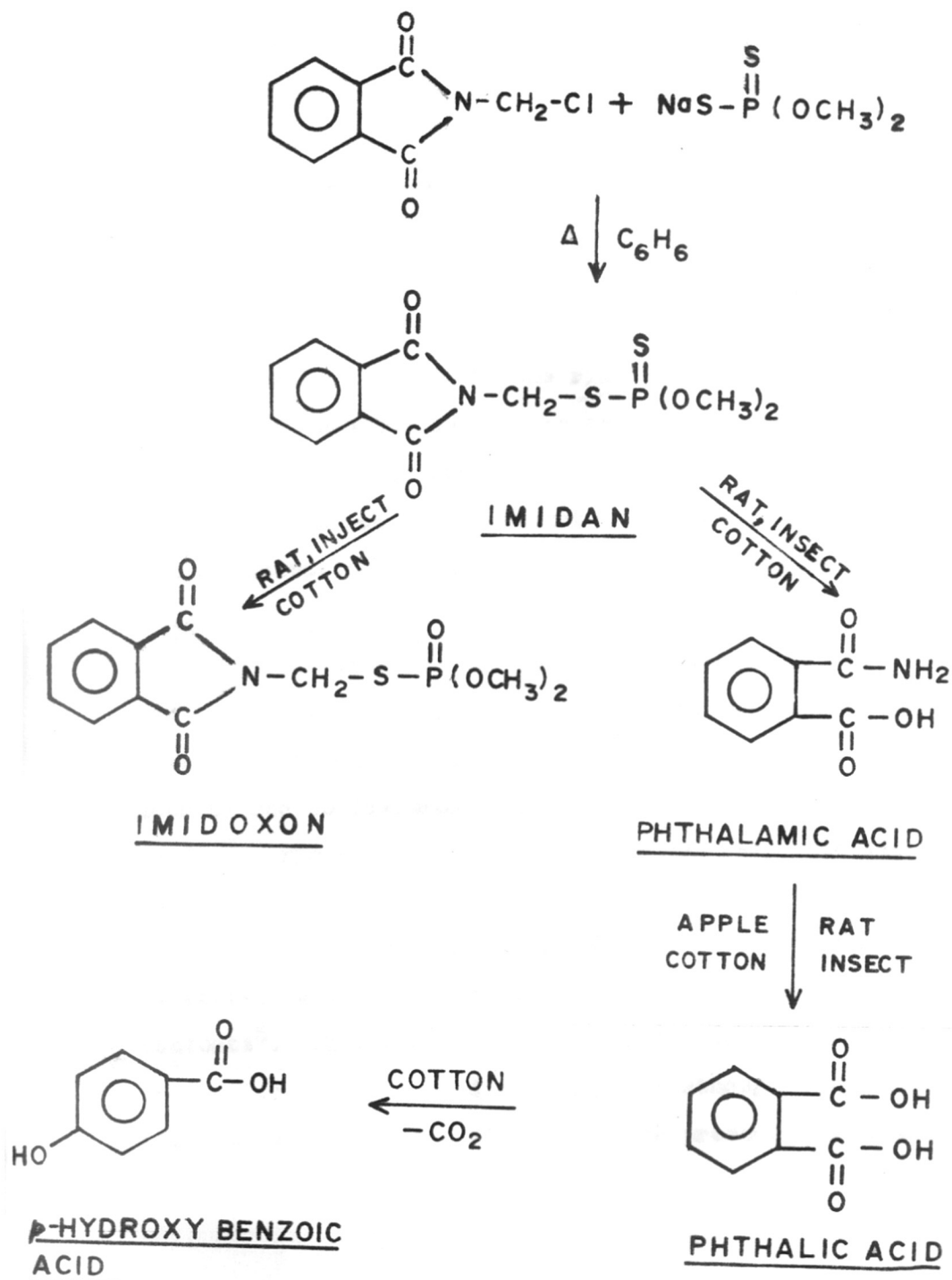


FIG. 1

via the urine⁴. The initial degradation in rat was via breaking C-S bond to form O,O-dimethyl phosphorodithioic acid and N-hydroxymethyl phthalimide followed by further degradation to phthalamic acid which in turn leads to phthalic acid^{5,6} (Fig. 1).

In plant metabolism^{7,8}, oxidation and hydrolysis occur causing cleavage in the ring system. The metabolites are rapidly absorbed but remain accumulated in leaves. Cotton plant metabolism⁷ is preferentially by hydrolytic pathways than oxidative one. The products are same as in case of animal metabolism^{5,6} but in addition, p-hydroxy benzoic acid, benzoic acid and benzamide were also isolated (Fig. 1). Though phthalimide was not actually isolated, its presence was deduced from the decarboxylation product i.e. p-hydroxy benzoic acid and evolution of CO₂. Imidan is also metabolised to Imidoxon (Fig. 1) in German cockroach⁶ and in fly⁹. Presence of the latter has not been established in rat, steer and cotton metabolism.

Persistence of Imidan in soil is very less due to hydrolysis which depends upon pH of soil and moisture contents⁵. In the absence of microorganisms, Imidan remains intact for longer period in soil. Eventhough hydrolysis products are not identified, they are presumed to be the

same as in plant metabolism⁷.

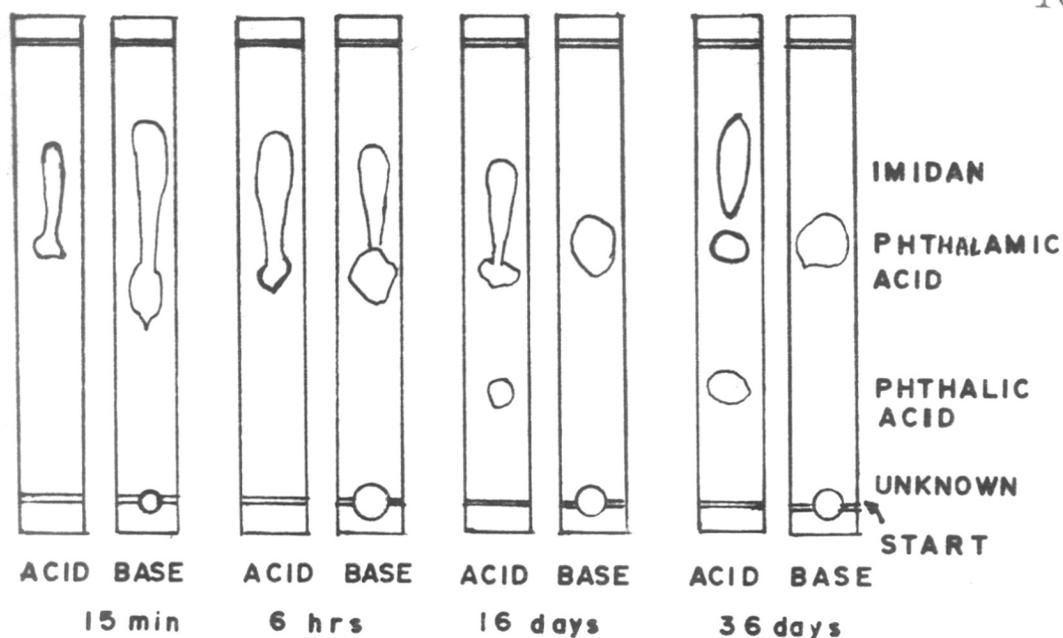
Stability of Imidan in aqueous solutions is varied. At 4.5 pH, it is quite stable (half life 13 days) while in alkaline medium (pH 8.3) its half life is only 4 hr resulting in phthalamic acid.⁵

4.2 THIN LAYER CHROMATOGRAPHY

4.2.1 INTRODUCTION

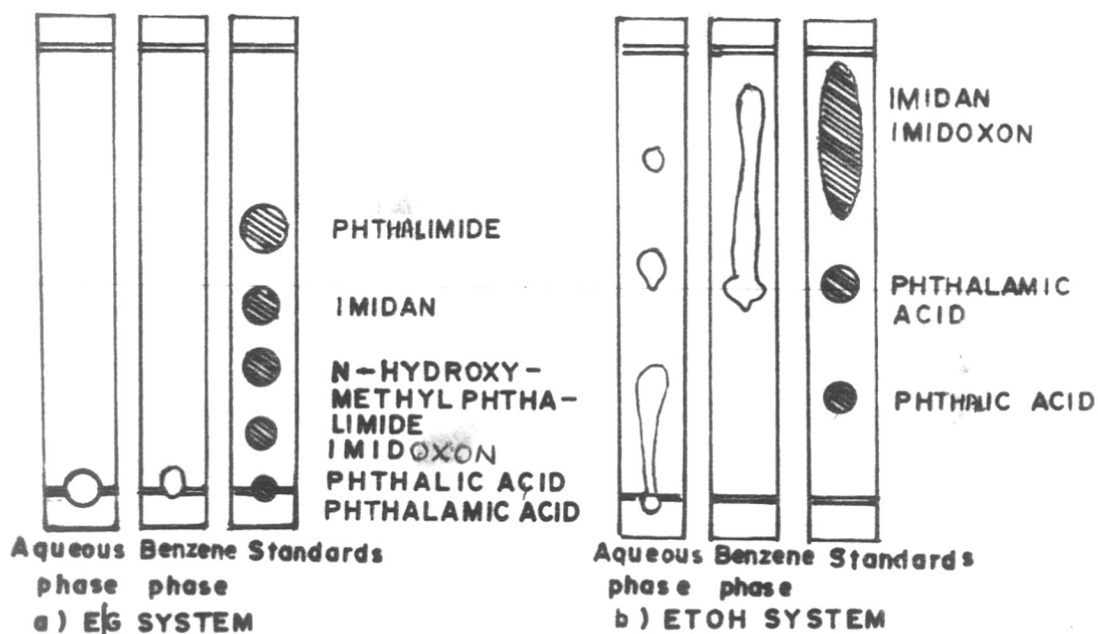
Metabolic studies of pesticides are essentially assisted by prompt analytical methods which are mainly categorised as spectroscopy and chromatography. The latter consists of thin layer chromatography (TLC) which has grown up rapidly in recent years and accepted as a quick and efficient technique for the detection of organo-phosphorus pesticides^{10,11,12}. Several special techniques have been developed in TLC for more precise determination of metabolites of the pesticides. They are: spray reagents¹³, fluorescent silica gel¹⁴ and mixing with cholinesterase¹⁵. Eventhough it has no automation, it gained wide applicability in this field due to its suitability over other analytical methods¹⁶, simplicity and precision.

Mainly TLC and paper chromatography to lesser extent have been used to study Imidan metabolism. Menn et al.⁵ have reported the hydrolysis of Imidan C¹⁴. They resolved the metabolites; phthalamic acid and phthalic acid on Whatman No.4 filter paper, using EtOH system [Ethanol:water: ammonium hydroxide (80:15:5)]⁷, (Fig. 2). Same authors while studying cotton plant metabolism⁷, applied paper



AUTORADIOGRAM OF IMIDAN HYDROLYSIS DEVELOPED
IN THE ETHANOL SYSTEM

FIG 2



AUTORADIOGRAPHY OF RAT URINARY METABOLITES OF
IMIDAN C^{14} DEVELOPED IN ETHANOL AND E/G SYSTEM

FIG 3

chromatography and detected phthalamic acid, phthalic acid as major end products along with benzoic acid and its derivatives. E/G solvent system (stationary phase: 12% glutaronitrile in acetone, mobile phase: isopropyl ether saturated with glutaronitrile) has also been applied to resolve Imidan, Imidoxon, N-hydroxymethyl phthalimide and phthalimide but resolution of phthalamic and phthalic acids has not been observed (Fig. 3). Additionally, use of isopropyl ether in glutaronitrile system is complicated¹⁷. Schumacher¹⁸ reported metabolic studies of thalidomide C¹⁴. He used paper chromatography with PAW solvent system [pyridine:n-amyl alcohol: water (7:7:6)] to resolve components similar to Imidan metabolites.

McBain and Menn⁶ have studied metabolism of Imidan C¹⁴ in rat and cockroaches. They have applied several solvent systems in paper and thin layer chromatography. They used EtOH system⁵ to identify phthalamic acid and phthalic acid (Fig. 3), while E/G paper chromatographic system⁷ was used to resolve Imidan, Imidoxon, phthalimide and N-hydroxymethyl phthalimide from benzene extract of rat urine. According to them, the PAW system¹⁸ provided best resolution of eight metabolites but only three of them i.e. phthalamic acid, phthalic acid and possibly pyridine salt of phthalic acid were identified. They have also applied paper chromatography with BAW system [n-Butanol:Glacial acetic acid:

water (2:1:1)] which resolves phthalic acid from insect tissue-hydrolyzates. TLC (silica gel G slurried in 0.1 M boric acid) with BAW system, separates phthalic acid and m-hydroxyphthalic acid but fails to resolve phthalic acid and p-hydroxyphthalic acid. They have reported that TLC using silica gel G and EB solvent system [ethyl acetate: benzene (9:1)] separates Imidan, Imidoxon and other metabolites from microsome-NADPH₂ in vitro system but does not resolve phthalamic acid, phthalic acid and hydroxy derivatives of phthalic acid. Reviewing the efforts taken by many workers all over the world to develop a suitable analytical method for the degradation products, still there is a good scope to develop a quick and simple analytical method. It may be useful in process development studies. It can also be utilised to control and monitor environmental pollution, particularly in waterways. Additionally the method may be suitable to analyse stored technical product and the formulation.

