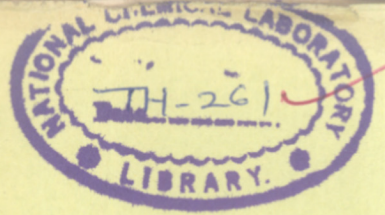


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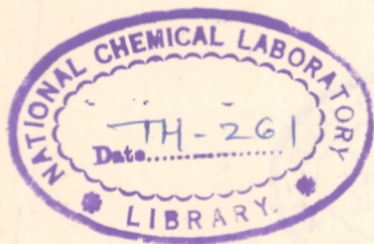
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AUGUST 1983

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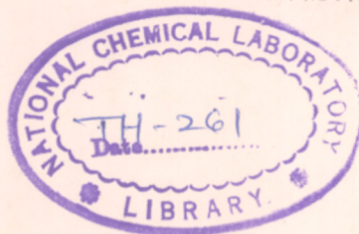
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*A Thesis Submitted to the
University of Poona
for the degree of*

MASTER OF SCIENCE

(Partly by Papers, Partly by research)

IN CHEMISTRY



BY

Mrs. NALINI VINAYAK BHALERAO

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CHAPTER – 1
INTRODUCTION

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

An organic chemist has used specific techniques and physical methods for the isolation, purification and identification of different compounds. Previous methods such as refractive index, boiling point, sodium fusion, solubility test, functional group test, molecular weight determination, combustion-analysis etc., for identification of organic compounds require much more quantity of sample and time. The spectroscopic techniques, widely used now a days, are rapid and require only milligram quantities. Widely used techniques are:

1. Infrared Spectroscopy
2. Ultraviolet Spectroscopy
3. Nuclear magnetic resonance
4. Mass Spectrometry

1. An infrared radiation when absorbed by a molecule, certain group of atoms give rise to absorption bands at or near the same frequency regardless of the structure of the remaining part of the molecule. The frequency of absorption depends on the relative masses of atoms involved and the force constant of the concerned bond in addition to geometry around the bond.

2. The Ultraviolet Spectroscopy deals with the electronic structure of the molecules. Conjugated systems are effectively studied with the help of this technique.

3. In nuclear magnetic resonance studies, the behaviour of nuclei having odd spin numbers (for e.g. ^1H , ^{19}F , ^{13}C , ^{31}P) when placed in a strong magnetic field and irradiated by radiofrequency corresponds to characteristic signals attributed to flipping of protons under specific chemical environment present in molecule.

4. In mass spectrometry an electron beam bombards the compound under investigation in high vacuum and positive ion fragments of the molecule are channelized to a detector where relative masses are recorded quantitatively as a spectrum. Separation of these positively charged fragments is strictly on the basis of mass/charge ratio.

All the above spectroscopic methods are useful for identification of different compounds. However the problem of getting pure compounds from intricate mixtures remains to be solved. This is achieved by chromatographic methods. Two mutually immiscible phases are brought into contact with each other wherein one is made to flow over a static stationary phase (solid support).

The sample is introduced into the above system which is partitioned between two phases and thus separated into pure components which are being eluted.

HISTORY OF CHROMATOGRAPHY

Day¹ and Tswett² are credited for the discovery of Chromatography in 1903-1906, although the word 'Chromatography' was used since 1731. Tswett recognised that chromatographic process consists of sorption-desorption interactions from his experiments of separation of plant pigments which were separated as coloured bands on calcium carbonate a column. The name "Chromatography" (Colour bands) given to this process is a misnomer in the present context.

In 1931 Kuhn and Lederer^{3,4} reported the separation of Carotene and Xanthophyll isomers by liquid solid chromatography proving it as an analytical tool. They used displacement and frontal analysis techniques. Wilson⁵ in 1940, was the first to describe the chromatographic method mathematically. Following the suggestion of Martin⁶ and Synge in a study for which they were later awarded the Nobel Prize, James and Martin⁷ introduced gas liquid chromatography in 1952. Most of the scientists who helped to enhance the knowledge of chromatographic method were not the analytical chemists but were from various disciplines like Botanists, Physical Chemists, Biochemists, Organic Chemists, Physicians, Chemical Engineers and Petroleum Chemists.

Classification of chromatographic methods

There are in all four types of chromatographic methods depending upon the nature of the stationary phases.

1. Liquid-Solid
 - a. Adsorption Chromatography
 - b. Thin-layer chromatography
 - c. Ion-exchange chromatography
2. Gas-Solid
 - a. Gas-solid chromatography
3. Liquid-Liquid
 - a. Partition chromatography
 - b. Paper Chromatography
4. Gas-Liquid
 - a. Gas-Liquid chromatography
 - b. Capillary column chromatography

Essentially chromatography is a method of separation where the components of the mixture are separated as a result of their partitioning between two immiscible heterogeneous phases. The amount of a particular substance adsorbed by the stationary phase, depends upon the concentration of that component in the mobile phase.

1a. Adsorption Chromatography

In this method column packed with activated solid phase such as alumina or silica gel, is used as stationary phase while the solvent flowing through it (eluent) as a mobile phase. The mixture to be separated is dissolved in a small quantity of the same solvent and passed on the column. The components of the mixture are carried with the solvent through the column. The weakly adsorbed component travels more rapidly than a strongly adsorbed one. The equilibrium is established between stationary and mobile phase.

Perfect selection of a mobile phase is an important factor. For polar adsorbents such as alumina or silica gel, the strength of adsorption increases with the polarity of the adsorbate. The adsorbent is called active provided that the surface area is more as well as the strength of adsorption is higher. Sometimes it is necessary to deactivate the adsorbent if very slow movement of the adsorbate is observed on the column. Adsorbents can be deactivated by addition of small quantities of solvents such as water.

1b. Thin-layer chromatography

In this method a mixture of adsorbent viz. Silica gel and a binder (Plaster of paris) is coated as a thin layer on a glass plate. The sample to be analysed is spotted on the

plate with glass capillary and the plate is kept in the required solvent system in a closed jar. It should be dipped just upto the spots. The separation depends on the polarity and adsorption of the component on the solid phase.

1c. Ion-exchange chromatography

In this chromatography ion-exchange resins are useful as column material. Cellulose sheets impregnated with ion-exchange resins, Zirconium phosphate and ammonium phosphomolybdate can be used. Synthetic resins commonly used include polystyrene and polymethacrylic acid. Cross linking in the polymer is an important factor as less cross linking leads to swelling because of large liquid uptake whereas too much linking reduces the ion exchange capacity because of steric hindrance.

There are two fundamental types of ion-exchange materials.

1. Cationic exchangers
2. Anionic exchangers

In cation exchangers the polar exchange groups are acidic ones like $-SO_3H$ or $-CO_2H$ while in anion exchangers basic polar exchanger groups are like NH_2 or HNH_2 . For ideal results the resin which is used in chromatography must have controlled cross linking and particle size must be as small as possible.

2a. Gas-solid chromatography

Gas solid chromatography represents a special part of chromatography where samples, mostly permanent gases and low molecular weight hydrocarbons, are separated by adsorption on an active solid rather than a liquid phase. Some of these active solids are alumina, charcoal, silica gel and various molecular sieves.

Alumina is used extensively for the separation of the hydrocarbon gases. Deactivation with required amount of water improves separation. Charcoal/^{activity}depends upon the source and prior treatment (activation).

Dry silica gel is used for most applications while in specific separations partially deactivated (using water or some liquid phases) silica is useful. The molecular sieves are synthetic zeolites. They are capable of separating materials based on their molecular shapes and configurations. They adsorb molecules in the intramolecular cavity according to the specific size.

3. Liquid-liquid chromatography

3a. Partition chromatography

The separation of components of a mixture by partition chromatography is a function of difference in distribution ratios of different components. In this technique the column is filled with substances like silica gel, activated charcoal,

kieselguhr, alumina or cellulose powder etc. which are saturated with a stationary solvent. This solvent forms a thin film on the surface of the column material. Common stationary liquid phases are polar solvents such as water, methanol, ethanol. Then the nonpolar solvent, such as chloroform, butyl alcohol or amyl alcohol etc. which forms a mobile phase, is passed through the column for elution. If there is difference in the distribution of components in these two solvent phases, then the components will move through the column at different rates.

$$\frac{C_A}{C_B} = \frac{C_{Mov}}{C_{Stat}} = K, \text{ the partition coefficient}$$

Where C_A = concentration of the component in solvent A (Moving)

C_B = concentration of the component in solvent B (Stationary)

The component with highest distribution ratio will be eluted out before the components with lower distribution ratio.

3b. Paper Chromatography

The adsorbent in this case is a specially treated paper, which is a thin layer of cellulose. Whatman No.1 filter paper is mostly used for separation of small quantities as it is cheaper and comparatively fast flow rate is obtained.

For preparative purpose special papers made up of fibres of polyamide, polyvinyl, glass etc. are used.

Each component, in a mixture to be separated, should be present as a single molecular or ionic species.

The component shows some specific Rf value which is a function of partition coefficient.

$$R_f = \frac{\text{Distance travelled by component}}{\text{Distance travelled by solvent}}$$
$$= \frac{L_K}{L_S}$$

The mixture to be separated is applied on the filter paper by capillary as small spots in a line. The spots are then allowed to dry. The paper strip is then suspended in a glass chamber saturated with a suitable solvent or solvent mixture. The paper when just dipped in the solvent upward flow of the solvent by capillary action permits separation of the components similar to thin layer chromatography. The paper is allowed to run to a specific length and then treated with suitable developers.

The developers used are (1) bromocresol green for acids (2) solution of ninhydrin in butyl alcohol for amino acids (3) solution of aniline and phthalic acid in butyl alcohol for aldehydes. The coloured bands are observed visibly and identified.

4. Gas-liquid chromatography (GLC)

Martin and Synges⁶ introduced the partition column chromatography in 1941. They had predicted the use of a gas

as the moving phase. In 1952 James and Martin⁷ reported their results where they had, for the first time, used the gas liquid chromatography technique. There was an improvement step by step in the sensitivity of detectors.

Apparatus and techniques for GLC

The apparatus is shown in the figure 1. The important part is a column, which is a long tube packed with an inert solid support, coated with the liquid stationary phase. The column is maintained at required constant temperature and a current of an inert gas is passed through it. A small quantity of the volatile mixture to be separated is introduced in the front end of the column with the help of hypodermic syringe.

Separation of components depends upon solubility in liquid phase. At the ^{other} end of column there is a device, the detector, which detects the individual components as they emerge out one by one.

Separation of complex mixtures of volatile substances with wide range in boiling points (-200° to 400° C) can be achieved in very short time by GLC technique. Both qualitative as well as quantitative analysis is possible with the introduction of GLC technique for separation of fatty acid esters⁸⁻⁹. It has now been used almost in all the branches of chemistry including steroids¹⁰, alkaloids¹¹, carbohydrates¹², lipids^{13,14}, carotenoids¹⁵, amino acids¹⁶, pesticides¹⁷,

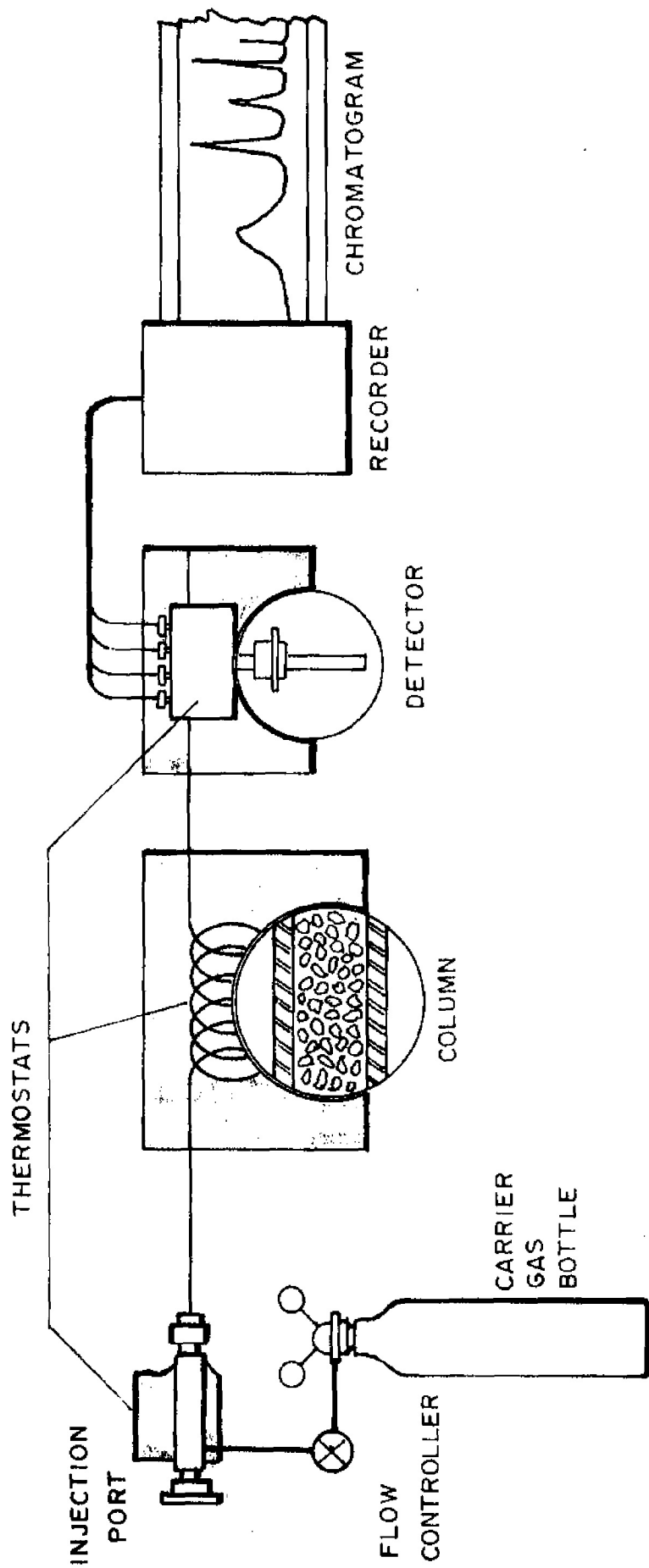


FIG. 1 . SCHEMATIC DRAWING OF A GAS CHROMATOGRAPHIC SYSTEM⁶⁷

drugs¹⁸, inorganic alloys¹⁹, clinical studies²⁰ etc.

Over 20,000 papers have been published dealing with almost every aspect of the application of this technique to analytical problems. GLC is thus a powerful analytical tool in the hands of scientists.

Mobile Phase (The carrier gas)

Inert gases like Nitrogen, hydrogen, helium, carbon dioxide are the gases which are commonly used as mobile phases. The selection of the carrier gas is done on the basis of its cost, availability and detector system employed. Hydrogen and helium have lower densities and higher thermal conductivities than nitrogen and therefore increase the sensitivity of the detectors. Hydrogen has been shown to have lower separation efficiency than nitrogen²¹. For good results the carrier gas is purified²² by passing through simple molecular sieve and fine filter to remove the impurities.

The Sampling Devices

For good resolution, quantity of the sample injected must be very small and the sample should be introduced as quickly as possible. Liquid samples are injected with the help of hypodermic syringes²³ while gas samples are introduced with by-pass gas pipettes or mercury burettes^{24,24a}.

The Chromatographic Column

The columns used for this purpose are in various shapes as straight, coiled or U-shaped. Generally the internal diameter of the column is 4-6 mm and the length is 1-3 meters. The common materials for these columns are glass, copper, aluminium and stainless steel. At high temperature copper column reacts with some samples such as amines, acetylenes, and terpenes. To avoid this defect glass columns are used for high temperature work. The liquid stationary phase is coated on a porous solid support. The solid support used should be inert, thermally stable and having high crushing strength. Also the particles should be uniformly spherical having large surface area. The size of the particles used is 60/80, 80/100, 100/120 mesh. Commonly used supports are celite, chromosorb, embasel and crushed fire brick. The optimum particle size should be between 80-80 mesh although smaller the particle size, greater the efficiency of the packed column. Porous supports are preferable over nonporous²⁵ ones.

The liquid phase is dissolved in a volatile solvent. Then it is coated (as a slurry) over the solid support material. The solvent is removed from the slurry by agitating under reduced pressure at low temperature (50° to 60°C). The solid support thus impregnated with the liquid phase is evenly packed in the column. Tapping or vibrating the column continuously

ensures uniform packing. The column, before use needs a period of conditioning to ensure the complete removal of moisture, solvent or other volatile contaminants in the preparation. The column should be heated 20° - 30° C above its proposed operating temperature for 20 hrs with stream of carrier gas passing through it.

4b. Capillary Column Chromatography

Martin²⁶ is given the credit for invention of capillary columns. In this type a liquid stationary phase is coated over the inner surface of a capillary tube.

Such columns, having low resistance to mobile phase flow, provide higher resolution and faster analysis. It requires highly sensitive detectors as flame ionisation detector. The liquid phase holding capacity of these columns is very small. Now a days it is possible to remove this defect by chemical treatment and these columns can hold more liquid phase.

Detectors

The chromatographic detector is a device which indicates and measures the amount of separated components in the carrier gas. There are two major classes of detectors.

1. Integrating detectors
2. Differentiating detectors

1. Integrating detectors give a response proportional to the total mass of component in the eluted zone, e.g. titrating burette.(2) Differentiating detector gives a response proportional to the concentration or mass flow rate of the eluted component e.g. Thermal conductivity detector, Flame Ionization detector

1. Selectivity
2. Sensitivity
3. Response
4. Noise and minimum detectable quantity
5. Linear range

These are the main characteristics to be considered for the usefulness of the detector. If possible, the detector should be simple, inexpensive, rugged and insensitive to changes in flow rate and temperature.

Thermal conductivity detector or Katharometer

This detector is based on the principle that a hot body will lose heat at a rate which depends upon the composition of the surrounding gas. Thus, the rate of heat loss can be used as a measure of the gas composition. The TC detector was introduced into gas chromatography by Claesson²⁷ in 1946 and has remained major detector ever since. A TC cell consists of a spiral filament wire, supported inside a cavity within a

metal block. The filament is made up of a material whose electrical resistance varies greatly with temperature i.e. it is having a high temperature coefficient of resistance. A constant current is passed through the filament causing its temperature to rise. When pure carrier gas is flowing, the heat loss is ^{constant} and thus the filament temperature is also constant. If the carrier gas composition changes because of the sample vapour saturation, the filament temperature changes causing a corresponding change in electrical resistance. It is this resistance change which is measured by a Wheatstone bridge circuit. The filament metal is chosen on the basis of high temperature coefficient of resistance and resistance to chemical corrosion. Common filament metals are platinum, tungsten and nickel. Thermal conductivity detectors are widely used because of their simple design, low cost, general applicability and good sensitivity.

Flame ionisation detector

This type of detector was introduced in 1956 for the first²⁸ time. The effluent gas from the column is mixed with hydrogen and burnt in air or oxygen. Ions and electrons formed in the flame enter the electrode gap, decrease the gap resistance, thus permitting a current to flow to the external circuit. The changes in the electrical conductivity of the

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flame are recorded as signals. The conductivity of pure effluent gas is just negligible while when it is mixed with sample vapour the flame produces ions carrying current. Flame ionisation detectors are very sensitive. The lack of response to air and water makes this detector suitable for the analysis of air pollutants, aqueous samples, biological materials etc. As there is no response for (CS₂) carbon disulphide, it makes carbon disulphide, a convenient solvent in use with FID.

Electron Capture Detector

This is an extremely sensitive detector to certain molecules, such as alkyl halides, conjugated carbonyls, nitriles, nitrates and organo metals but ^{is} virtually ⁱⁿ sensitive to hydrocarbons, alcohols, ketones etc. This detector is particularly useful for the analysis of pesticides because of its selective sensitivity.

The electron capture detector measures the loss of signal rather than a positively produced electrical current. The carrier gas should be inert. Generally nitrogen is the choice. The radioactive foil made up of Tritium or (Ni⁶³) produces the electrons which ionizes the carrier gas molecules producing electrons. These electrons migrate to the anode under the fixed voltage and produce a steady current. When the sample containing electron absorbing molecules is

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introduced, this steady current is reduced. Thus loss of current is a measure of the amount of the sample.

Recorder

The recorder does the main function of reproducing the output of the detectors graphically as accurately as possible to obtain a permanent record of the results in the form of chromatograms.

Conventional potentiometric strip chart recorder having 0-1 mV range with variable chart speed and 1 second or less, full scale response time, were the common recorders.

Now recently with the help of electronic integration systems and computerised circuits it is possible to get the full data printed on chart paper effortlessly.

Development of the theory

The actual separation of two closely spaced components of a mixture on a particular column is related to two factors.