From this point of view present studies were taken up.

4.2.2 EXPERIMENTAL

4.2.2.1 PREPARATION OF PURE SAMPLES: Authentic samples were prepared for comparison. They are described as follows:

1) Phthalimide²⁰ (Fig. 4. IV): Commercial phthalimide is a pale pink powder, m.p. 235°-240°C. It was recrystallised from water to get glittering white crystals, m.p. 238°C.

TLC : EtOH solvent system and silica gel, single spot,
R_f value 0.90.

IR : 3330 cm⁻¹ (-NH), 1760 and 1730 cm⁻¹ (>C=O)²².

Elemental Analysis : Calculated for C₈H₅NO₂. C, 65.31%; H, 3.40%; N, 9.52%. Found: C, 65.39%; H, 3.42%; N, 9.57%.

2) N-Hydroxymethyl phthalimide^{2,19} (Fig. 4. III): Phthalimide (1, 5.1 g, 0.35 M), formaline (3 ml) and water (20 ml) were refluxed until a clear solution was obtained. The reaction mixture was filtered while hot and the filtrate was cooled overnight. The white crystalline product thus obtained was filtered and dried in air for 48 hr. Yield 5.63 g (90%), m.p. 137°-141°C. It was ^{re-}crystallised from rectified spirit.

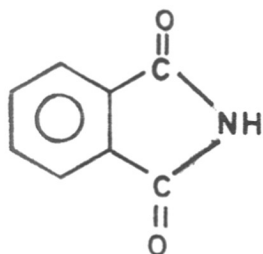
On keeping overnight white crystals were obtained, m.p. 139°C.

TLC : EtOH solvent system and silica gel, single spot,
R_f value 0.85.

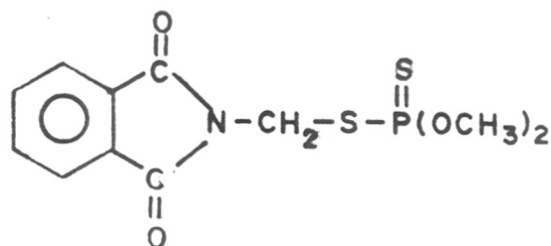
IR : 3770 cm⁻¹ (-OH), 1780 and 1720 cm⁻¹ (>C=O)²¹.



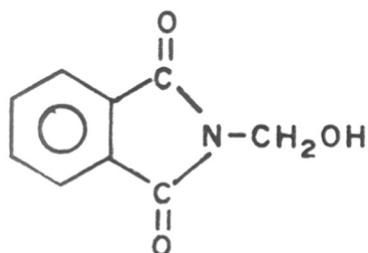
PREPARATION OF IMIDAN, (I)



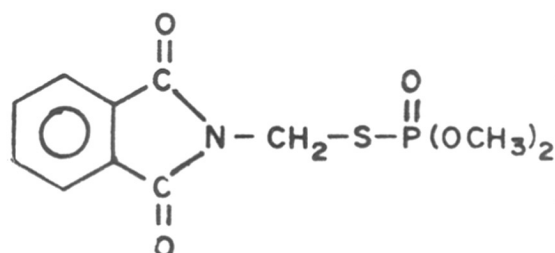
1) PHTHALIMIDE, (IV)



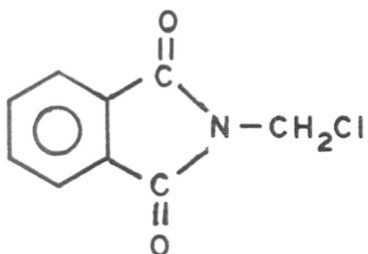
5) IMIDAN, (I)



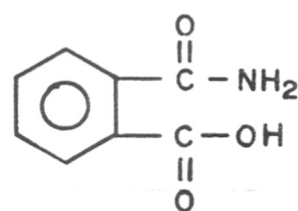
2) N-HYDROXYMETHYL
PHTHALIMIDE, (III)



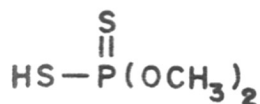
6) IMIDOXON, (II)



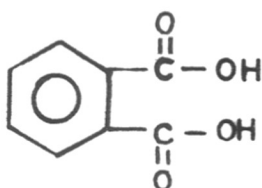
3) N-CHLOROMETHYL
PHTHALIMIDE



7) PHTHALAMIC ACID, (V)



4) O,O-DIMETHYL
PHOSPHORODITHIOIC ACID



8) PHTHALIC ACID, (VI)

FIG. 4

Elemental Analysis

: Calculated for $C_9H_7NO_3$. C, 61.01%;
H, 3.96%; N, 7.91%. Found: C, 61.13%;
H, 3.93%; N, 7.89%.

3) N-Chloromethyl phthalimide^{2,23} (Fig. 4): N-hydroxymethyl phthalimide (2, 7.08 g, 0.04 M) was added to 30% HCl (30 ml) and heated over a waterbath for 2 hr. A white crystalline material was obtained on keeping overnight. It was filtered and washed with cold water, yield 5.88 g (75%), m.p. 132°-133°C.

IR : 1785 and 1740 cm^{-1} ($>C=O$)²¹

4) O,O-Dimethyl phosphorodithioic acid^{24,25} (Fig. 4): Powdered phosphorus pentasulfide (20 g) was suspended in distilled benzene (20 ml) and the mixture was heated at 64°C with stirring. Methanol (16 ml) was added dropwise over a period of 1 hr at 64°C. H_2S evolved was allowed to escape through an outlet tube. The reaction mixture was further refluxed for 2 hr. The initial yellow coloured mixture turned into dark brown liquid at the end of the reaction. The reaction mixture was filtered to get blackish benzene solution of the acid. It was extracted with NaOH solution (10%, 40 ml). The clear aqueous layer was acidified, and saturated with sodium chloride to get colourless layer of the acid which was separated and dried over sodium sulphate. Yield 3.30 g (82%).

Mass spectrum: 27 (50), 43 (60), 61 (55), 77 (40),
91 (30), 93 (25), 110 (5), 124 (85),
141 (5), 157 (100).

Elemental Analysis : Calculated for $C_2H_7O_2PS_2$. C, 15.19%;
H, 4.43%. Found: C, 14.59%; H, 4.37%.

5) Imidan^{1,2} (Fig. 4, I): A solution of N-chloromethyl phthalimide (3, 3.91 g, 0.02 M) in benzene was placed in 250 ml three necked round bottom flask equipped with stirrer, reflux condenser and a dropping funnel. A solution of sodium salt of O,O-dimethyl phosphorodithioic acid (4, 3.16 g in 8% NaOH, 10 ml) was added dropwise over a period of 30 min and the reaction mixture was refluxed for 3 hr. Benzene was then distilled off. The residue was kept overnight to get a white precipitate. It was filtered and washed with water. Yield 5.89 g (90%), m.p. 70°-71°C.

Mass spectrum: 63 (15), 76 (18), 77 (20), 93 (19), 104 (10),
105 (6), 125 (4), 130 (5), 132 (5),
160 (100), 161 (20), 317 (10)²⁶.

Elemental Analysis : Calculated for $C_{11}H_{12}NO_4PS_2$. C, 41.65%;
H, 3.79%; N, 4.42%. Found: C, 41.41%;
H, 3.93%; N, 4.32%.

6) Imidoxon^{27,28} (Fig. 4, II): Imidan (5, 1 g) was stirred with conc. HNO_3 (5 ml, $d = 1.5$) in chloroform (50 ml) at room temp. for 5 hr. The reaction mixture was then washed several times with water till washings were neutral to litmus. The solvent layer was dried over Na_2SO_4 and evaporated to give Imidoxon, m.p. 70°C .

TLC : Benzene solvent system and silica gel,
single spot, R_f value 0.50.

Mass spectrum : 60(36), 76(65), 77(56), 91(13), 92(25),
93(30), 94(38), 95(33), 130 (34), 132 (13),
133 (33), 147 (17), 148 (11), 160 (100),
161 (43), 301 (17).

Elemental Analysis : Calculated for $\text{C}_{11}\text{H}_{12}\text{NO}_5\text{PS}$. C, 43.85%
H, 3.99%; N, 4.65%. Found: C, 44.52%;
H, 3.85%; N, 4.47%.

7) Phthalamic acid³⁰ (Fig. 4, V): Phthalic anhydride (6 g) was gradually added with stirring to warm aqueous ammonia (9 ml, $d = 0.88$). The material dissolved readily with considerable evolution of heat and ammonium phthalamate separated into fine, white crystals; the amount increased on cooling. Phthalamic acid was obtained by treating the ammonium salt in cold water (10 ml) with slightly excess of equivalent quantity of conc. HCl (4 ml). The crystalline paste was washed with cold water until it was free from chloride. The pure product was obtained by recrystallization from ethanol. Yield 4.4 g (81%), m.p. 148°C .

TLC : EtOH solvent system and silica gel,
single spot, R_f value 0.53.

IR : 3500 and 3300 cm^{-1} ($-\text{NH}_2$), 1690 and 1650 cm^{-1}
($>\text{C}=\text{O}$)²¹

Elemental Analysis : Calculated for $\text{C}_8\text{H}_7\text{NO}_3$. C, 58.19%;
H, 4.24%; N, 8.48%. Found: C, 57.95%;
H, 4.32%; N, 8.53%.

8) Phthalic acid³¹ (Fig. 4. VI): Commercial phthalic acid was ash coloured amorphous material. It was recrystallized from water to get colourless needles, m.p. 200°C.

TLC : EtOH solvent system and silica gel,
single spot, R_f value 0.48.

IR : 2900-2200 cm^{-1} ($-\text{OH}$), 1685 cm^{-1} ($>\text{C}=\text{O}$)³².

Elemental Analysis : Calculated for $\text{C}_8\text{H}_6\text{O}_4$. C, 57.82%; H, 3.62%
Found: C, 57.91%; H, 3.57%.

4.2.2.2 PURIFICATION OF SOLVENTS: Commercially available solvents were purified³³ as described below.

1) Acetone: Commercial acetone (1 L) was refluxed over potassium permanganate (10 g) for 3 hr till the colour of permanganate persisted. The fraction boiling at 55-56°C was collected.

2) Benzene: Commercial benzene (1 L) was washed with three portions of conc. H_2SO_4 (60 ml) and finally with water till washings were neutral. It was kept over CaCl_2 for 3 days and distilled to get pure benzene at $78-80^\circ\text{C}$.

3) Chloroform: Commercial chloroform (1 L) was dried over CaCl_2 for 2 days. Pure chloroform was obtained by distillation (b.p. $60-61^\circ$).

4) Ethanol: Commercial solvent (1 L) was refluxed with good quick lime (50 g) for 3 hr under anhydrous conditions and distilled to get absolute alcohol (b.p. $77-78^\circ\text{C}$).

4.2.2.3 PREPARATION OF TLC PLATES: Silica gel (National Chemical Laboratory, Pune-8, mesh 200) was used for preparing TLC plates. Silica gel: plaster of paris³⁴: water (5:1:50, w/w/v) were mixed and stirred with a rod for two minutes to get an uniform slurry. Glass plates were cleaned with detergent and washed thoroughly with water, then with acetone-pet.ether mixture and dried. The plates were coated with silica gel slurry using applicator method³⁵. The plates were dried, activated in an oven at 120°C for 4 hr and used immediately.

4.2.2.4 GENERAL PROCEDURE OF ANALYSIS: Clear concentrated solutions of samples (Fig. 4, I-VI) were prepared and spotted using capillaries (1 mm I.D.) 2 cm away from the

edge in a horizontal straight line. The spots were allowed to dry. The plate was then kept in a presaturated solvent chamber. The solvent was allowed to ascend upto 0.9 of the height. It was then removed, dried and developed in a presaturated iodine chamber. R_f values were calculated³⁶ as the ratio of length from the centre of spot to the origin and the distance between solvent front and the origin.

$$R_f \text{ value} = \frac{\text{Dist. travelled by component}}{\text{Dist. travelled by solvent system}}$$

4.2.2.5 DETAILS OF EXPERIMENTS: The above mentioned procedure was followed for all experiments. Following compounds were tried, using different solvent systems. They are Imidan (I), Imidoxon (II), N-hydroxymethyl phthalimide (III), phthalimide (IV), phthalamic acid (V) and Phthalic acid (VI) (Fig. 4). The results are tabulated in Table No.1.