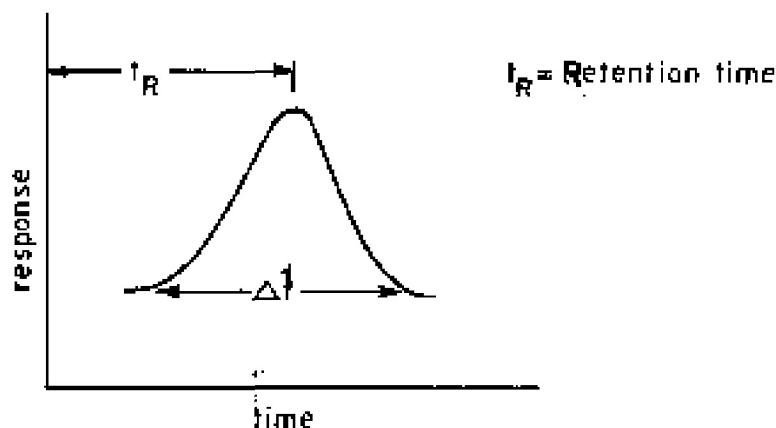
1. Column efficiency
2. Solvent efficiency

Column efficiency includes so many parameters

(a) length of column (b) diameter (c) inlet and outlet pressure ratio (d) nature and particle size of support (e) nature and flow rate of the carrier gas (f) temperature of the column.

Some of the fundamental theories for GLC are enumerated here e.g. (1) The plate theory of Martin and Synge⁸ modified by Glueckauf²⁹ (2) The rate theory of Van Deemter²⁸ and (3) The theory developed by Golay²⁰ for capillary columns.

The GC column efficiency is measured in terms of the number of the theoretical plates. The chromatographic column can be considered to be made up of a number of theoretical plates. Theoretical plate is defined³¹ as the length of column required to establish an equilibrium between the vapour pressure of the solute in the gas phase to the vapour pressure of the solute in the liquid phase. More the number of theoretical plates greater the efficiency of the column which can be increased by increasing the length of the column to a certain extent. However, with the increased length the ratio of inlet to outlet pressure also increases and thus tends to reduce the efficiency. Overall separation depends upon the separation per plate and total number of theoretical plates.



No. of theoretical plates (N) are calculated by

$$n = 16 \left(\frac{t_R}{\Delta t} \right)^2$$

$$HETP = \frac{\text{column length in centimeters}}{n}$$

HETP or Height Equivalent to a theoretical plate, $h = L/n$

HETP represents the peak broadening as a function of retention time. Low values of HETP correspond to efficient column operation. According to Van Deemter's²⁰ kinetic approach, three factors responsible for the column broadening are

- (1) Eddy diffusion (2) Molecular diffusion, and
- (3) Resistance to mass transfer.

1. Eddy diffusion

This factor results from the irregular paths the gas takes through the packed column. These paths have different lengths, cross sections and directions. Uniformity of particle size and proper packing helps to increase the efficiency of the column.

2. Molecular diffusion

Increasing molecular weight of carrier gas increases the contribution of this term to HETP.

3. Resistance to mass transfer

Components with greater solubility in the liquid phase will lead to somewhat smaller HETP.

$$HETP = 2 \lambda d_p + \frac{2Y D_{gas}}{\mu} + \frac{2}{\pi^2} \frac{K^1}{(1+K)^2} \frac{d_f^2 \mu}{D_{Liquid}}$$

Where

- λ = a quantity characteristic of packing
- d_p = average particle diameter of the solid support
- Y = a correction factor tortuosity of the inter particle spaces
- D_{gas} = diffusion coefficient in the gas phase
- μ = linear gas velocity
- K^1 = capacity factor defined as fraction of sample in liquid phase divided by the fraction in vapour phase
- d_f = thickness of liquid film
- D_{Liquid} = diffusion coefficient in the liquid film

Simplified equation is

$$HETP = A + \frac{B}{\mu} + C\mu$$

A = Eddy's diffusion or multiple path effect term.

B = Molecular diffusion term

C = Resistance to mass transfer term

μ = linear gas velocity

- a) Smallest particle size of solid support, (upto the limit as very fine powder cannot be packed evenly)
- b) Optimum flow rate,
- c) High molecular weight gas
- d) Liquid phase of low viscosity having low vapour pressure and good solubility for the sample at 1 to 10% loading.

e) Low inlet to outlet pressure ratio ,
 f) Adjustable temperature ,
 g) Narrowest possible internal diameter of the column
 together increase the efficiency of the column and good
 separation is achieved. Lewis³² has reviewed the
 combination of "rate" and "plate" theories which helps in
 better understanding of numerous parameters operating in gas
 chromatography. Rate theory supersedes the plate theory which
 suggests the ways in which the performance of a column can be
 improved³³. Golay's³⁰ theory describes the performance of
 capillary columns in terms of "HETP" and the average carrier
 gas velocity ' μ ' as

$$HETP = h = \frac{L}{N} = \frac{C_L}{\mu} + \sqrt{C_G} + C_D \sqrt{\mu}$$

where C_L = resistance in the liquid phase

C_G = resistance in the gas phase

Since solid support is not present in capillary columns,
 Eddy's diffusion term (i.e. term A) is vanished.

Factors responsible for separation

Several factors have influence on the separation on GLC
 columns. These factors are classified into two parts.
 Part one covers parameters of column like (1) length
 (2) diameter (3) inlet and outlet pressure ratio (4) the nature
 of supporting material (5) particle size of supporting material.

(6) temperature of the column (7) flow rate of the carrier gas.

Part two includes the most important factor that is nature of a stationary phase.

Stationary phase

The stationary phase plays an important role in GLC.

1. The ideal stationary phase should be thermally stable, when it is in close contact with supporting material.
2. It should not react with the components of the mixture at operating temperatures.
3. It should be a good solvent.

The chemical nature of the sample to be analysed and operating temperature are the main factors to be considered while selecting the stationary phase. There are three major categories of stationary phases depending upon the chemical structure.

(i) Polar (ii) Non-polar (iii) Semi-polar.

(i) Polar phases consist of polymeric materials, such as polyethylene glycols (carbowaxes) with molecular weights ranging from 200 to 15,000 or other simple organic polar compounds such as pentaerythritol, diglycerol etc.

(ii) The ^{stationary} stationary phases such as squalane, apieson-, silicone greases etc. are most non-polar in character.

(iii) Polyesters are the medium polar stationary phases. The polarity of liquid phase is defined in terms of 'polarity index'³⁴ or polarity scale³⁵. For this purpose the nonpolar phase squalane has ^{been} assigned the polarity index value zero and for polar phase, β, β' -oxydipropionitrile as 100. The difference in the retention volumes for a homologous series of nonpolar solutes is greater on a nonpolar than on a polar column. This forms the basis of Littlewood's polarity scale³⁵.

Common liquid phases

1. Liquid paraffin: Keulemans³⁶ used this phase to separate the C_3 - C_6 hydrocarbons. Separation using these phases is more or less by differences in boiling points. This can be used only upto 100°C temperature.

2. Phthalates³⁷ and halogenated phthalates

These have been used for the separation of numerous aromatic isomeric mixtures. These are all medium polarity stationary phases commonly used esters are dimethyl, diethyl, dibutyl and tetrachlorophthalates.

3. Polyglycols

Adlard has studied in details polyglycols or carbowaxes as stationary phases in the temperature range of 130-170°C for the separation of aromatic hydrocarbons³⁸. These are polar phases and can be used selectively for high boiling alcohols, esters and phenols.

4. Liquid phases containing inorganic electrolytes
 solution of silver nitrate in any glycol or benzyl cyanide^{39,40} has proved to be selective for the separation of olefins⁴¹.

Amines and hydrazines have been separated on polyethylene glycol containing potassium hydroxide⁴². Metal halides, sulphate and nitrate salts dissolved in the liquid phase have proved useful for the separation of isomeric phenols, cresols, nitro and bromo compounds⁴³.

5. Liquid Crystals

These columns are particularly useful for the separation of positional isomers which exhibit different solubilities on the crystal lattice. Richmond used three liquid crystals coated on 60/80 mesh chromosorb w, for the separation of twenty five di-substituted benzenes.

6. Silicones

Silicones are widely used as stationary phases because of their excellent thermal stability at temperatures upto 250°C and in some cases upto 400°C. These are suitable for the analysis of high boiling compounds like fluorooesters of cephoric acid, fatty acid esters, alcohols, amines etc. Previously only non-polar methyl and phenyl-silicones were available. Now a days selective fluorosilicones and polar cyanoethylsilicone are commonly used.

silicones can be attached directly to the solid supports by polymerisation involving chemical bonding. The thermal stability and overall efficiency of such phases is superior to other phases.

7. Polymer Substrates

These phases are thermally stable upto 250°C and give excellent separations for polar compounds. Fuller⁴⁴ has prepared porous co-polymers of divinylbenzene, ethylvinylbenzene and styrene bonded to support. Vinylformal propionitrile, a new polar liquid phase which remains stable at temperatures upto 300°C has been used for the separation of polyols, fatty acid esters, and multifunctional alcohols⁴⁵.

8. Mixed liquid phases

Weiser et al.⁴⁷ and Hilderbrand and Heilley⁴⁸ have studied the mixed liquid phases. When only single liquid phase cannot separate the complex mixtures, such mixed liquid phases are very useful. Mixed phase of carbowax-400 and silicone oil has been used for the separation of methyl iodide, ethyl iodide, cyclohexane and cyclohexane mixture.

9. Polyesters

The demand of substantially stable stationary phases of medium polarity is fulfilled by the polyester type plasticizers giving better resolution. These polyesters can be synthesised from number of dibasic acids and different glycols, while their

polarity can be readily varied by introducing various cross-linking agents. These phases are having low viscosity.

Grr and Calien used polyester plasticizer Neoplex 400⁴⁸ as ^{stationary} stationary phase which gave excellent separation of methyl esters of fatty acids compared to Apieson L and silicone greases liquid phases which were used before that. Lipaky⁴⁹ et al. tried adipate polyesters of neopentyl glycol, butylene glycol and diethylene glycol to achieve rapid and complete resolution of saturated and unsaturated fatty acid esters. Craig has tested phthalic acid polyesters for the analysis of fatty acid esters⁵⁰.

Adipate polyester of diethylene glycol treated with 2% phosphoric acid on celite⁵¹ ^{was} used to analyse fatty acids upto chain length C₂₂. A new polyester liquid phase prepared by the condensation of maleic acid with diethylene glycol has been found to be useful for the separation of methyl esters of fatty acid, including ^{isomers} cis-trans of octadecadienoic acid.

Naathi⁵² and co-workers have used five polyesters neopentyl glycol-adipate, succinate, ethylene glycol-succinate, adipate and isophthalate as liquid phase for the separation of steroids. Lipaky and Landowne⁵³ studied steroids by using polyester phases of succinate, dipate and sebacate with neopentyl glycol, 1,4-butanediol, 1,4-pentanediol and 2-ethylhexane 1,3 diol.

These semipolar polyester phases are advantageous over the other phases. A systematic investigation of these useful

polyester stationary phases have been carried out in this laboratory⁵⁴⁻⁵⁷ with very interesting results.

Preparative application of gas chromatography

Gas chromatography has the special device for the actual separation of the components along with the identification of the same. Spectroscopic methods are useful only in detecting the different components of a mixture. Preparative gas chromatography is the technique where you can isolate the components of a complex mixture. With automatic injection and collection device, analysis of sample size as large as 200-300 gms is reported⁵⁸.

The preparative gas chromatography functions in four different operations:

- 1) Injection of a sample by a pneumatic injection system
- (ii) Isothermal and temperature programmed control for the separation of individual components followed by a collection system to trap the ensuing vapours. (iii) Elevation of oven temperature to remove residual or unwanted components as rapidly as possible (iv) lowering of the oven temperature for the repetitive cycles.

Temperature Programmed GC

If the components of a complex mixture having wide boiling range are to be analysed then the isothermal conditions are not enough. For achieving separation in such cases the

temperature programming is required⁵⁹. The column temperature is gradually increased at a fixed rate so that more volatile components are eluted first and strongly retained components are resolved as sharp peaks at higher temperature.

Gas Chromatography/Mass Spectrometry

Gas chromatography and Mass spectrometry are two powerful instruments in the analysis of organic compounds. These two techniques in combination were used for the first time by Holmes and Morrell⁶⁰ in 1957 for the analysis of city gas. In 1960 Lindeman et al.⁶¹ successfully recorded the entire spectrum on an oscillograph recorder. The use of the two instruments simultaneously allows the optimum utilization of separating ability of one with identification possibility of other.

As the combined instrument is more sensitive, care must be taken to obtain optimal results. The column, the carrier gas and the flow rate must be so chosen that the efficiency of the separator is high but the pressure in the ion source and the vacuum system is relatively low.

Finkle et al. have established gc/ms reference data system for identification of drugs⁶². Eldjarn et al. have reviewed gc/ms application in routine and clinical research chemistry⁶³.

High performance liquid chromatography (HPLC)

HPLC is most recent development in liquid chromatography. Usual gas chromatography cannot be used for the analysis of ionic compounds, polymers etc. because of their thermal instability and insufficient volatility. HPLC is very much useful for these type of compounds.

Columns made up of stainless steel or glass with 2 to 3 mm internal diameter and 50 to 100 cms in length are used⁶⁴.

The column support is Zipax or Cerasil having particle size 20 to 60 microns. Polymeric silicone^{is} material used extensively for column packings now a days. As the column material is very fine, you have to push the solvent with the help of pressure pump. High sensitivity detectors⁶⁵ such as Refractive index, Ultraviolet, Microabsorption detector, with detection capacity of the order of 10^{-9} g or less, are incorporated in HPLC unit as standard fittings. All these detectors continuously and quantitatively monitor the analysis. Recently it is possible to transfer the pure sample from GLC directly on the plate or on a porous membrane⁶⁶ for IR analysis (1) Diethyl malonate (2) 2-hydroxydodecanoic acid are examples of such analysis.

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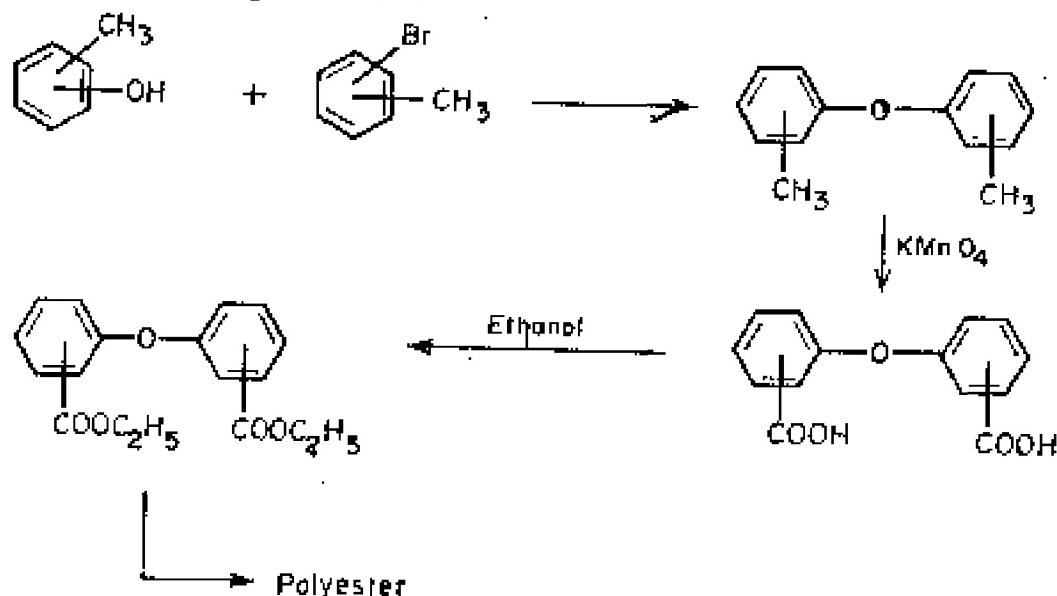
CHAPTER - 2
EXPERIMENTAL

EXPERIMENTAL

Seven new polyesters were synthesized for investigation of their solvent capability as stationary liquid phases. These polyesters were prepared from the following dicarboxylic acids and diol.

1. *p,p'*-Oxydibenzic acid - 1,3-propanediol
2. *o,p'*-Oxydibenzic acid - 1,3-propanediol
3. *m,p'*-oxydibenzic acid - 1,3-propanediol
4. *o,m'*-oxydibenzic acid - 1,3-propanediol
5. *o,o'*-Oxydibenzic acid - 1,3-propanediol
6. *m,m'*-Oxydibenzic acid - 1,3-propanediol
7. Diphenic acid - 1,3-propanediol

The oxydibenzic acids No.1 to 6 were prepared following the same method¹. Initially, corresponding ditolyl ethers were prepared from cresols and bromotoluenes and oxidized to the corresponding dicarboxylic acids. These acids were esterified to their diethyl esters.



The diphenic acid was prepared by another method² which then was esterified to it's diethyl ester. All these esters were converted to their respective polyesters.

"Synthesis of Ditolyl ethers"

o-Cresol (15 gms) was taken in a three necked (100 ml) RBF and potassium hydroxide pellets (9 gms) were dissolved in it. Heated the mixture on an isometric under reflux. Then the temperature was slowly raised upto 150°C to remove the water formed in the reaction. The reaction mixture was then cooled upto ~ 100°C and p-bromotoluene (25 gms) was added slowly in presence of Cu-bronze catalyst (0.25 gms) in the reaction mixture. The reaction mixture was again refluxed and heated until it reached the temperature of 210° to 220°C. The heating was continued for 3-4 hrs. at this temperature.

The reaction mixture was cooled and extracted with ether. The ether extract was washed with 5% sodium hydroxide solution first and then with water until the washings were neutral. The ether solution was dried over anhydrous sodium sulphate, ether was removed and the crude product was distilled under vacuum.

o,p'-Ditolyl ether -

B.P. 121°-23°/7 mm, yield 12 gms (41.37%)

Analysis - Found: C, 84.65; H, 7.07%

C₁₄H₁₄O requires C, 84.10; H, 6.9%

Similar procedure was followed for the preparation of all other isomers of ditolyl ether using the appropriate derivatives as given below:

1. p-Bromotoluene and p-cresol - p,p'-ditolyl ether;
Yield: 12 gms (44%); M.P. 43°C Lit. M.P. 49°C
2. m-Cresol and p-bromotoluene - m,p'-ditolyl ether;
Yield: 14 gms (49%); B.P. $173^{\circ}\text{C}/17\text{ mm}$. Lit. B.P. $175^{\circ}\text{C}/17\text{ mm}$.
3. o-Bromotoluene and m-cresol - o,m'-ditolyl ether
Yield: 13 gms (41.37%); B.P. $162-63^{\circ}\text{C}/17\text{ mm}$. Lit. B.P. $170^{\circ}\text{C}/17\text{ mm}$
4. o-Bromotoluene and o-cresol - o,o'-ditolyl ether
Yield: 19.5 gms (46.85%); B.P. $118^{\circ}\text{C}/4\text{ mm}$, Lit. B.P. $120^{\circ}\text{C}/4\text{ mm}$
5. m-Bromotoluene and m-cresol - m,m'-ditolyl ether;
Yield: 12 gms (41.37%); B.P. $274-79^{\circ}\text{C}$.

Microanalyses of all these five isomers show results within the limits of calculated values. Purity is checked by GLC and confirmed by IR, PMR data.

Preparation of oxidibenzic acid

In a three necked RBF (500 ml) a suspension of ditolyl ether (5 gms) in water (200 ml) was heated to reflux and the reaction mixture was stirred with mechanical stirrer. Under constant stirring potassium permanganate (20 gms) was added in small lots. The time required for addition was about 2 hrs. The reaction mixture further refluxed for 20 hrs after which it was filtered off to remove MnO_2 formed in the reaction.

The MnO_2 residue was boiled with a little fresh water and again filtered it. The combined filtrate was then extracted with ether to remove the unreacted ditolyl ether. Acidified the filtrate with conc. hydrochloric acid and cooled the solution. White precipitate obtained was filtered, washed with water and dried in oven at 115°C temperature.

1. p,p'-Oxydibenzic acid

Yield: 1.9 gms (89%); M.P. $225-26^\circ\text{C}$ Lit. M.P. 225°C .

ANALYSIS Found: C, 65.00; H, 4.00%

$\text{C}_{14}\text{H}_{10}\text{O}_2$ required C, 65.12; H, 3.90%

2. o,p'-Oxydibenzic acid,

Yield: 1.925 gms (89.6%); M.P. 209°C . Lit. M.P. 210°C .

3. m,p'-Oxydibenzic acid,

Yield: 1.5 gms (83%); M.P. 279°C Lit. M.P. 281°C .

4. o,m'-Oxydibenzic acid,

Yield: 0.45 gms (7%); M.P. 200°C Lit. M.P. 200°C .

5. o,o'-Oxydibenzic acid

Yield: 1.5 gms (83%); M.P. $216^\circ-217^\circ\text{C}$, Lit. M.P. 217°C

6. m,m'-Oxydibenzic acid,

Yield: 0.47 gms (7%); M.P. $235-40^\circ\text{C}$. Lit. 237°C

Microanalyses of all the acids are found within the range of calculated values.

Acids are confirmed by IR data, Fig. No.3.