4.2.2.6 STANDARD PROCEDURE: Trial 12 was standardised further and recommended for the use. It is as follows.

A glass plate (25 cm x 2 mm thickness) was taken. It was cleaned first with detergent and water and then with pet.ether-acetone mixture. The dried plate was coated with uniform slurry of silica gel [silica gel (200 mesh): Plaster of Paris:water (5:1:50, w/w/v)]. The plate was then dried,

Table 1

No. Solvent system	R _f values					Remarks	
	I*	II*	III*	IV*	V*		VI*
1. Acetone	1.00	1.00	1.00	1.00	0.85	0.80	PR
2. Benzene	0.60	0.50	0.30	0.07	0.00	0.00	PR
3. Acetone:benzene (1:3)	1.00	1.00	0.80	0.90	0.50	0.50	PR
4. Chloroform:benzene (1:1)	0.75	0.70	0.10	0.15	0.00	0.00	PR
5. EtOH	1.00	1.00	0.85	0.90	0.53	0.48	GR
6. EtOH:chloroform (1:4)	1.00	1.00	0.75	0.85	0.60	0.60	PR
7. EtOH:chloroform (4:1)	-	-	-	-	0.73	0.66	GR
8. EtOH:chloroform (1:1)	-	-	-	-	0.60	0.50	GR
9. Chloroform	1.00	1.00	0.80	0.85	-	-	PR
10. Benzene:chloroform (1:4)	0.83	0.80	0.50	0.55	-	-	GR
11. Benzene:chloroform (1:9)	0.98	0.94	0.38	0.56	-	-	
12a. Benzene:chloroform (1:9)	0.98	0.94	0.38	0.56	0.00	0.00	a & b on same plate
12b. EtOH:chloroform (1:1)	0.98	0.94	0.38	0.56	0.20	0.17	(R _f) _b = 1/3 (R _f) _a

* Please refer Fig. 4

PR = Poor resolution

GR = Good resolution

and activated at 120°C for 4 hr. It was removed, cooled and the samples were spotted in horizontal straight line 2 cm above the edge. Benzene:chloroform (1:9) solvent system was allowed to run on the plate in a presaturated solvent chamber through a distance of 21 cm. The plate was removed, dried and allowed to run in EtOH:chloroform (1:1) solvent system in another solvent chamber through a distance of 7 cm. The plate was allowed to dry and developed in a presaturated iodine chamber. The particulars are mentioned in Table 2 (Fig. 5c).

Table 2

Component	Position at	R _f value	Identification mark
I	20.58 cm	0.98	dark brown spot
II	19.24 cm	0.94	dark brown spot and edge merged with spot I
III	7.98 cm	0.38	yellow spot
IV	11.76 cm	0.56	yellow spot with minor trailing
V	4.20 cm	0.20	colourless spot
VI	3.57 cm	0.17	colourless spot with trailing

4.2.3 RESULTS AND DISCUSSION

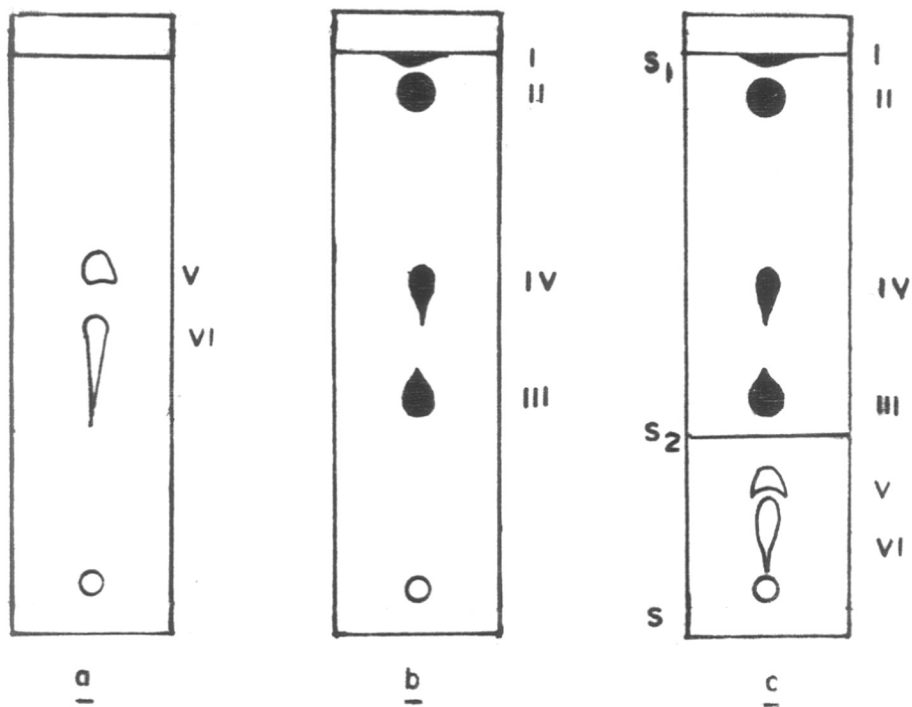
4.2.3.1 Development of Technique: Several studies in degradation of Imidan namely plant metabolism^{7,8}, animal metabolism^{4,5,6}, metabolism in soil⁵ and non-enzymatic hydrolysis⁵ reveal that Imidan (I), Imidoxon (II), N-hydroxymethyl phthalimide (III), phthalimide (IV), phthalamic acid (V) and phthalic acid (VI) (Fig. 4) are major products of the degradation. Therefore these compounds were chosen for the development of thin layer chromatographic technique (TLC). A characteristic of these compounds is their polar nature which differ from each other. Naturally the requirements for their chromatography at a time are very difficult to judge. Therefore present investigations were aimed to develop an handy but suitable technique where analysis of these compounds may be achieved on a single chromatogram, with efficient resolution.

For this purpose, numerous trials were taken using different solvent systems. Some of the representative trials are as follows:

It has been observed that systems with single solvent such as benzene, acetone and chloroform fail to resolve all the components at a time (Table 1). Therefore mixed phases were tried. Acetone:benzene (1:1) and chloroform:benzene (1:1)

systems also found inefficient in resolving the components (I-VI) (Table 1). These trials clearly showed that satisfactory analysis may not be achieved in single stage using only one solvent system. Therefore on the basis of polar nature of compounds and their behaviour observed during the experiments, they were divided into different groups for two stage analysis. The acids V and VI were considered in one group. Phthalimides (III and IV) and pesticides (I and II) were considered as another group. As ethanol system is shown efficient for the acids in earlier attempts, more improvements were tried with it. Among many combination trials, EtOH:chloroform (1:1) system was able to give optimum resolution. R_f values for V and VI are 0.60 and 0.50 respectively (Fig. 5a). For another group of compounds, in addition to many solvent systems various combinations of benzene and chloroform were tried. Finally benzene:chloroform (1:9) system found to give the required resolution. Eventhough Imidan (I) and Imdoxon (II) have not been resolved efficiently, they can be individually identified and total pattern was more promising. R_f values for I, II, III and IV are 0.98, 0.94, 0.38 and 0.56 respectively (Fig. 5b).

For the development of whole chromatogram, the phases were used in two stages. Namely benzene:chloroform (1:9) used initially and EtOH:chloroform (1:1) then followed upto one third of total height. The spots were developed in iodine



a) $\text{EtOH} : \text{CHCl}_3 (1:1)$ SYSTEM

b) $\text{BENZENE} : \text{CHCl}_3 (1:9)$ SYSTEM

c) $S - S_1$ AS IN b

$S - S_2$ AS IN a

FIG. 5

chamber. They have characteristic shape and colour for their individual identifications (Table 2). The R_f values for I-VI are 0.98, 0.94, 0.38, 0.56, 0.20 and 0.17 respectively (Fig. 5c).

After various trials, the standardised procedure has been recommended for the use. It is described in experimental part (4.2.2.).

Thus a biphasic TLC technique has been developed successfully to resolve the compounds I-VI on a single chromatogram with distinguished pattern.

4.2.3.2 COMPARISON WITH EXISTING METHODS: Eventhough present studies principally differ in aims, it has many advantages over existing methods mentioned earlier. Use of EtOH system by Menn et al.^{5,6} resolves only I, V and VI (Fig. 2). This method on the other hand resolves not only I, V and VI but also Imidoxon (II) from Imidan (I) and the cyclic amides (III and IV). The E/G system^{6,7} developed for plant metabolism studies, separates I, II, III and IV but resolution of acids (V and VI) was not reported (Fig. 3). Additionally the solvent system requires the critical composition¹⁷. While present method covers all the compounds at a time and the solvent systems are simple to be included in routine analysis.

Eventhough McBain and Menn⁶ have achieved best resolution with PAW system¹⁸ due to failure in identifying the components, (it is insufficient) The BAW system⁶ has specific requirements. Therefore it has limited use. The MB system⁶ fails to resolve the major metabolities; phthalamic acid (V) and phthalic acid (VI). Thus these existing methods have a limited use only for specific studies. Therefore they have limited applications. They are also inadequate for the separation of I-VI as no report has mentioned TLC analysis of I-VI at a time on a single chromatogram. Present technique includes the major metabolites (I-VI) reported earlier in all types of metabolic studies such as soil⁵, animal⁶ and plant⁷ metabolism. Therefore the technique is more useful for general purpose. It is particularly applicable for studies such as decomposition in aquatic media where possibility of S-C fission and oxidative conversions to Imidoxon (II) are anticipated, decomposition of technical product on storage as it is necessary to estimate it before formulation. The reports mentioned earlier can be repeated for the identification of major metabolites, also using the developed technique.

Apart from autoradiographic identifications, many selective developing agents were mentioned in the literature.

2,6-Dibromo-N-chlorop-quinoneimine in cyclohexane was used to identify Imidan (I) and Imidoxon^{7,37} (II) while phthalic acid (VI) and its hydroxy analogues were identified as blue and white spots using ultraviolet light (2537 Å)⁶. The potential metabolites were detected as red and yellow spots using AgNO₃ solution and U.V.^{6,38}. Phthalic acid was detected as yellow spot on a blue background using bromophenol blue in ethanol⁶ but in present investigations no specific reagent is used and plates were developed simply using iodine which is easily available in any laboratory. Still the appearance and colour of the spots is characteristic enough so that they can be identified individually e.g. the phosphorus moieties (I and II) are brown spots. The cyclic amides (III and IV) are yellow and the acids (V and VI) are colourless spots on yellowish background. The spot of dicarboxylic acid (VI) has significant trailing (Fig. 5c). Thus the chromatogram is distinguished enough for identification purposes.

Merit of present investigations over existing methods is use of common chemicals such as silica gel and iodine and simple procedure which can be performed in any laboratory in a very short time. It also has wide applicability for all types of decomposition studies. It is quite efficient and reliable to resolve and identify the components with varied polarities at a time on a single chromatogram.

4.3 HYDROLYSIS STUDIES

4.3.1 INTRODUCTION

A great majority of organophosphorus pesticides are ester derivatives. The polarized phosphoryl group in them creates a positive charge on the phosphorus atom which consequently becomes highly electrophilic and reacts with nucleophiles. Thus they are susceptible to hydrolysis.

Menn et al.⁵ reported the hydrolysis of Imidan C¹⁴. It was carried out in aqueous solutions buffered at pH 4.55 and 8.26. Imidan C¹⁴ suspensions were vigorously shaken, stoppered and stored at room temperature. At predetermined intervals, samples were taken out from each solution and chromatographed on Whatman No.4 filter paper using EtOH solvent system [ethanol:water:ammonium hydroxide (80:15:5)]⁷. The autoradiograms indicated that Imidan C¹⁴ hydrolysed to phthalamic acid in basic medium and to phthalic acid via phthalamic acid in acidic medium. Imidan dissolved in water proceeded to phthalamic acid³⁹. McBain et al.⁶ have reported comprehensive studies of Imidan metabolism. They have proposed the pathways of metabolism and hydrolysis in alkaline medium.

It is well-known fact that metabolic reactions occurring in plants, animals and soil have similarities with chemical

reactions, particularly hydrolysis. Therefore chemical reactions have great significance in elucidating and confirming mode and nature of metabolic pathways. As the products obtained are identical in majority of cases^{18,40}, empirical information obtained from hydrolysis studies provided a basis for predicting potential in-vivo metabolites of the pesticides. Thus there exists an identity relationship between metabolism and hydrolysis^{18,40}. To be more precise, it is the alkaline hydrolysis.

If this theory is applied to Imidan degradation, there are many intermediate metabolites such as N-hydroxymethyl phthalimide, phthalimide, benzoic acid and its derivatives^{5,6,7} which are not isolated in nonenzymatic hydrolysis so far. Additionally role of biochemicals is also not clarified.

Biodegradation reactions have mostly hydrolytic pathways which occur in such a manner that the end products are harmless to mammals and are excreted out without any danger⁴¹. Thus enzymatic hydrolysis is a naturally occurring detoxication reaction. As the identity of metabolism and nonenzymatic hydrolysis has already been proved by Schumacher¹⁸ and Plapp et al.⁴⁰, the latter may serve as a good method for artificial detoxication of the pesticide. Now-a-days the use of organophosphorus pesticides has been increased steeply, so also pollution hazards⁴². Therefore development and

detailed studies of hydrolysis may be very useful to lower the environmental pollution which is the greatest threat to mankind at present.

On this backgroundⁿ, present studies were taken up for the following reasons. It may evaluate a good detoxication method which can be employed to effluent treatment of waste water from the pesticide industry. It may serve to confirm the identity between metabolism and chemical hydrolysis. Detailed studies may also be useful to identify unknown hazardous metabolites.