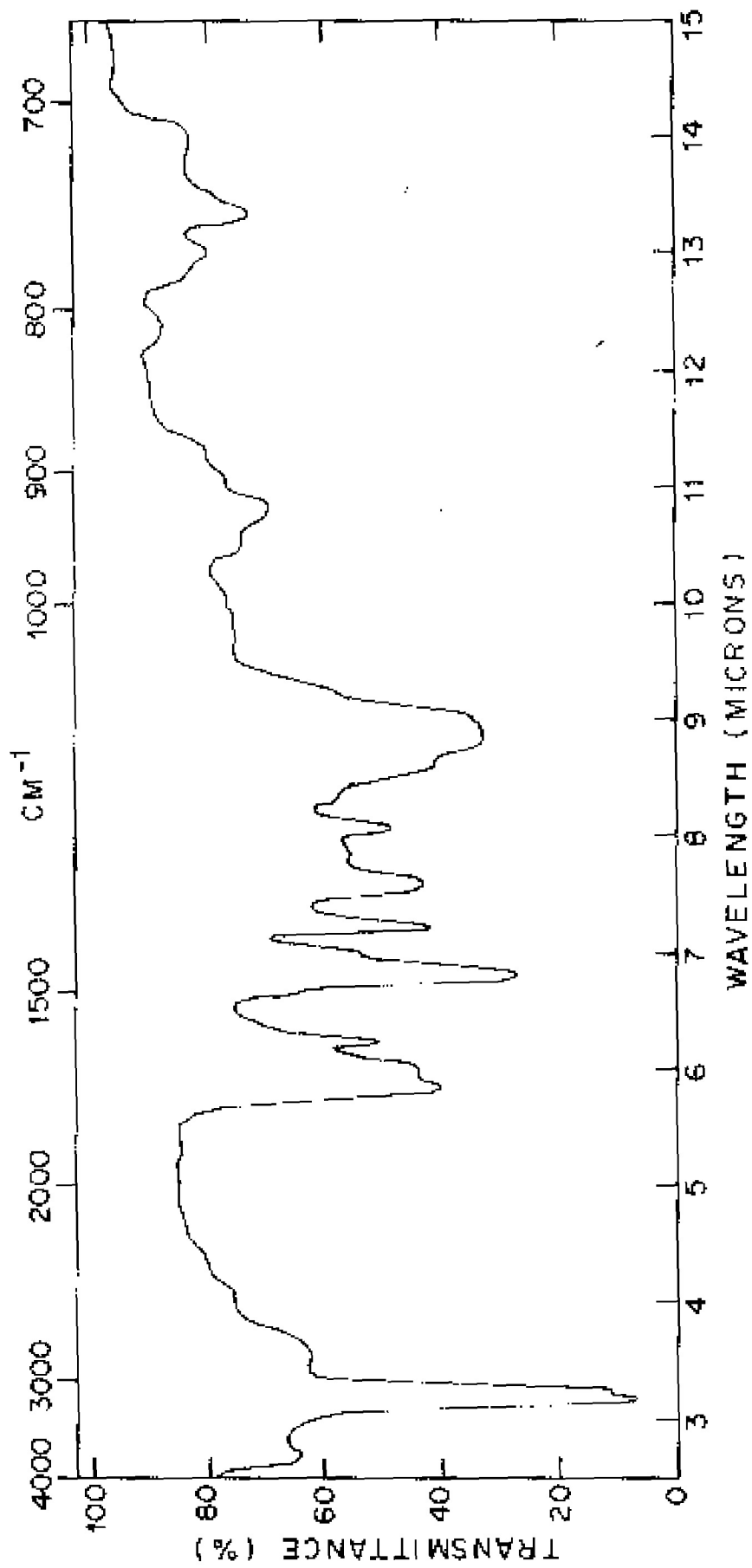


FIG. NO 2. IR SPECTRUM OF *m,m'*-OXYDIBENZOIC ACID

Preparation of diphenic acid

The diphenic acid is prepared by the following method².

In a three necked (100ml) RBF, phenanthrene (22 gms) was dissolved in glacial acetic acid (250 ml) and the solution was warmed on a water bath upto 85°C with continuous stirring. From the side neck H₂O₂ solution (90 ml of 30%) was run in dropwise over 40 minutes. After the addition was complete, the mixture was further heated for 3-4 hrs on a water bath. The volume of the reaction mixture was reduced to half by distilling out acetic acid under vacuum. The reaction mixture when cooled the precipitate settled down. The precipitate was collected by filtration on a buchner funnel, crystallized from acetic acid and dried at 120°C for one hour.

Yield: 9 gms (30%); M.P. 228°C, Lit. 230°C

Analysis: Found: C, 69.37; H, 4.09%

C₁₄H₁₀O₄ requires C, 69.42; H, 4.14%

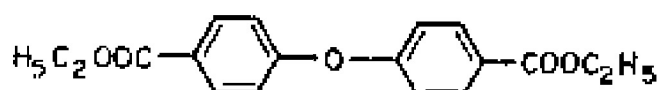
The acid is confirmed further by IR data.

Preparation of diethyl ester

All the seven acids were esterified to their diethyl esters by following the same procedure³. The acid (2 g) was taken in (100 ml) RBF, fitted with the reflux condenser and CaCl₂ guard tube. Dry ethyl alcohol (30 ml) and conc. sulphuric acid (0.75 ml) were added to it. The reaction mixture was

refluxed for 8 hrs. Excess ethyl alcohol was removed by distillation and 5% sodium bicarbonate solution (20 ml) added to neutralise the acid. The mixture was diluted with water (500 ml) and then extracted with ether. The ether layer after drying over anhydrous sodium sulphate was subjected to heating to remove ether and the residue distilled under vacuum.

1. Diethyl ester of *p,p'*-oxydibenzoic acid

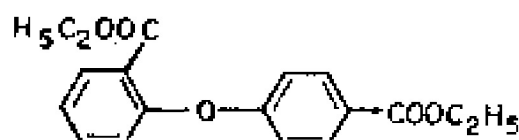


Yield: 1.2 gm (40%); B.P. 220°C/15 mm Lit. B.P. 170°C/4 mm.

Analysis: Found: C, 68.62; H, 6.74%.

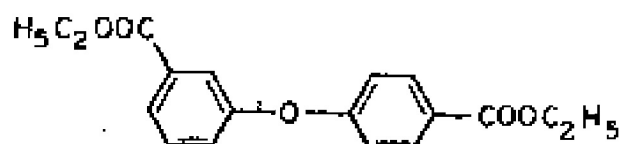
$C_{16}H_{12}O_6$ requires C, 68.73; H, 6.77%.

2. Diethyl ester of *o,p'*-oxydibenzoic acid

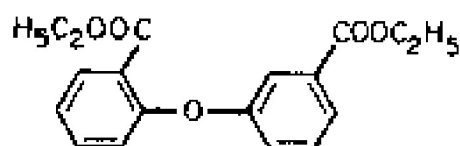


Yield: 1.7 gm (87%); B.P. 220°C/19 mm Lit. B.P. 220°C/19 mm.

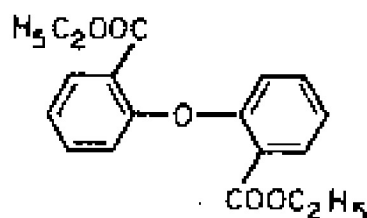
3. Diethyl ester of *m,p'*-oxydibenzoic acid.



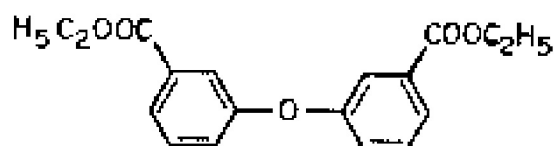
Yield: 1.7 gm (87%); B.P. 190°/5 mm.

4. Diethyl ester of *o,o'*-oxydibenzoic acid

Yield: 1.8 gm (60%); B.P. 179°C/15 mm, Lit. B.P. 181°C/15 mm.

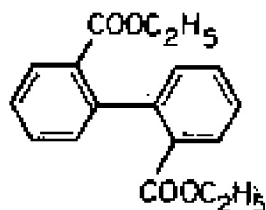
5. Diethyl ester of *o,o'*-oxydibenzoic acid.

Yield: 1.668 gm (55.8%); B.P. 185°C/15 mm (bath temp.)

6. Diethyl ester of *m,m'*-oxydibenzoic acid

Yield: 1.8 gm (50%); B.P. 220°C/17 mm.

7. Diethyl ester of diphenic acid



Yield: 1.8 gm (60%); B.P. 220°C/12 mm, Lit. B.P. 220°C/12 mm.

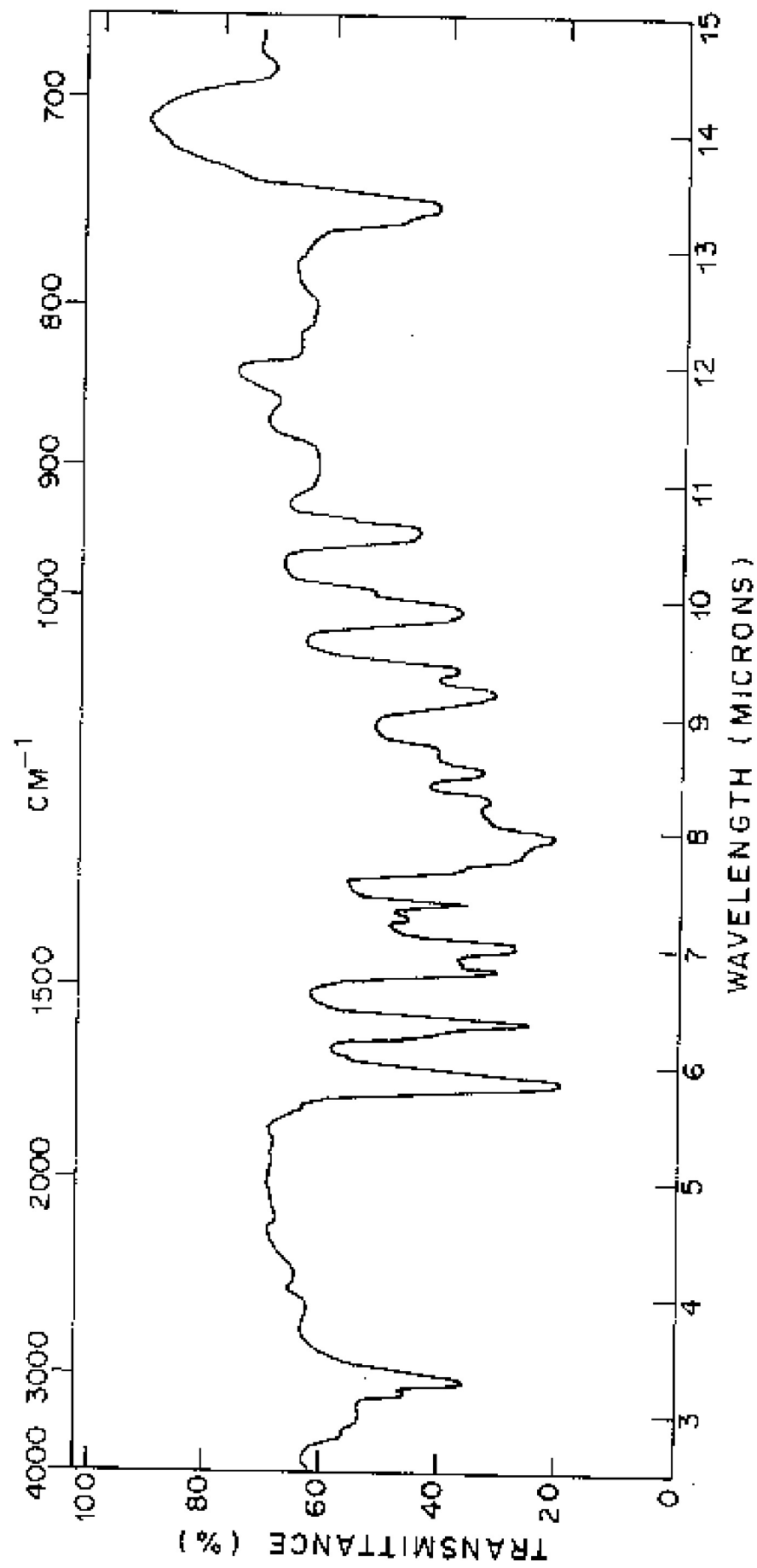


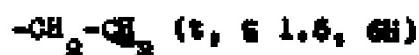
FIG. NO. 3. IR SPECTRUM OF DIETHYL ESTER OF *m,m'*-OXYDIBENZOIC ACID

The esters were confirmed by microanalysis, IR (Fig.No.3) and NMR data.

As the NMR data was not traceable in the literature, the spectra of some of the diethyl esters are described (Fig.No.5,6,7). The NMR spectra are recorded on T-60 NMR spectrometer operating at 60 MHz radiofrequency. The NMR spectra of diethyl esters of oxydibenzoic acids are shown in the figure. The chemical shifts of the protons are summarized below:

The TMS is used as an internal standard to measure the chemical shifts. The chemical shifts are given in δ value down to the TMS.

Fig. No.5 NMR spectrum of diethylester of p,p'-oxydibenzoic acid (Solvent: TFAA)



It is a symmetrical molecule. It is giving only one pattern. In aromatic system it gives A_2B_2 type pattern i.e. perturbed quartet, centered at 7.5 δ . The AA protons from phenyl ring which are ortho to ether linkage have appeared at higher field centered at (6.9 δ) and BB protons ortho to ester group have some little down field centered at (7.9 δ) in the aromatic region.

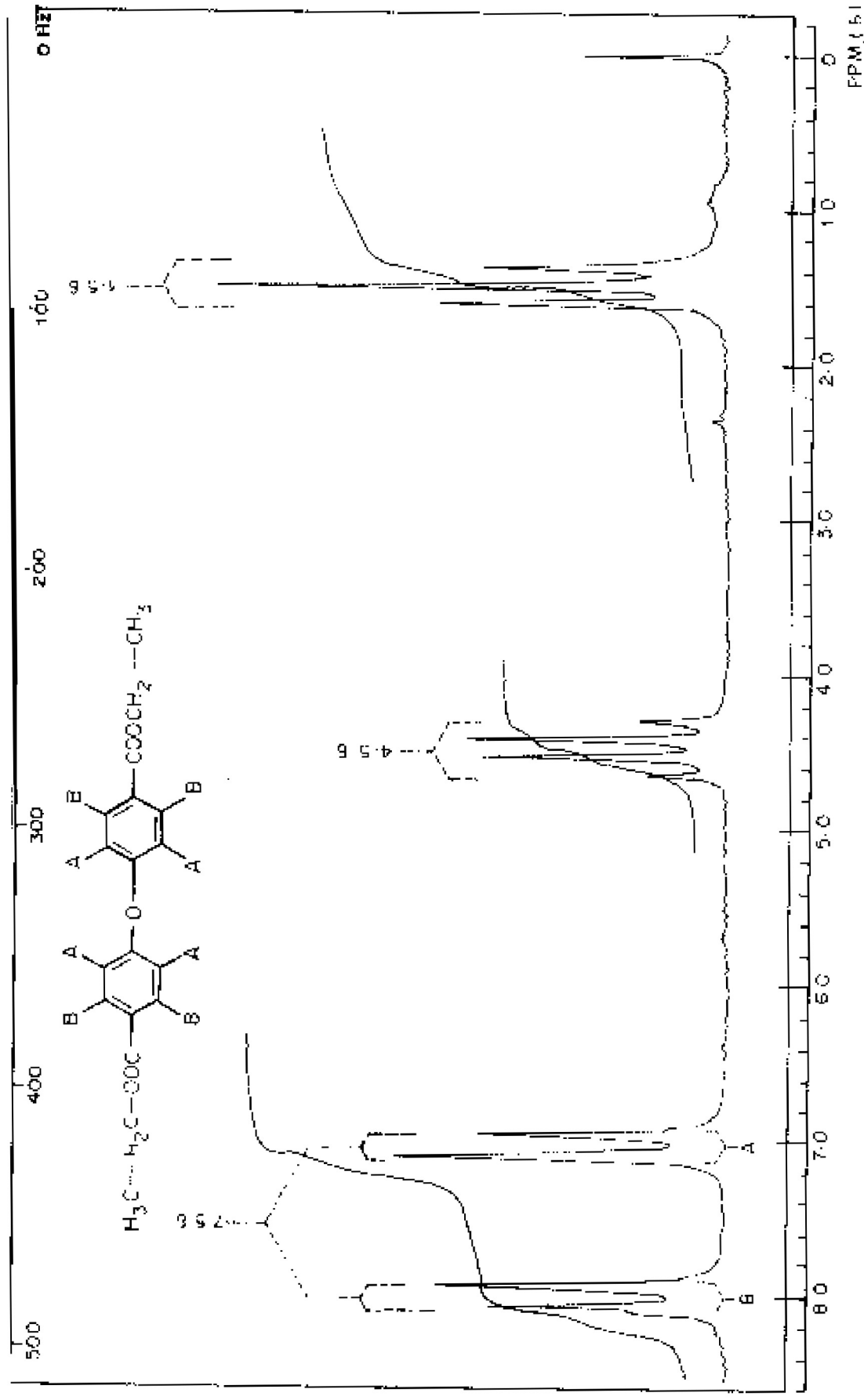


FIG. NO. 5. PMR SPECTRUM OF DIETHYLESTER OF P,P'-OXYDIBENZOIC ACID

Fig No.6 FMR spectrum of diethylester of
 m,p'-oxydibenzic acid (solvent: CCl₄)

$-\text{H}_4-\text{C}_6-\text{O}-\text{C}_6\text{H}_4$.. (m, δ 7.4, 8H)
$-\text{Ph}-\text{COOCH}_2$.. (q, δ 4.3, 4H)
$-\text{CH}_2-\text{CH}_2$.. (t, δ 1.4, 6H)

This system is not symmetric. Out of the two aromatic rings, ring A gives A₂B₂ pattern i.e. quartet, centered at 7.4 and ring B gives little complex pattern.

Fig. 7 FMR spectrum of diethyl ester of o,o'-oxydibenzic acid
 (Solvent: CCl₄)

$-\text{H}_4-\text{C}_6-\text{O}-\text{C}_6\text{H}_4-$	(m, δ 7.3, 8H)
$-\text{Ph}-\text{COOCH}_2$	(q, δ 4.23, 4H)
$-\text{CH}_2-\text{CH}_2$	(t, δ 1.27, 6H)

This is a symmetrical system. As it is 1,1'-disubstituted aromatic system it is giving a complex pattern showing multiplets

"Preparation of polyester liquid stationary phase"

Sankpal et al.⁴ prepared the polyesters of isomeric phthalic acids by heating their dimethyl esters with diethylene glycol (BHM quality) at 130°C for 2 hrs and further heating for 1 hr under vacuum at 4-6/mm. In the present work all the diethyl esters of oxydibenzic acids and diethyl ester of diphenic acid were reacted with 1,3-propane diol. A ratio