4.3.2 EXPERIMENTAL

The hydrolysis was carried out using different catalysts i.e. (1) NaOH, (2) HCl and (3) Na_2CO_3 . The experimental details are as follows:

4.3.2.1 HYDROLYSIS USING NaOH (Fig. 6)

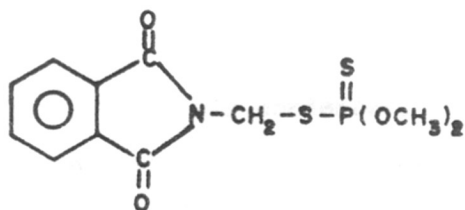
Expt. One: Powdered Imidan (I, 3.17 g, 0.01 M) was added to 30% aqueous solution of NaOH (50 ml) in 100 ml round bottom flask equipped with an air condenser. The heterogeneous pink reaction mixture was refluxed for 1 hr. Evolution of ammonia gas was tested using moist turmeric paper. The evolution ceased completely at the end. The clear pale brown liquid was cooled under tap, neutralised using 1N HCl and allowed to stand overnight. White precipitate thus obtained was filtered, washed with water and dried at 25°C for 48 hr. Yield 1.5 g, m.p. 201°C .

TLC : EtOH: chloroform (1:1) system and silica gel,
single spot, R_f value 0.50

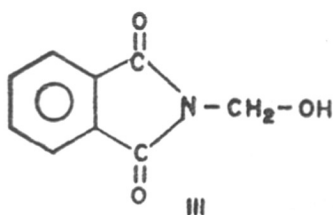
IR : $2900\text{--}2200\text{ cm}^{-1}$ (-OH), 1685 cm^{-1} ($>\text{C}=\text{O}$)

It was identified as phthalic acid³¹ (Fig. 6, VI) by TLC, superiaposable IR³² (Section 4.2) and mixed m.p.

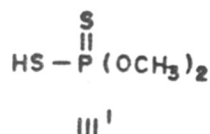
Expt. Two: Imidan (I, 3.17 g, 0.01 M) was powdered and added to 1% aqueous solution of NaOH (50 ml) in 100 ml round bottom



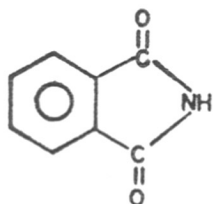
I



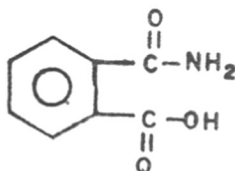
III



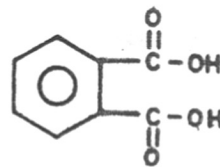
III'



IV



V



VI

EXPT. ONE	I	$\xrightarrow[100^{\circ}\text{C}, 1 \text{ hr}]{\text{NaOH}}$	VI
EXPT. TWO	I	$\xrightarrow[25^{\circ}\text{C}, 2.5 \text{ hr.}]{\text{NaOH}}$	V + VI
EXPT. THREE	I	$\xrightarrow[0^{\circ}\text{C}, 15 \text{ min.}]{\text{NaOH}}$	IV + III'
EXPT. FOUR	III	$\xrightarrow[0^{\circ}\text{C}, 2 \text{ min.}]{\text{NaOH}}$	IV
EXPT. FIVE	IV	$\xrightarrow[0^{\circ}\text{C}, 1 \text{ hr.}]{\text{NaOH}}$	V
EXPT. SIX	III	$\xrightarrow[25^{\circ}\text{C}, 1 \text{ min}]{\text{NaOH}}$	VI
EXPT. SEVEN	V	$\xrightarrow[100^{\circ}\text{C}, 20 \text{ min}]{\text{NaOH}}$	VI
EXPT. EIGHT	III'	$\xrightarrow[100^{\circ}\text{C}, 1 \text{ hr.}]{\text{NaOH}}$	INORGANIC PHOSPHATES

FIG 6

flask equipped with an air condenser. The heterogeneous mixture was shaken at 25°C for 2.5 hr. It was then filtered to get pale pink solution which was neutralised using 1N HCl. On standing overnight, a white precipitate was obtained. It was filtered, washed with water till washings were neutral and dried at room temperature (25°C) for 48 hr. Yield 0.141 g, m.p. 178° - 185°C (range).

TLC : EtOH: Chloroform (1:1) system and silica gel, two spots, R_f value (i) 0.60 and (ii) 0.50.
 IR : 3500 and 3300 cm^{-1} ($-\text{NH}_2$); 2900 - 2200 cm^{-1} ($-\text{OH}$); 1690 , 1685 and 1650 cm^{-1} ($>\text{C}=\text{O}$).

The components in the mixture were identified as (i) phthalamic acid³⁰ (ii) phthalic acid³¹ (Fig. 6, V and VI res.) by TLC, IR^{21,32} (Section 4.2).

Expt. Three: Imidan (I, 3.17 g, 0.01 M) was suspended in 2% aqueous solution of NaOH (40 ml) in 100 ml round bottom flask equipped with an air condenser. The mixture was shaken at 0°C in an ice bath for 15 min. The heterogeneous reaction mixture was filtered. The filtrate was neutralised using 1N HCl, saturated with NaCl and left overnight. It was then extracted with ether. The ethereal layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated to get a brown gummy material. Yield 0.270 g. It was extracted with benzene (3 x 30 ml). The reddish solvent layer was dried over anhydrous Na_2SO_4 and

the solvent was evaporated in vacuum (60°C/50 mm) to get a red syrupy liquid. Yield 0.133 g.

Gas chromatography: S.E. = 30 (3%), temp. 140°C, single peak, R_t 2'05".

Mass spectrum : 45(63), 61 (56), 77(41), 91(30), 93(28), 124 (78), and 157 (100).

It was identified as O,O-dimethyl phosphorodithioic acid²⁴ (Fig. 6, III') by comparison on GC and superimposable mass spectrum (Section 4.2).

Benzene insoluble pale yellow solid was washed with benzene (10 ml) and dried at 25°C for 48 hr. Yield 0.128 g, m.p. 225-230°C.

TLC : Benzene:Chloroform (1:9) system and silica gel, single spot, R_f value 0.56.

IR : 3300 cm^{-1} (-NH), 1760 and 1730 cm^{-1} (>C=O).

It was identified as phthalimide²⁰ (Fig. 6, IV) by comparing TLC, superimposable IR²² (Section 4.2) and mixed m.p.

Expt. Four: To a solution of aqueous NaOH (0.35 g, 50 ml) in 100 ml conical flask, N-hydroxymethyl phthalimide¹⁹ (III, 2 g) was added in one lot at 0°C in an ice bath. After 2 min, the insoluble material was filtered off. The filtrate was neutralised using 1N HCl. The precipitated solid was filtered, washed with water till washings were neutral to

litmus and dried at 25°C for 48 hr. Yield 1.53 g, m.p. 225°C.

It was characterised as phthalimide²⁰ (Fig. 6, IV) as described in Expt. Three.

Expt. Five: Phthalimide²⁰ (IV, 1.47 g, 0.01 M) was added to 4% aqueous solution of NaOH (10 ml) in 50 ml conical flask in lots at 0°C over 1 min with occasional shaking. The reaction mixture was kept at 0°C for 1 hr. It was then neutralised using 1N HCl. On standing overnight, a white precipitate was obtained. It was filtered and washed with water till washings were neutral to litmus and dried at room temp. (25°C) for 48 hr. Yield 1.57 g, m.p. 148°C.

TLC : Ethanol:chloroform (1:1) system and silica gel,
single spot, R_f value 0.60
IR : 3500 and 3300 cm^{-1} ($-\text{NH}_2$), 1690 and 1650 cm^{-1}
($\text{C}=\text{O}$)

It was identified as phthalamic acid³⁰ (Fig. 6, V) by TLC, superimposable IR²¹ (Section 4.2) and mixed m.p.

Expt. Six: N-Hydroxymethyl phthalimide¹⁹ (III, 1.77 g, 0.01 M) was added to 30% aqueous NaOH solution (30 ml) in 100 ml conical flask at room temp. (25°C). After 1 min clear reaction mixture was obtained which was neutralised using 1N HCl. On keeping at 0°C for 5 hr, a white precipitate was obtained. It was filtered, washed with cold water and dried

at 25°C for 48 hr. Yield 1.63 g, m.p. 200°C.

It was identified as phthalic acid³¹ (Fig. 6, VI) as described in Expt. one.

Expt. Seven: Phthalamic acid³⁰ (V, 1.65 g, 0.01 M) was added to 30% aqueous NaOH solution (10 ml) in 50 ml round bottom flask equipped with an air condenser and refluxed for 20 min. Evolution of ammonia was tested using moist turmeric paper. The cooled reaction mixture was neutralised using 1N HCl and kept overnight at 25°C. White precipitate thus obtained was filtered, washed with cold water and dried at 25°C for 48 hr. Yield 1.60 g, m.p. 198°C.

It was identified as phthalic acid³¹ (Fig. 6, VI) as described in Expt. one.

Expt. Eight: O,O-Dimethyl phosphorodithioic acid²⁴ (III', 1.58 g, 0.01 M) was dissolved in 30% aqueous NaOH solution (20 ml) in 50 ml round bottom flask equipped with an air condenser. It was refluxed for 1 hr. The persistent bad odour disappeared completely at the end. It was cooled, neutralised using 1N HCl and saturated with NaCl. After standing for 2 hr, it was exhaustively extracted with solvent ether (100 ml), dried over anhydrous Na₂SO₄ and the solvent was evaporated off. It left behind no residue. The aqueous solution was tested with ammonium molybdate and showed the presence of inorganic phosphates.

4.3.2.2 HYDROLYSIS USING HCL (Fig. 7)

Expt. One: Imidan (I, 3.17 g, 0.01 M) was powdered and refluxed with 1N HCl (60 ml) in 100 ml round bottom flask attached with an air condenser. Samples were taken out time to time and were analysed by TLC.

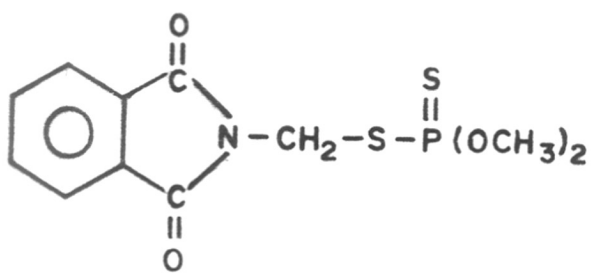
a) A sample of the reaction mixture was taken out at the end of 1st hr. It was filtered hot. The residue was washed with water till washings were neutral to litmus and dried at 25°C for 48 hr, m.p. 65°C (i). The filtrate did not afford any precipitate on standing at 25°C for 48 hr.

b) A sample was removed at the end of 3rd hr and filtered while hot. The residue was washed with water and dried at 25°C for 48 hr, m.p. 62°C (ii).

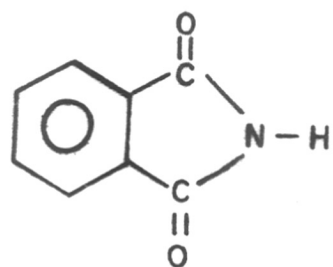
On standing overnight, a pale yellow precipitate was obtained from the filtrate. It was filtered, washed with cold water (10 ml) and dried at 25°C for 48 hr. Yield 0.5 g, m.p. 115°-220°C (range) (iii).

The latter filtrate afforded a pale yellow precipitate on standing for 72 hr. It was filtered, washed with cold water and dried at 25°C for 48 hr. Yield 0.2 g, m.p. 120°-130°C and 170°-210°C (range) (iv).

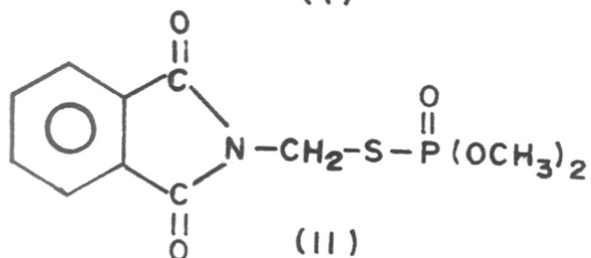
Sample was taken out at the end of 6th hr. The reaction mixture was filtered while it was hot to remove undissolved material (V). The filtrate was saturated with NaCl and allowed



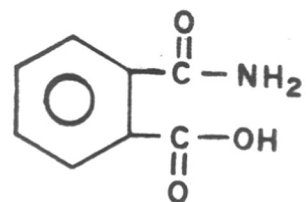
(I)



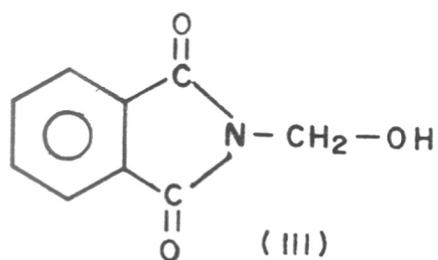
(IV)



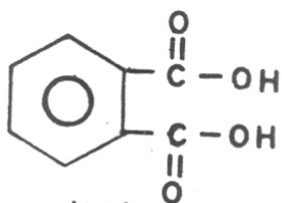
(II)



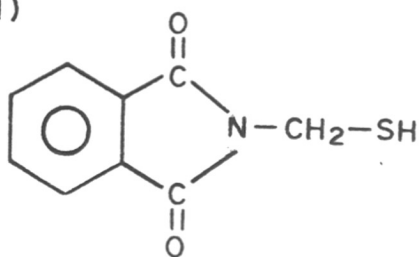
(V)



(III)



(VI)



(VII)

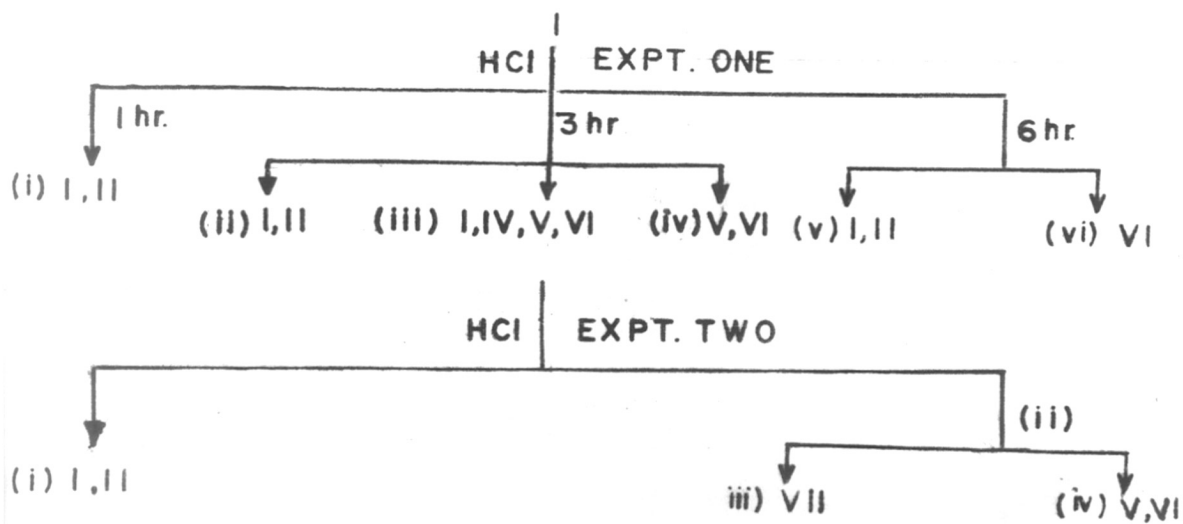


FIG. 7.

to stand overnight. Pale yellow solid precipitated was filtered and washed with cold water till washings were neutral to litmus. It was dried at 25°C for 48 hr, m.p.195°C (vi).

The compounds were identified by comparing their R_f values with that of authentic samples on TLC (Section 4.2). The results are summarised in Table 3.

Expt. Two: Powdered Imidan (I, 4.74 g, 0.015 M) was refluxed with 1N HCl (25 ml) in 100 ml round bottom flask equipped with an air condenser. After 6 hr, the heterogeneous reaction mixture was filtered while hot to separate undissolved material. Yield 4.26 g (90%), m.p.65°C (i). The filtrate was saturated with NaCl and allowed to stand overnight. The precipitate thus obtained was filtered, washed with cold water till washings were neutral to litmus and dried at 25°C for 48 hr to get yellow solid. Yield 0.521 g, m.p.190°C (ii).

The yellowish solid was exhaustively extracted with chloroform (50 ml). The solvent layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated to get a gummy material which solidified slowly. It was recrystallised from ethyl acetate. Yield 0.113 g, m.p.135°C (iii). The insoluble white material was washed with cold water and dried at 25°C for 48 hr. Yield 0.4 g, m.p.200°C (iv).

Table 3

No.	Solvent system [*]	Components	R _F value	Name of the Compound (Fig. 7)
1	BC	1	0.98	Imidan (I)
11		2	0.94	Imidoxon (II)
v				
111	BC	1	0.98	Imidan (I)
		2	0.56	Phthalimide (IV)
		3	0.00	?
	EC(0.33)	3'	0.20	Phthalamic acid (V)
		3"	0.17	Phthalic acid (VI)
1v	EC	1	0.60	Phthalamic acid (V)
		2	0.50	Phthalic acid (VI)
v1	EC	1	0.50	Phthalic acid (VI)

Table 4

1	BC	1	0.98	Imidan (I)
		2	0.94	Imidoxon (II)
11	BC	1	0.38	N-Hydroxymethyl phthalimide (III) ?
	EC(0.33) →	2	0.00	?
		2'	0.20	Phthalamic acid (V)
		2"	0.17	Phthalic acid (VI)
111	BC	1	0.38	N-hydroxymethyl phthalimide (III) ?
1v	EC	1	0.60	Phthalamic acid (V)
		2	0.50	Phthalic acid (VI)

^{*}BC: Benzene:chloroform (1:9), EC: Ethanol:chloroform (1:1)

The results based on TLC analysis are mentioned in Table 4.

(iii) IR	: 2650-2220 cm^{-1} (-SH), 1760 and 1710 cm^{-1} ($>\text{C}=\text{O}$) (Fig. 8).
Mass Spectrum	: 76 (58), 93 (80), 105 (80), 122 (56), 149 (100), 160 (45), 177 (68) and 193 (68) (Fig. 9).
Elemental Analysis	: Calculated for $\text{C}_9\text{H}_7\text{NO}_2\text{S}$, 55.96%, H, 3.65%; N, 7.25%; S, 16.60%. Found: C, 54.84%; H, 4.05%; N, 7.37%; S, 16.21%.

It was characterised as N-mercaptomethyl phthalimide⁴³ (Fig. 7, VII), by comparing IR⁴³, elemental analysis and mass fragmentation pattern. Therefore component (ii-1) is N-mercaptomethyl phthalimide⁴³ (m.p. 135-137°C and 138°-139°C)⁴³.

4.3.2.3 HYDROLYSIS USING Na_2CO_3 (Fig. 10)

Expt. One: Imidan (I, 1 g) was refluxed with 5% aqueous solution of Na_2CO_3 (25 ml) in 50 ml round bottom flask attached with an air condenser for 5 hr. The clear solution was cooled and neutralised using 1N HCl. On standing overnight at 25°C, a white precipitate was obtained. It was washed with cold water till washings were neutral to litmus and dried at 25°C for 48 hr. Yield 0.47 g, m.p. 198°C.

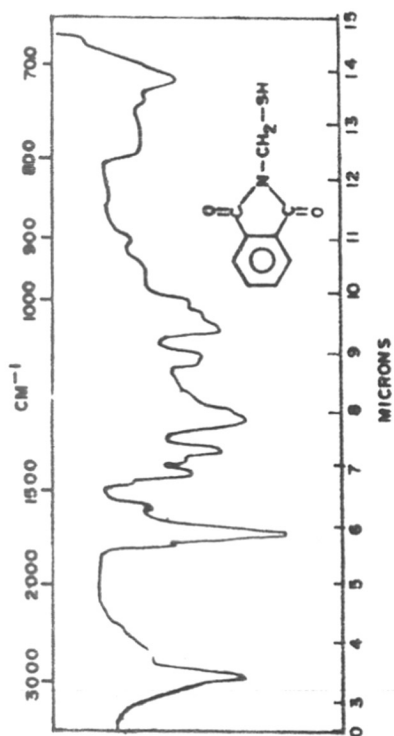


FIG. 8 IR SPECTRUM OF N-MERCAPTOMETHYL PHTHALIMIDE, (VII)

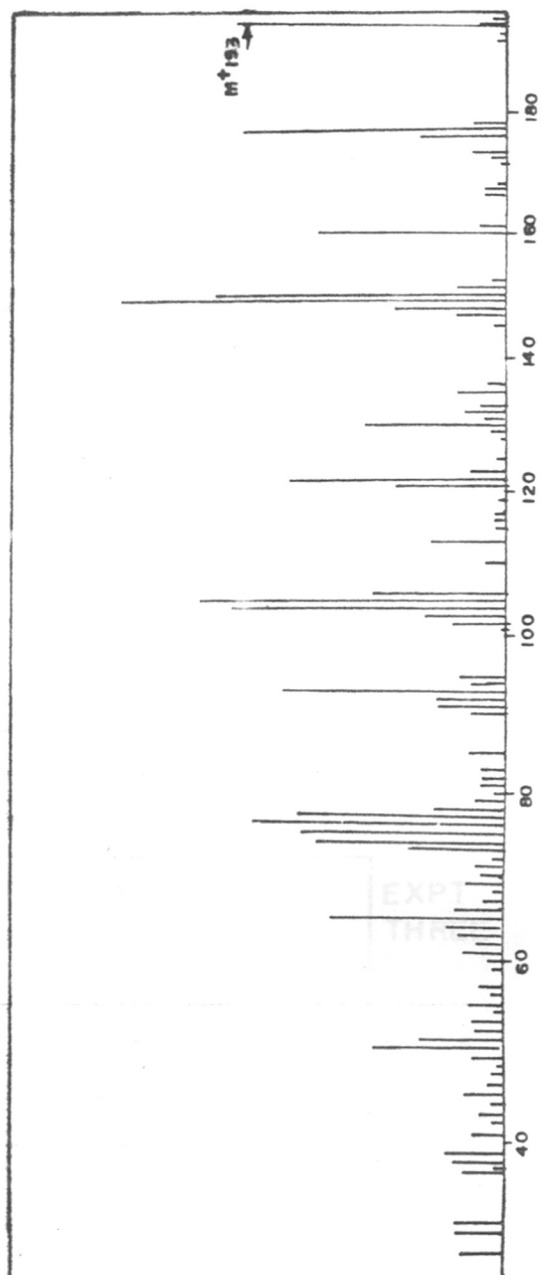


FIG. 9 MASS SPECTRUM OF N-MERCAPTOMETHYL PHTHALIMIDE, (VII)

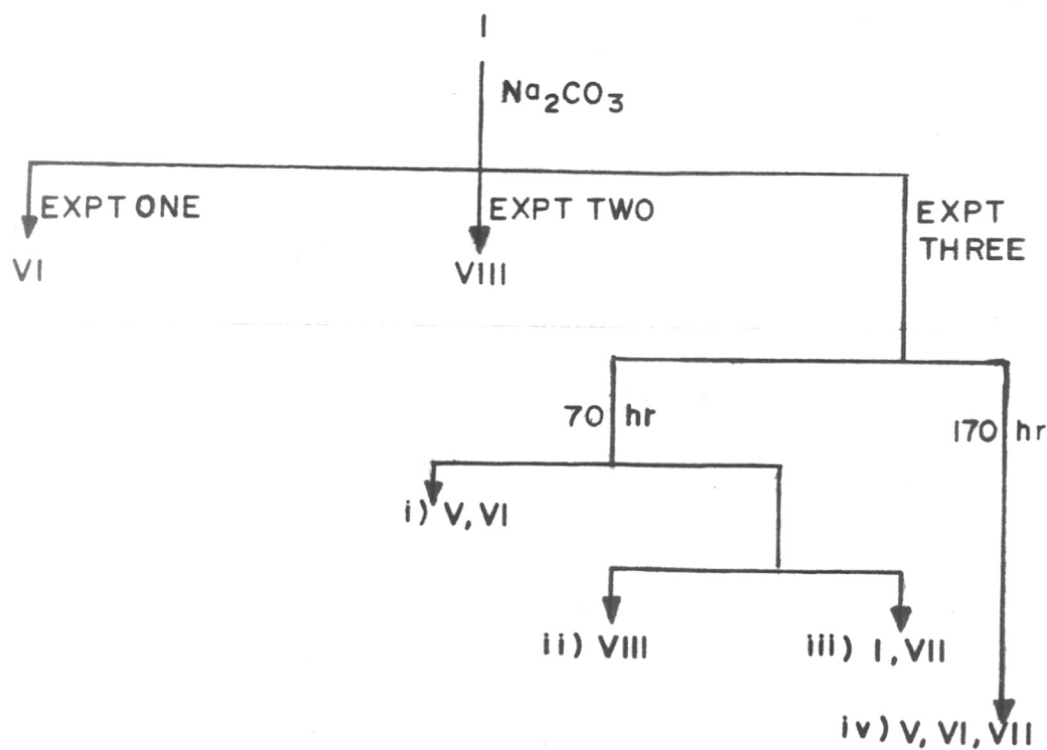
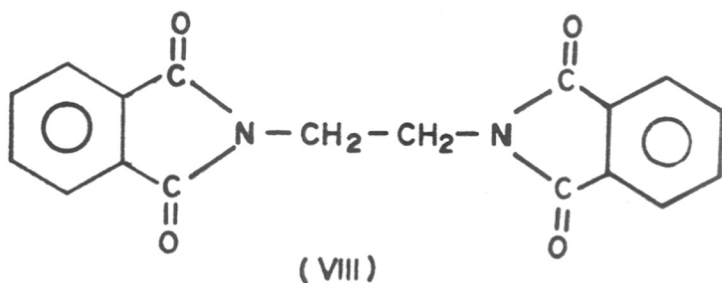
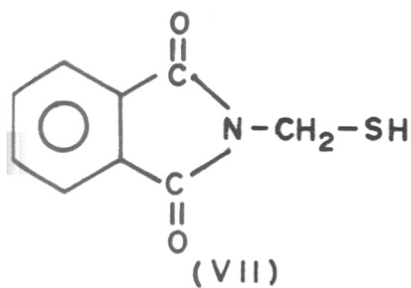
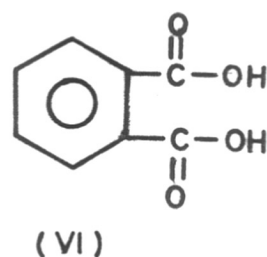
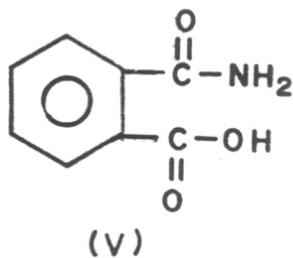
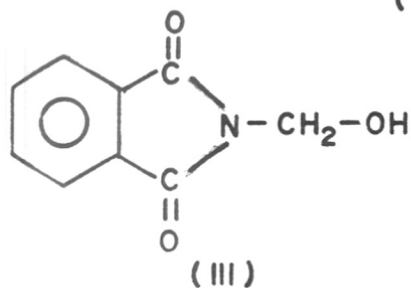
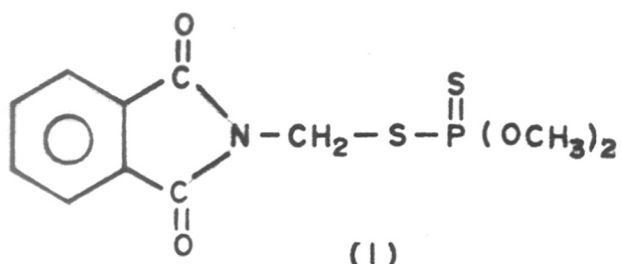


FIG. 10

It was identified as phthalic acid³¹ (Fig. 10, VI) as described in 4.3.2.1-Expt. One.