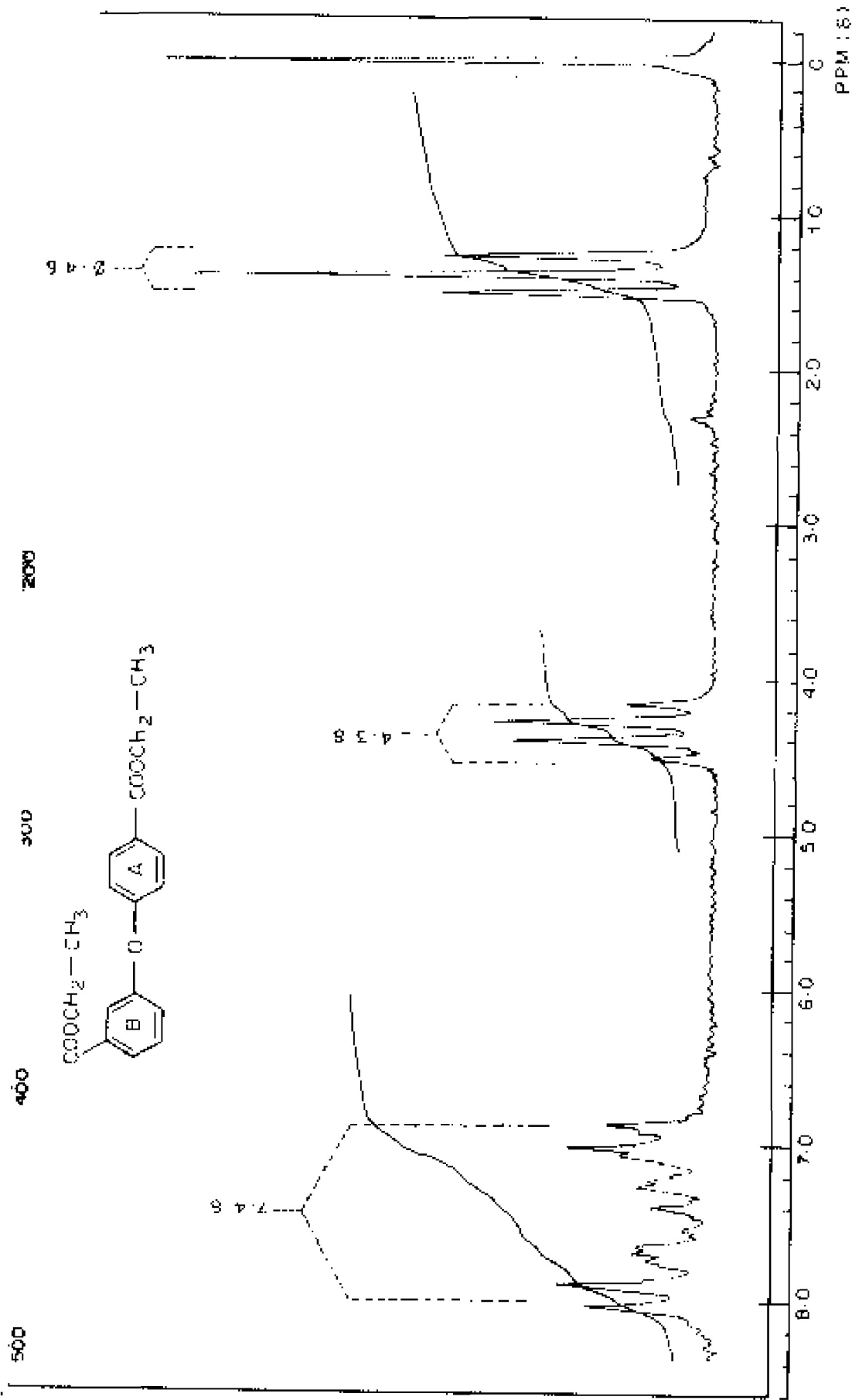


FIG. NO. 6. PMR SPECTRUM OF DIETHYLESTER OF *m,p'*-OXYDIBENZOIC ACID

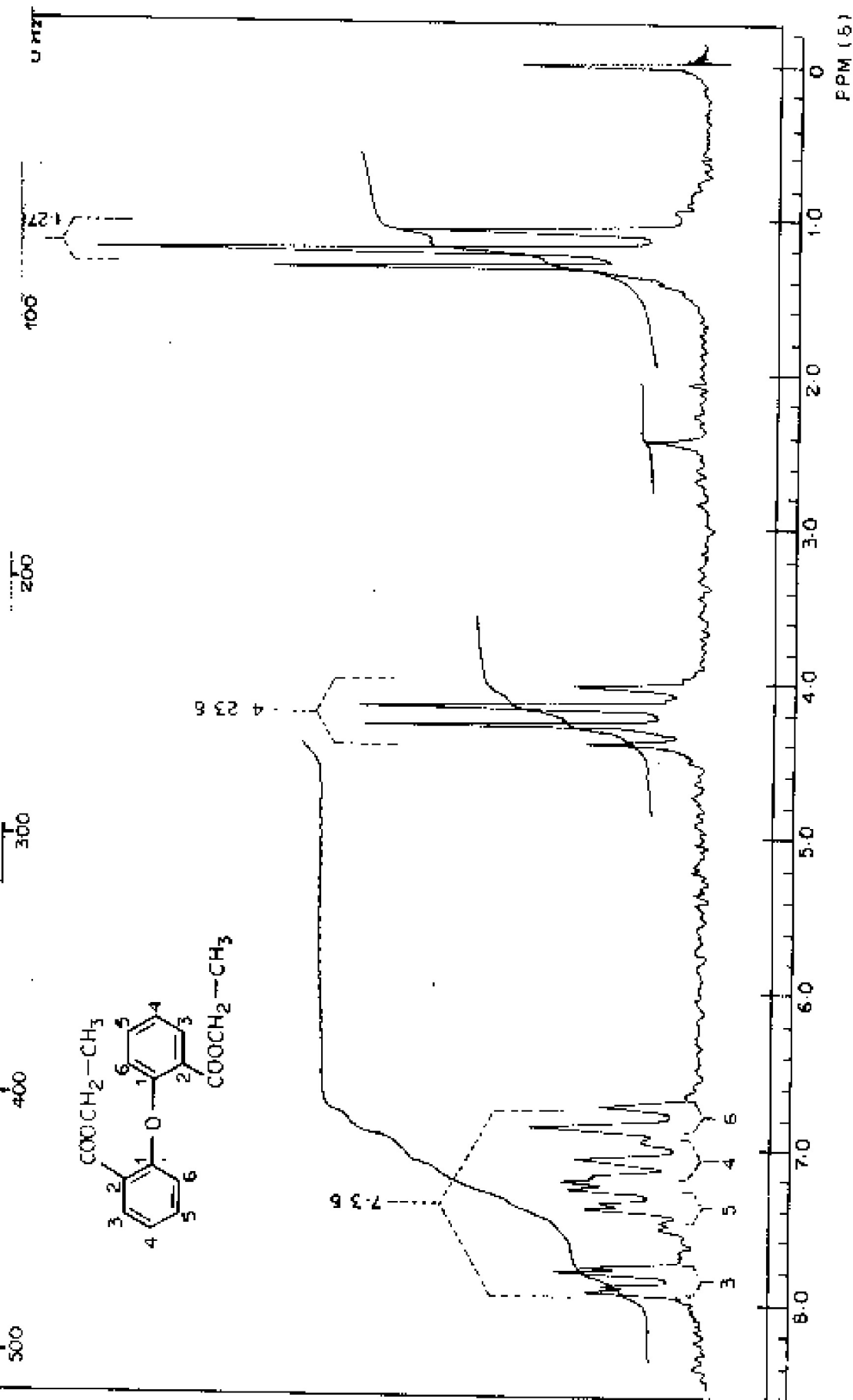


FIG. NO. 7. PMR SPECTRUM OF DIETHYLESTER OF *o,o'*-OXYDIBENZOIC ACID

of diethyl ester of 1,3-propane diol was maintained at 1:2. The purity of 1,3-propane diol was checked before use by GLC. Perotolyl-sulphonic acid (0.5 gm) was employed as catalyst. In a 50 ml RBF the mixture was refluxed in an oil bath at a temperature of 130-140°C continuously for 4 hrs. The reaction mixture was then cooled down and excess 1,3-propane diol distilled out under vacuum (4-5/mm) using air condenser. The reaction mixture was then heated to 140°C for 5 hrs at 4-5/mm pressure. After cooling the product was extracted with chloroform. The chloroform layer was washed with 5% sodium bicarbonate solution followed by water. Chloroform was removed by distillation of the solution and the polyester was dried under vacuum.

General properties of the polyesters

All the polyesters, except diphenic acid polyester, are very thick, viscous liquids. Diphenic acid polyester is comparatively a mobile liquid. The IR spectrum of diphenic polyester is shown in Fig. 4. The approximate molecular weights were determined by end group titration method.

No.	Name of the polyester	Molecular weight
1.	p,p'-Oxydibenzoic acid-1,3-propanediol	5,000 to 6,800
2.	m,p'-Oxydibenzoic acid-1,3-propanediol	15,000 to 16,100
3.	m,p'-Oxydibenzoic acid-1,3-propanediol	8,000 to 8,400
4.	o,m'-Oxydibenzoic acid-1,3-propanediol	10,000 to 10,300
5.	o,o'-Oxydibenzoic acid-1,3-propanediol	4,000 to 4,500
6.	m,m'-Oxydibenzoic acid-1,3-propanediol	5,000 to 5,100

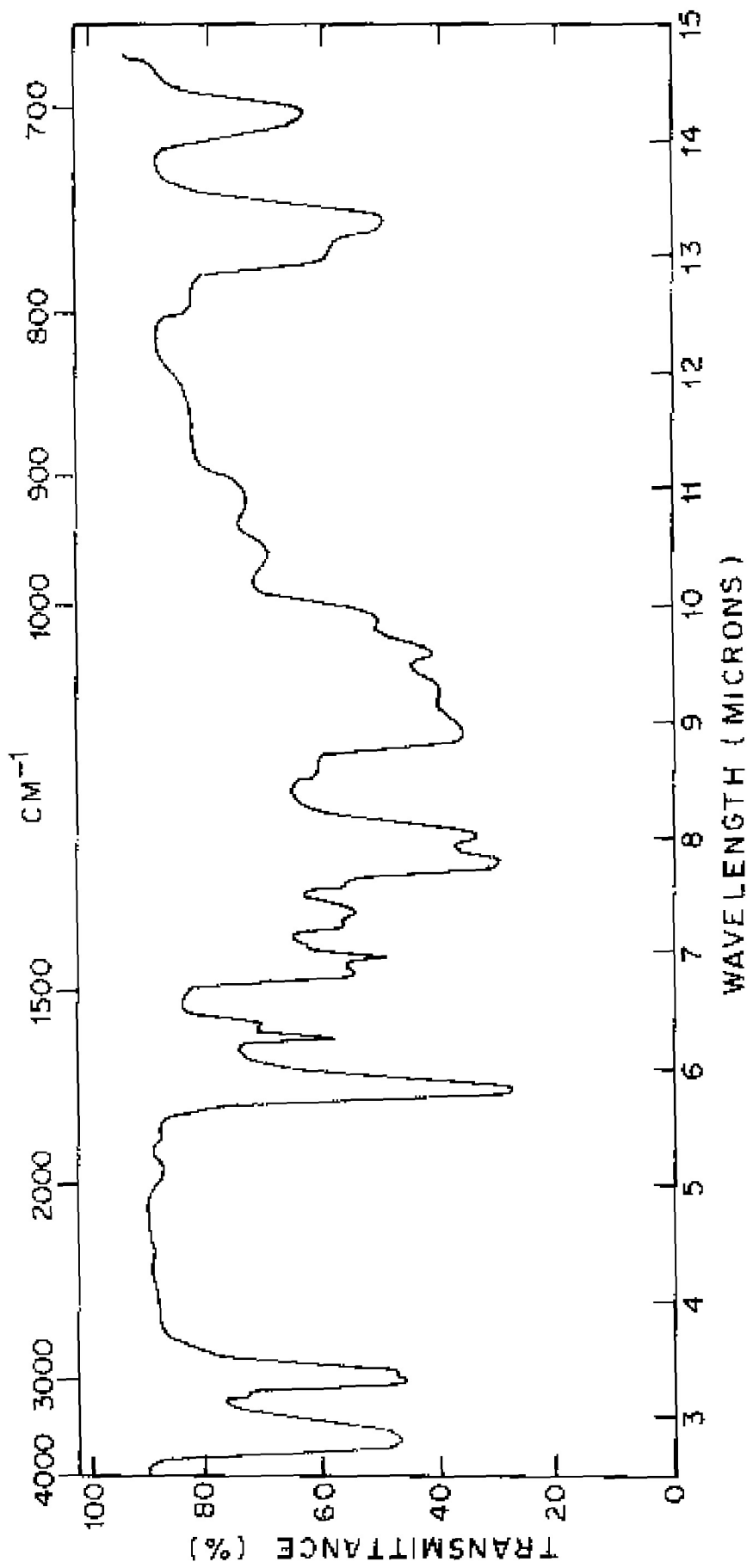


FIG. NO. 4. IR SPECTRUM OF POLYESTER OF DIPHENIC ACID

Collection of Retention Time data

In order to collect retention time data Mark II Gas chromatograph Serial No.70080 supplied by M/s. Associated Instruments manufacturers (India) was used.