Expt. Two: In a 250 ml conical flask provided with a stopper, Imidan (I, 1 g) was added to 5% aqueous Na_2CO_3 solution (100 ml) and kept at 25°C for 180 hr. The heterogeneous reaction mixture was filtered to get clear solution which was then neutralised using 1N HCl. It was saturated with NaCl, allowed to stand at 25°C for 24 hr and extracted with ether to get gummy material which was repeatedly washed with benzene to get a white material as insoluble portion. It was recrystallised from ethanol. Yield 0.2 g, m.p. 230°C .

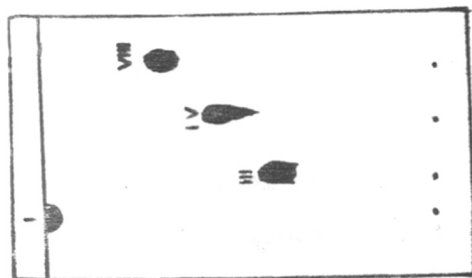
TLC : Benzene:chloroform (1:9) system and silica gel, single spot, R_f value 0.70 (Fig.11).

IR : 1770 and 1730 cm^{-1} ($>\text{C}=\text{O}$), (Fig. 12).

Mass spectrum : 93 (16), 104 (25), 105 (16), 125 (32), 130 (25), 133 (30), 160 (100) and 320 (50) (Fig. 13).

Elemental Analysis : Calculated for $\text{C}_{18}\text{H}_{12}\text{N}_2\text{O}_4$, C, 67.5%; H, 3.75%; N, 8.75%.
Found: C, 67.32%; H, 3.82%; N, 8.47%.

The compound was characterised as N,N'-ethylene diphthalimide⁶³ (Fig. 10, VIII), m.p. 233°C ⁴⁵, 243° - 244°C ^{44,46} by IR⁴⁶, mass fragmentation pattern⁶⁴ and elemental analysis.



BENZENE: CHLOROFORM
(1:9) SYSTEM

FIG 11 TLC OF EXPT. TWO

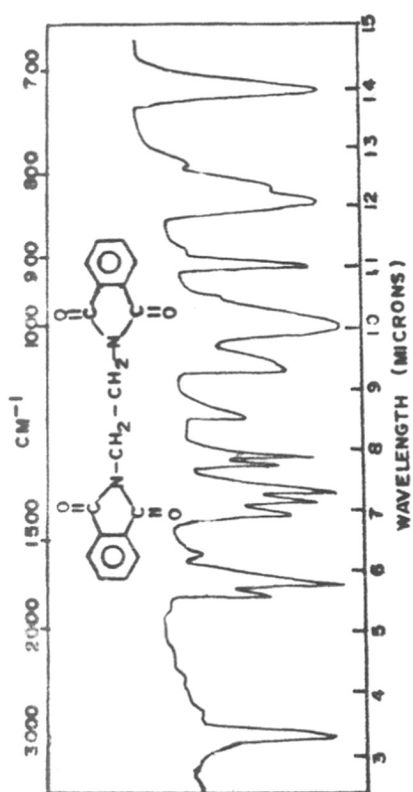


FIG 12 IR SPECTRUM OF N,N'-ETHYLENE DIPHTHALIMIDE, (VIII)

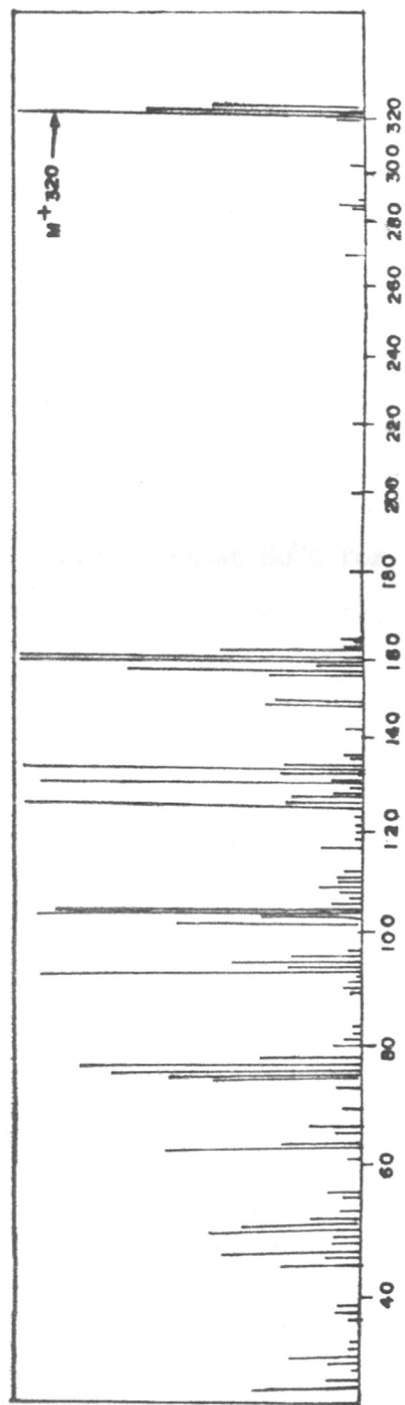


FIG 13 MASS SPECTRUM OF N,N'-ETHYLENE DIPHTHALIMIDE, (VIII)

Expt. Three: Powdered Imidan (I, 3.17 g, 0.01 M) was shaken with 3% aqueous solution of Na_2CO_3 (100 ml) to 250 ml conical flask provided with a stopper and allowed to stand at 25°C . Samples were drawn out time to time, processed and analysed by TLC.

- a) A sample was drawn out at the end of 70th hr. The heterogeneous reaction mixture was filtered. The clear solution was neutralised using 1 N HCl. It was saturated with NaCl and kept at 0°C for 24 hr. The precipitated material was extracted with ether and a residue was obtained by usual workup, m.p. 195°C . It was warmed with chloroform at 50°C for 30 min. A white solid was separated as undissolved material, m.p. 198°C (i). The chloroform layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated to get a gummy solid which was recrystallised from ethanol, m.p. 230°C . (ii). The mother liquor was concentrated to get gummy liquid which was repeatedly washed with cold water, dried at 25°C for 48 hr. It solidified on keeping, m.p. $80-90^\circ\text{C}$ (range) (iii).
- b) At the end of 170th hr the reaction mixture was filtered to remove undissolved material. The filtrate was neutralised using 1N HCl, saturated with NaCl and allowed to stand at 0°C for 24 hr. The precipitate after usual workup afforded yellow solid, m.p. 170°C (iv).

The total unreacted portion was a white solid.
Yield 2.26 g (68%), m.p. 68°C (v).

The components were identified by comparing their R_f values with that of authentic samples (Section 4.2). The results are summarised in Table 5.

Components iii-2 and iv-1 were isolated in pure form by preparative TLC, m.p.135°C. Both were identified as N-mercaptomethyl phthalimide⁴³ (Fig. 10, VII) as described in 4.3.2.2-Expt. Two.

Table 5

No.	Solvent system*	Component	R_f value	Name of the compound (Fig. 10)
1	EC	1	0.60	Phthalamic acid (V)
		2	0.50	Phthalic acid (VI)
ii	BC	1	0.70	N,N'-Ethylene diphtalimide (VIII)
iii	BC	1	0.98	Imidan (I)
		2	0.38	N-Hydroxymethyl phthalimide (III) ?
iv	BC	1	0.38	" " ?
		2	0.00	?
	EC (0.33)	2'	0.20	Phthalamic acid (V)
		2"	0.17	Phthalic acid (VI)
v	BC	1	0.98	Imidan (I)

*BC: Benzene:chloroform (1:9), EC:Ethanol:chloroform (1:1)

4.3.3 RESULTS AND DISCUSSIONS

Hydrolysis of Imidan was carried out using different reagents such as NaOH, HCl and Na_2CO_3 . Due to low solubility, the reactions were carried in heterogeneous medium which resembles with existence of the pesticide and its metabolites in environment. Therefore the predictions may be more reliable. The products were analysed by thin layer chromatography (TLC) as described in section 4.2. They were identified by their R_f values which were in good agreement with R_f values of authentic samples. Compounds isolated in pure form were further characterised by Infrared, mass spectroscopy and elemental analysis.

The results are as follows:

4.3.3.1 HYDROLYSIS USING NaOH: Number of reactions carried, are shown in Fig. 6. The reactions were chosen in such a way so that a stepwise process of the hydrolysis can be unveiled. Minimum time required to destroy the potent pesticide into nontoxic phthalic acid was also determined. Evolution of ammonia served as a criteria for the same.

Spontaneous hydrolysis of Imidan to phthalic acid occurs at elevated temperature. Therefore the intermediates are not isolable. So the reactions were carried at milder conditions i.e. dilute alkaline medium and low temp. They

are (i) Imidan $\xrightarrow[25^{\circ}\text{C}]{1\% \text{ NaOH}}$ phthalamic acid (V) + *phthalic acid* (VI)

(ii) Imidan $\xrightarrow[0^{\circ}\text{C}]{2\% \text{ NaOH}}$ phthalimide (IV) and O,O-dimethyl phosphorodithioic acid (III')

N-Hydroxymethyl phthalimide (III) was not actually isolated but its presence has been presumed by characterising the other fragment: O,O-Dimethyl phosphorodithioic acid (III'). For confirmation of its presence, some reactions were carried on the authentic compound which results in phthalimide (IV) and phthalic acid (VI). Other well-known reactions were repeated in support of the mode of stepwise hydrolysis.

Eventhough N-hydroxymethyl phthalimide (III) does not have prime place in metabolism studies⁴⁻⁹, it has been included in present studies because it is the initial cleaved product and it also may be present in environmental decomposition, particularly on storage.

Probable mechanism of the hydrolysis is as follows (Fig. 14). The electron deficient carbon atom which is flanked by two electronegative atoms viz. sulfur and nitrogen is readily attacked by the nucleophile OH^- . It results in N-hydroxymethyl phthalimide and O,O-dimethyl phosphorodithioic acid. The former then eliminates the sidechain as HCHO . The remainder cyclic amide is very

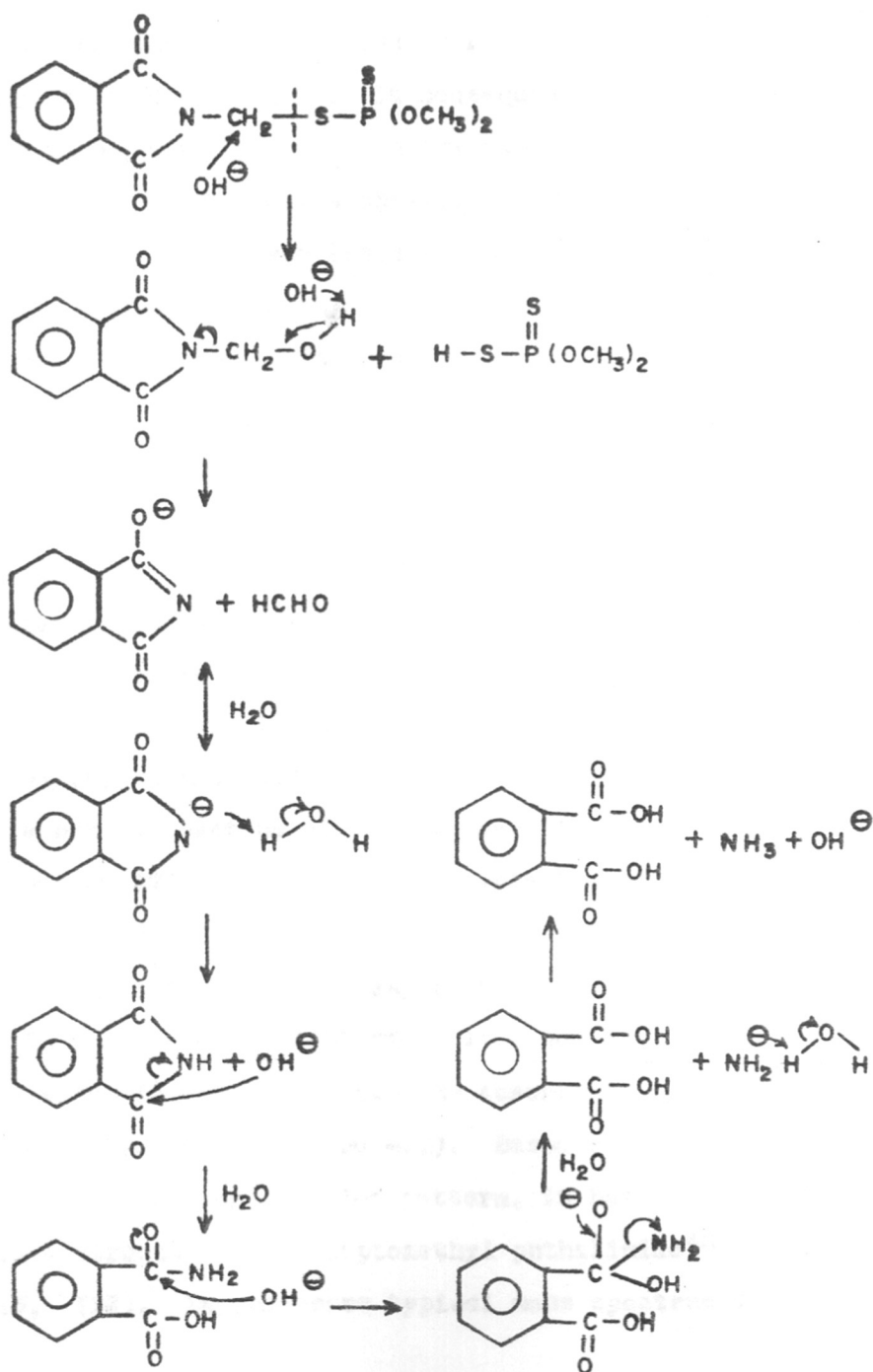


FIG. 14

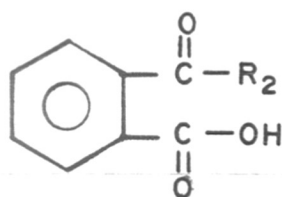
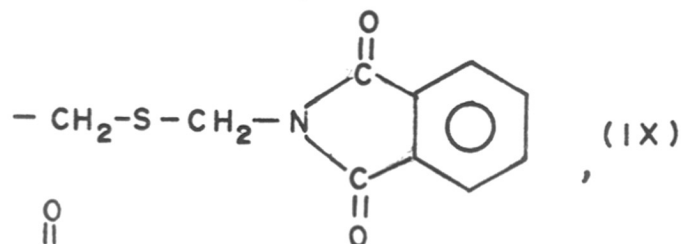
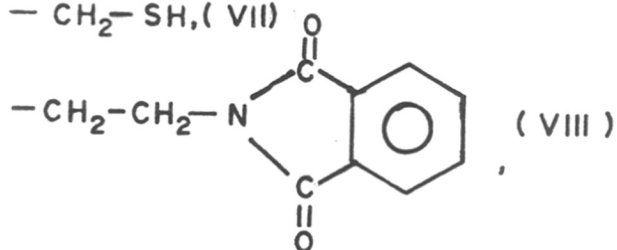
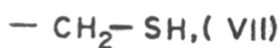
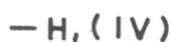
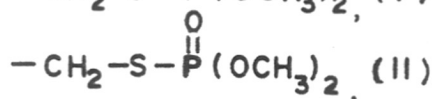
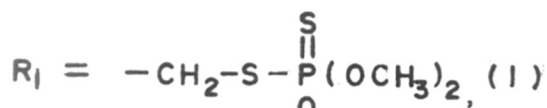
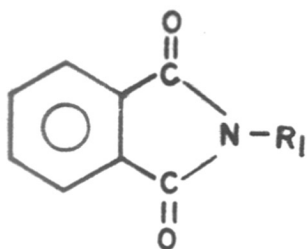
susceptible to the base which causes ring cleavage, resulting in phthalamic acid. It consequently gets converted into phthalic acid with the expulsion of ammonia.

On the basis of results obtained in the experiments, stepwise nature of the hydrolysis is as follows.

Imidan (I) $\rightarrow$ $\overline{N}$ -hydroxymethyl phthalimide (III) $\rightarrow$ phthalimide (IV) $\rightarrow$ phthalamic acid (V) $\rightarrow$ phthalic acid (VI) (Fig. 15a).

4.3.3.2 HYDROLYSIS USING HCl: In one experiment phthalimide (IV), phthalamic acid (V) have been detected. Unreacted Imidan (I) and Imidoxon (II) were identified from the insoluble portion. All of them were also detected in another experiment carried at different dilution.

The compound 11- 1 (Table 4) was believed to be $\overline{N}$ -hydroxymethyl phthalimide (III) as the R_f values were agreeable but IR spectrum of the mixture (11) did not show absorptions at 3770 cm^{-1} which is a typical absorption for the compound (III)²¹ (Section 4.2). Therefore its presence was doubtful. The purified component, 111-1 (Table 4) absorbs IR radiations at 2650-2200, 1760 and 1710 cm^{-1} (Fig. 8) which do not agree with the absorption of III; 3770, 1780, 1720 cm^{-1} (Section 4.2). Based on the elemental analysis and mass fragmentation pattern, it has been assigned a structure; $\overline{N}$ -mercaptomethyl phthalimide⁴³ (Fig. 15, VII). It possesses typical mass spectrum which



a) HYDROLYSIS USING NaOH



b) HYDROLYSIS USING HCl



c) HYDROLYSIS USING Na_2CO_3



FIG 15

is different from III i.e. VII- 76(58), 93 (80), 105 (80), 122 (56), 149 (100), 160 (45), 177 (68) and 193 (68) (Fig. 9). III - 51 (100), 75 (62), 77 (61), 78 (65), 103 (28), 104 (54), 105 (68), 106 (50), 132 (34), 150 (22), 162 (20) and 177 (23).

Phthalimide (IV), N-mercaptomethyl phthalimide (VII) and Imidoxon (II) have been reported for the first time as nonenzymatic hydrolysis products. Occurrence of II has been attributed due to oxidative desulfuration reaction⁴⁷. Presence of VII clearly indicates initial cleavage of Imidan at P-S linkage. Its co-occurrence has been reported with bis(phthalimidomethyl)sulfide (Fig. 15, IX)⁴³. Therefore the same may also be the next hydrolysis product which further leads to phthalimide (IV).

The experimental data reveals that the mode of acidic hydrolysis may be as follows. Imidoxon (II) ← Imidan (I) → N-mercaptomethyl phthalimide (VII) → $\bar{\text{L}}$ bis (phthalimidomethyl)sulfide (IX)⁷ → phthalimide (IV) → phthalamic acid (V) → Phthalic acid (VI) (Fig. 15b).

4.3.3.3 HYDROLYSIS USING Na_2CO_3 : Phthalamic acid (V) and phthalic acid (VI) are the major hydrolysis products. As N-hydroxymethyl phthalimide (III) and N-mercaptomethyl phthalimide (VII) possess identical R_f values on TLC, identification of components iii-2 and iv-1 (Table 5) was

rather difficult. Further analysis of the purified samples revealed that it was N-mercaptomethyl phthalimide (Fig. 15, VII).

Second experiment resulted in an abnormal product which has R_f value 0.70 (Fig. 11). It does not agree with any compounds mentioned earlier. Therefore further investigations were carried out. IR spectrum (Fig. 12) does not show presence of any functional groups except carbonyls. Mass spectrum (Fig. 13) indicates it to be a dimer of a compound (mole.wt. 160). Based on elemental analysis and characteristic mass fragmentation pattern⁶⁴, it has been assigned structure: N,N'-ethylene diphthalimide⁶³ (Fig. 15, VIII) which has identical IR absorptions⁴⁶. Both VII and VIII are reported for the first time in the alkaline hydrolysis. The latter has been reported as a thermally interchangeable product in some phthaloyl derivative studies⁴⁶.

As Na_2CO_3 is a weak alkali, it required more time to hydrolyse imidan completely into phthalic acid. Eventhough the phthaloyl ring cleaved at elevated temperature, at room temperature the reaction is sluggish resulting in the dimer (VIII). Therefore temperature plays an important role in determining mode of the hydrolysis. As Expt. Two and Three yielded different products, they are good examples of impact of dilution over course of the reaction.

Thus imidan initially hydrolysed at P-S linkage to get VII which may further lead to bis (phthalimidomethyl) sulfide⁴³ (Fig. 15, IX). However, the conversion is not confirmed. Mechanism of formation of the dimer (VIII) is also not clear. Further there may occur ring cleavage initially than $N-CH_2$ or H_2C-CH_2 fission to get V and VI.

Thus mode of the hydrolysis is as follows. Imidan (I) $\rightarrow$ N-mercaptomethyl phthalimide (VII) $\rightarrow$ bis (phthalimidomethyl) sulfide (IX) $\rightarrow$ N,N'-ethylene diphthalimide (VIII) $\rightarrow$ phthalamic acid (V) $\rightarrow$ phthalic acid (VI) (Fig. 15 C).

4.3.3.4 APPLICATION OF THE TLC METHOD: The analytical technique described in 4.2 has been developed to assist different types of decomposition studies. It has been employed in the hydrolysis studies so that its usefulness may be justified.

It has been observed that the method is satisfactory to detect the major decomposition products (I-VI). As N-hydroxymethyl phthalimide (III) and N-mercaptomethyl phthalimide (VII) possess identical R_f values, their presence could not be confirmed by TLC. The method is also useful to detect abnormal products such as N,N'-ethylene diphthalimide (VIII) as they possess nonagreeable R_f values. Thus the TLC technique is suitable for hydrolysis studies. Therefore its applicability is fully justified in this case.

4.3.3.5 BEHAVIOUR OF P-S-C-N BRIDGE: Important feature of Imidan molecule is the phosphoryl bond containing phosphorus atom which is Sp^3 hybridised and possesses nitrogen oxide type tetrahedral structure⁴⁷. The bond arises from the donation of the non-bonding 2p electrons in the polarised P-S and/or P-O bonds. The electrons return to phosphorus atom through the orbitals and the phosphoryl group has partial $p\pi-d\pi$ bonding along with σ bond. Orbitals of sulfur or oxygen having unshared electrons, partially overlap with 3d orbitals of phosphorus. Due to this, all phosphorus linkages have double bonding character⁴⁸.

Another typical feature of the molecule is P-S-C-N bridge. It is highly polarised as electronegative and electro-positive atoms are placed sidebyside. Due to the double bonding nature of phosphoryl bond and high electronegativity of sulfur and nitrogen atoms, only carbon atom is susceptible for nucleophilic attack of OH^- . This results in S-C bond fission, yielding N-hydroxymethyl phthalimide (Fig. 15, III) and O,O-dimethyl phosphorodithioic acid (Fig. 6, III'). This is the behaviour of the bridge with NaOH. Eventhough the same is expected in the hydrolysis using Na_2CO_3 , it was not observed. N-mercapto-methyl phthalimide (Fig. 15, VII) has been isolated leading to N,N'-ethylene diphtalimide (Fig. 15, VIII) presumably via bis[^](phthalimidomethyl)sulfide (Fig. 15, IX).

It indicates P-S cleavage in the molecule. Above mentioned assumptions fail in this case and the behaviour may be explained due to octet expansion of phosphorus atom which is attacked by the nucleophile⁴⁹. Thus the double bonding character of phosphoryl bond seems to be nonrigid and partial.

Acid hydrolysis of Imidan occurs due to protonation of sulfur atom in the bridge, further weakening the P-S bond. Phosphorus atom is then readily attacked by water molecule. It results in N-Mercaptomethyl phthalimide (Fig. 15, VII) leading to phthalimide (Fig. 15, IV).

Thus, behaviour of the P-S-C-N system is rather uncertain and predominantly depends upon basic nature of reagents.

4.3.3.6 COMPARISON WITH EARLIER STUDIES: It has been reported that Imidan is more stable in acidic medium (half life 13 days at 4.5 pH) than in alkaline medium (half life 4 hr at 8.3 pH)⁵. Similar reactivity has been observed in present studies. At elevated temperature, NaOH completely destroys the pesticide within 1 hr. The weaker alkali namely Na_2CO_3 requires 5 hr, while 90% of the material remains unreacted at the end of 6th hr in acidic medium.

As present studies are aimed to study the modes of the reactions, it not only covers previous studies but provides additional valuable information. Eventhough the

NaOH hydrolysis leads to phthalamic acid (Fig. 15, V), it finally results in phthalic acid (Fig. 15, VI) via phthalimide (Fig. 15, IV) as an intermediate. Mode of hydrolysis using NaOH determined here is concurrent with that one proposed by McBain et al.⁶

Though Menn et al.⁵ have reported hydrolysis of Imidan C¹⁴ in acidic medium, they have identified only phthalamic acid (V) and phthalic acid (VI) but less polar compounds remain unidentified. In present investigations, in addition to above mentioned compounds, phthalimide (IV) and N-mercaptomethyl phthalimide (Fig. 15, VII) have been characterised. It provides information about initial cleavage of the pesticide and stepwise mode of acid catalysed hydrolysis.

Hydrolysis of Imidan using Na₂CO₃ has been reported for the first time.

The fate of phosphorus moiety is found to be identical with the studies reported by Plapp⁴⁰, Dauterman⁵⁰ and Uchida⁵¹ i.e. O,O-Dimethyl phosphorodithioic acid was converted into inorganic phosphates.

Principle of identity relationship between metabolism and alkaline hydrolysis has been mentioned by Plapp et al.⁴⁰ in case of organophosphorus pesticides. It has also been proved by Schmach^uer¹⁸ in the studies of a drug 'thalidomide'.

McBain et al.⁶ in his detailed report have shown the same for imidan. As more information has been gathered in the present studies, it is necessary to mention that experiments using Na_2CO_3 gives altogether different products. Therefore the identity pertains to hydrolysis only using NaOH . The relationship is confirmed in these investigations.

4.3.3.7 COMPARISON WITH METABOLIC STUDIES: Based on various reports⁴¹ including the identity relationship (section 4.3.3.6) and formation of identical products in major portion, metabolism of imidan in plants^{7,8}, animal^{5,6} and soil⁵ is preferentially hydrolytic than oxidative. Presence of the metabolite: Imidoxon (Fig., 15, II) was due to oxidative pathways^{6,7}. Benzoic acid and its hydroxy derivatives⁷ have been originated from complex biochemical reactions. Their presence was suggested due to decarboxylation and minor hydroxylation which occurs prior to hydrolysis⁶.

Eventhough generally catalytic role of enzymes is important in such type of biodegradation reactions^{53,54}, chemical hydrolysis can provide sufficient information to predict metabolic pathways which are predominantly hydrolytic and side reactions though occur are minor.

4.4 EFFLUENT TREATMENT

4.4.1 NEED OF APPLICATION

Nearly 900 pesticides are in use all over the world. Therefore pollution from their industrial waste poses a serious threat to the environment. Eventhough consumption of pesticides in India is low, it is ever increasing. So the problem will be very acute in coming years. Particularly aquatic pollution has immediate effects to cause large scale mortality of living things. The major source of water contamination is effluent water from pesticide process industries which dispose off huge amount of toxic material from reactor, equipment and container washings. Organo-phosphorus pesticides are very toxic at initial stages though ^{They} ~~it~~ leads to nontoxic products at the end⁵⁵. Their chronic side effects are already discussed in section 1.2.6. Therefore it is necessary to minimise water pollution by treating the waste water from these pesticide industries before disposing them into main streams.

4.4.2 METHODS OF APPLICATIONS

Recently Sharma⁵⁶ and Duttachowdhury⁵⁷ have summarised various methods of applications. They are mainly divided into three parts as physical, chemical and biological. First one mainly includes screening, degritting, clarification,

gravity separation, filtration, flotation and adsorption. Now-a-days scrubber units and adsorption techniques are most common for organophosphorus pesticides⁵⁸. Former consists of high pressure sprays projected against a membrane in the direction of gas flow. In latter, multiple columns loaded with activated carbon are arranged in series. Adjustment of pH in the range 6-8 by lime or acid is a preliminary treatment in chemical methods. Use of caustic lye has been found effective for organophosphorus waste⁵⁹. Oxidation methods though common for other pesticides, are avoided as they result in highly toxic products. Ion-exchange is also used less due to deterioration problems. In biological techniques, enzyme catalysed reactions are used to degrade organophosphorus pesticides to less toxic forms⁶⁰. The mode of degradation is mainly oxidative.

4.4.3 APPLICATION OF PRESENT INVESTIGATIONS

Our laboratory is extensively engaged in the development of processes for pest control agents. Related problems such as effluent treatment are discussed simultaneously. On this background of need of applying various methods, chemical hydrolysis has been suggested for further applications. It is because, detailed study of the reaction has been done and a good analytical method has also been developed to monitor

the treatment. Therefore the nature of the treatment is already known to facilitate in standardising the procedure.

Eventhough Imidan does not leave any residue in aquatic media⁶², the need of treatment has been necessitated because the transformations are highly toxic in initial stages³⁹.

Eventhough solubility of the pesticide is very less (25 ppm at 25°C), it is detectable by TLC. Presence of solvents such as methanol (5%) and benzene (15%) increases its solubility to the extent of 1.14% but 1% level may be considered as normal because organic solvents do not present in effluent water to that extent.

It has already shown in 'Hydrolysis Studies' (Section 4.3) that only NaOH reaction is useful to destroy the pesticide completely. Therefore the same is recommended for effluent treatment. Detailed reaction conditions are mentioned in 4.3.2.1 Use of 1% NaOH is enough to carry out detoxication of 1% Imidan. It also renders treated effluent alkaline after diluting it ten times (pH 8). Therefore it does not exceed the prescribed limit. The end product: phthalic acid is naturally occurring⁵², nontoxic⁶¹ substance. Therefore its isolation is not essential.

Eventhough the method has been proved satisfactory on laboratory scale, it needs more trials on industrial scale.

Following procedure has been suggested for its industrial applications. The effluent water is allowed in settling tanks or filtration beds which remove large portion of the undissolved pesticide. It can be treated separately. It also facilitates evaporation of solvents and water so that solubility of Imidan is decreased further and volume of the reaction mixture is reduced. Excess alkali ensures complete destruction of Imidan rendering in alkaline solution at the end. The treated aqueous layer may be analysed by TLC as a safety measure and can be drained out safely.

REFERENCES

1. Fancher, L.W., U.S. Patent 2,767,194 (1956).
2. Fancher, L.W., and Hallett, J.T., Belg.Patent 630,933 (1963).
3. Batchelder, G.H., Patchett, G.G. and Menn, J.J.,
Zweig, G., Ed., Analytical Methods for Pesticides,
Plant Growth Regulators and Food Additives, V,
258 (1967).
4. Ford, J.M., Menn, J.J. and Meyding, G.D.,
J.Agric.Food Chem., 14, 83 (1966).
5. Menn, J.J., McBain, J.B., Adelson, B.J. and
Patchett, G.G., J.Econ.Entomol., 58, 875 (1965).
6. McBain, J.B., Menn, J.J. and Casida, J.E.,
J.Agric.Food Chem., 16, 813 (1968).
7. Menn, J.J. and McBain, J.B., *ibid.*, 12, 162 (1964).
8. Batchelder, G.H. and Wise, J.J., Stauffer Chem.Co.,
Western Research Centre, Calif., Personal Communic.(1963).
9. Tsukamoto, M. and Casida, J.E., Nature, 213, 49 (1967).
10. Hamilton, D.J. and Simpson, B.W., J.Chromatogr.,
39, 186 (1969).
11. Abbott, D.C. and Egan, H., Analyst, 92, 475 (1967).
12. Getz, M.E. and Wheeler, H.G., J.Assoc.Offic.Anal.
Chemists, 51, 1101 (1968).

13. Watts, R.R., Res.Rev., 18, 105 (1967).
14. Villeneuve, D.C., Butterfield, A.G., Grant, D.L.
and McCully, K.A., J.Chromatogr., 48, 567 (1970).
15. Mendoza, C.E., Wales, P.J., McLeod, H.A. and
McKinley, W.P., Analyst, 93, 34 (1968).
16. Abbott, D.C. and Thomson, J., Res.Rev., 11, 1 (1965).
17. Menn, J.J., Patchett, G.G. and Batchelder, G.H.,
J.Econ.Entomol., 53, 1080 (1960).
18. Schumacher, H., Smith, R.L. and Williams, R.T.,
Brit.J.Pharmacol., 25, 324 and 338 (1965).
19. Buc, S.R., J.Am.Chem.Soc., 69, 254 (1947).
20. Liebermann, C., Ber., 19, 2283 (1886).
21. Dyer, J.R., Applications of Absorption Spectroscopy of
Organic Compounds, Prentice-Hall Inc. (USA), 1965.
22. Janzen, A.F. and Kramer, E.A., Can.J.Chem., 49,
1011 (1971).
23. Harbenind, I.G., German Patent 442744 (1927).
24. Christmann, L.J., U.S. Patent 1,893,018 (1928).
25. Fletcher, J.H., Hamilton, J.C., Hechenbleikner, I.,
Hoegberg, E.I., Sertl, B.J. and Cassaday, J.T.,
J.Am.Chem.Soc., 72, 2461 (1950).
26. Damico, J.N., J.Assoc.Offic.Anal.Chemists, 49(5),
1027 (1966).
27. Lorenz, W., Ger.Patent 930,446 (1955).

28. Schrader, G., Die Entwicklung neuer insektizider Phosphorsaure Ester, Verlag Chemie, Weinheim, 281 (1963).
29. Holmstead, R.L. and Casida, J.E., J.Assoc.Offic.Anal.Chemists, 57(5), 1050 (1974).
30. Chapman, E. and Stephens, H., J.Chem.Soc., 127, 1791 (1925).
31. Laurent, Ann., 19, 38 (1836).
32. Flett, M.ST., J.Chem.Soc., 962 (1951).
33. Vogel, A.I., Text Book of Practical Organic Chemistry 163 (1966).
34. Kirchner, J.G., Miller, J.M. and Keller, G.J., Anal.Chem., 23, 420 (1951).
35. Stahl, E., Chem-Ztg., 82, 323 (1958).
36. Kirchner, J.G., Thin Layer Chromatography, 2nd Ed., Wiley-Interscience, 9 (1978).
37. Menn, J.J., Erwin, W.R. and Gordon, H.T., J.Agric.Food Chem., 5, 601 (1957).
38. Mitchell, L.C., J.Assoc.Offic.Agric.Chemists, 41, 781 (1958).
39. Menn, J.J. and Miskus, R.P., Ref. 3,115 (1967).
40. Plapp, F.W. and Casida, J.E., J.Econ.Entomol., 51, 800 (1958).
41. O'Brien, R.D., Insecticides: Action and Metabolism, Academic Press, New York (1967).

42. Matsumura, F., Edwards, C.A., Ed., *Environmental Pollution by Pesticides*, Plenum Press, London, 494 (1973).
43. Henery-Logan, K.R. and Abdou-Sabet, S., *J.Org.Chem.*, 38(5), 916 (1973).
44. Beilstein's Handbuch der Organischen Chemie, Julius Springer Verlag, Berlin, XXI, 492 (1935).
45. Sakellarios, E.J., *Helv.Chim.Acta*, 29, 1675 (1946).
46. Saul, W. and Hasan, S.K., *Can.J.Chem.*, 48(22), 3566(1970).
47. Van Wazer, J.R., *Phosphorus and its Compounds*, Interscience, New York, I (1958), II (1961).
48. Hudson, R.F., *Structure and Mechanism of Organophosphorus Chemistry*, Academic Press, London (1965).
49. Traylor, P.S. and Westheimer, F.H., *J.Am.Chem.Soc.*, 87, 553 (1965).
50. Dauterman, W.C., Casida, J.E., Knaak, J.B. and Kowalczyk, T., *J.Agric.Food Chem.*, 7, 188 (1957).
51. Uchida, T., Dauterman, W.C., O'Brien, R.D., *ibid.*, 12, 48 (1964).
52. Cross, B.E., Galt, R.H.B., Hanson, J.R., Curitis, P.J., Grove, J.F. and Morrison, A., *J.Chem.Soc.*, 2937 (1963).
53. Aldridge, W.N. and Reiner, E., *Enzyme Inhibitors and Substrates*, North-Holland, Amsterdam (1972).
54. Oppenoorth, F.J. and Van, A.K., *Entomol.Exp.Appl.*, 4, 311 (1961).

55. Bender, M.E., Water Res., 3, 571 (1969).
56. Sharma, H.C. and Agarwal, R.A., Pesticides, 14(9),
3 (1980).
57. Duttachoudhury, S.R., Ghose, J.L., Lonsane, B.K.,
Raghavan, K.V. and Sandhu, J.S., J.Sci. and Ind. Research,
40(2), 126 (1981).
58. Bernardin, F.E. (Jr.) and Froelich, E.M.,
30th Ann. Purdue Ind. Waste Conf. (1975).
59. Powers, P.W., How to dispose of toxic substances and
industrial wastes, Noyes Data Corp., Park Ridge, N.J.,
388 (1976).
60. Coley, G. and Stutz, C.W., J. Water Pollut. Control. Fed.,
38, 1345 (1966).
61. Spector, W.S., Saunders, W.B., Ed., Handbook of
Toxicology, Philadelphia and London, 1, 236 (1956).
62. Frank, R., Holdrinet, M., Braun, H.E., Sirois, G.J.
and Ripley, B.D., 59th Chem. Inst. of Canada Conf.,
June (1976).
63. Gabriel, R., 20, 2225 (1887)
64. Sharvit, J. and Mandelbaum, A., Israel J. Chem.,
5(1), 33 (1967).