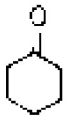


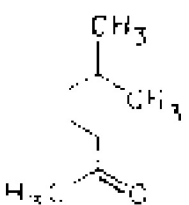
All column variables were normalised according to method given by Littlewood⁵.

Specifications of the column

1. Column material : Aluminium
2. Column length : 6'
3. Weight of the column liquid: 0.6 gm \pm 0.02 gm
4. Carrier gas flow rate : 2.8 L/hour
5. Temperature of the column: 65°C, 100°C, 140°C, 170°C, 180°C
6. Temperature of the injection chamber: 100-200°C
7. Temperature of the detector: 240-270°C
8. Inlet pressure : 8 lbs.
9. Bridge Current: 140 m.a.
10. Attenuation : 4
11. Sample size : 0.5 μ l
12. Carrier gas: Hydrogen
13. Solid support - Celite

The following charts depict the structure and the physical properties of the compounds which were used as probe materials. Purity of these samples was checked by melting points or boiling points and GLC.

CHART NO. 1

S. NO.	NAME OF COMPOUND	STRUCTURAL FORMULA	B.P. °C
COLUMN TEMP. 65 °C			
1	ACETONE	$\text{H}_3\text{C}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_3$	56
2	METHYL ETHYL KETONE	$\text{H}_3\text{C}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_2-\text{CH}_3$	80
3	METHYL PROPYL KETONE	$\text{H}_3\text{C}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_2-\text{CH}_2-\text{CH}_3$	102
4	METHYL ISOBUTYL KETONE	$\text{H}_3\text{C}-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_2-\overset{\text{H}}{\underset{\text{CH}_3}{\text{C}}}-\text{CH}_3$	116-65
COLUMN TEMP. 140 °C			
5	CYCLOPENTANONE		130
6	CYCLOHEXANONE		155
7	CYCLOHEPTANONE		179-80
8	CYCLOOCTANONE		195-97
9	2-METHYL HEPTANONE		173



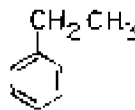
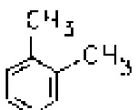
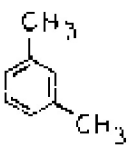

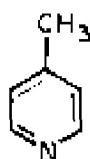
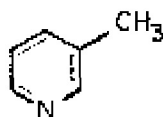
NAME OF COMPOUND	STRUCTURAL FORMULA	B.P. °C
METHANOL	CH_3OH	65
ETHANOL	$\text{CH}_3\text{CH}_2\text{OH}$	78
n-PROPANOL	$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$	97
ISO-PROPANOL	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{C}-\text{C}-\text{OH} \\ \\ \text{CH}_3 \end{array}$	83
n-BUTANOL	$\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{OH}$	116
ISO-BUTANOL	$\begin{array}{c} \text{H} \\ \\ \text{H}_3\text{C}-\text{C}-\text{CH}_2-\text{OH} \\ \\ \text{CH}_3 \end{array}$	108
SEC.-BUTANOL	$\begin{array}{c} \text{H} \\ \\ \text{CH}_3-\text{CH}_2-\text{C}-\text{OH} \\ \\ \text{CH}_3 \end{array}$	99
TERTIARY BUTANOL	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H}_3\text{C}-\text{C}-\text{OH} \\ \\ \text{CH}_3 \end{array}$	83
AMYL ALCOHOL	$\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{OH}$	131
BENZENE		80
TOLUENE		110
ETHYLBENZENE		136
o-XYLENE		144
m-XYLENE		139
p-XYLENE		138

CHART NO. III.
POSITIONAL ISOMERS STUDIED

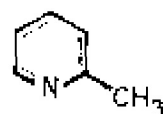
55



γ -PICOLINE
B.P. 143.1°C



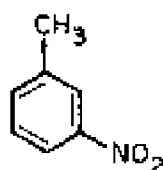
β -PICOLINE
B.P. 143.8°C



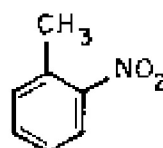
α -PICOLINE
B.P. 129°C



p-NITROTOLUENE
M.P. 54.5°C
B.P. 237.7°C



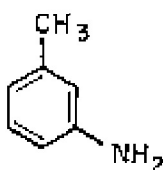
m-NITROTOLUENE
B.P. 227.5°C



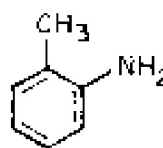
o-NITROTOLUENE
B.P. 222.3°C



p-TOLUIDINE
M.P. 42°C
B.P. 200.3°C



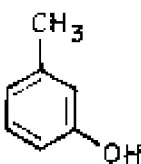
m-TOLUIDINE
B.P. 203°C



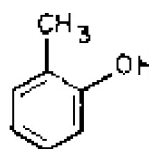
o-TOLUIDINE
B.P. 200.6°C



p-CRESOL
M.P. 34°C
B.P. 202.5°C



m-CRESOL
B.P. 202°C

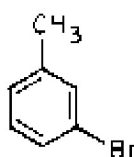


o-CRESOL
B.P. 191°C

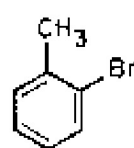
CHART NO. IV.
POSITIONAL ISOMERS STUDIED



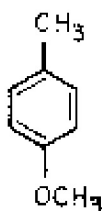
p-BROMOTOLUENE
M.P. 28° C
B.P. 185° C



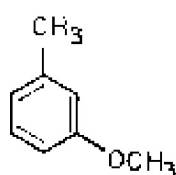
m-BROMOTOLUENE
B.P. 183° C



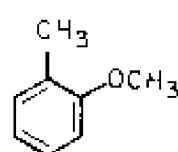
o-BROMOTOLUENE
B.P. 181° C



p-METHYLANISOLE
B.P. 178.5° C



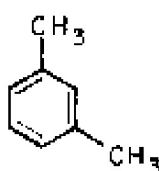
m-METHYLANISOLE
B.P. 177.2° C



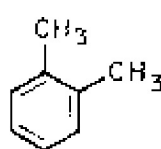
o-METHYLANISOLE
B.P. 171.3° C



p-XYLENE
B.P. 137° C



m-XYLENE
B.P. 139° C



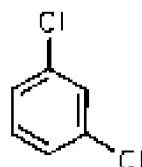
o-XYLENE
B.P. 142° C

CHART NO. V.
POSITIONAL ISOMERS STUDIED



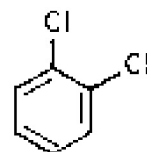
p-DICHLOROBENZENE

M.P. 53°C
B.P. 174°C



m-DICHLOROBENZENE

B.P. 172°C



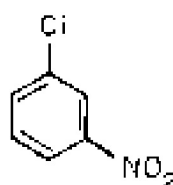
o-DICHLOROBENZENE

B.P. 180°C



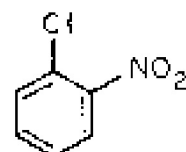
p-CHLORONITROBENZENE

M.P. 83°C
B.P. 242°C



m-CHLORONITROBENZENE

M.P. 46°C
B.P. 235°C



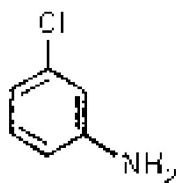
o-CHLORONITROBENZENE

M.P. 33°C
B.P. 245°C



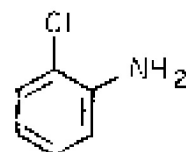
p-CHLOROANILINE

M.P. 70°C
B.P. 232°C



m-CHLOROANILINE

B.P. 230°C



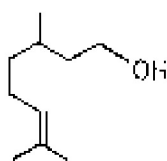
o-CHLOROANILINE

B.P. 208°C

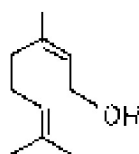
CHART NO. VI.
TERPENOIDS

58

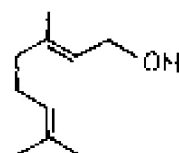
COLUMN TEMP. 180°C



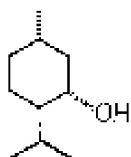
CITRONELLOL
 B.P. 225° / 764.5 mm



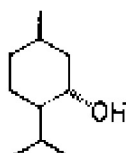
NEROL
 B.P. 224° / 755 mm



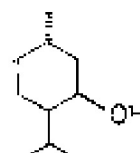
GERANIOL
 B.P. 230°



L-MENTHOL
 B.P. 216.5°C



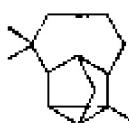
ISOMENTHOL
 B.P. 218.5°; M.P. 52°C



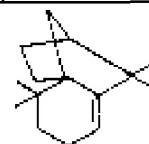
NEOMENTHOL
 B.P. 211.5°, M.P. 49.5°C



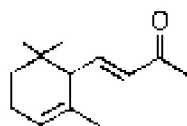
LONGIFOLENE
 B.P. 254° / 706 mm



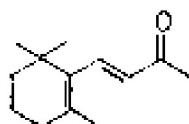
LONGICYCLENE
 B.P. 252° / 706 mm



SOLONGIFOLENE
 B.P. 107.9° / 7 mm



α-IONONE
 B.P. 127° / 12 mm



β-IONONE
 B.P. 134.6° / 12 mm



ψ-IONONE
 B.P. 135°-150° / 5 mm



α-PINENE
 S.P. 156°C



β-PINENE
 B.P. 162-64°C



α-TERPENEOL
 B.P. 219-21°C

REFERENCES

1. O.V. Schickh, *Riziska*, 52, 842 (1956).
2. A.I. Vogel, 'Practical Organic Chemistry',
Third Edition, p. 762.
3. C.A. Yale, 42, 11591⁶
4. T.K. Sankpal, M.Sc. Thesis
5. A.B. Littlewood, 'Gas Chromatography' Academic Press,
London, p.30 (1958).

CHAPTER – 3

DISCUSSION AND INTERPRETATION OF RESULTS

SUMMARY

In GLC polyester liquid phases are important, from the view of separation of different compounds because of their medium polar nature. The substituted aromatic positional isomers are very difficult to separate from each other under normal conditions. In terpenoids also, there are so many closely spaced compounds which need a good liquid stationary phase to separate them from each other. In the present work a new series of diaromatic polyesters has been prepared.

The polyesters prepared are:

1. p,p'-Oxydibenzic acid - 1,3-propane diol
2. o,p'-Oxydibenzic acid - 1,3-propane diol
3. m,p'-Oxydibenzic acid - 1,3-propane diol
4. o,m'-Oxydibenzic acid - 1,3-propane diol
5. o,o'-Oxydibenzic acid - 1,3-propane diol
6. m,m'-Oxydibenzic acid - 1,3-propane diol
7. Diphenic acid - 1,3-propane diol

All these polyesters have been prepared for the first time and evaluated for their solvent capabilities as substrates.

The results are very interesting. Some of the phases are showing arithmetical relationship for homologous series of compounds including carbonyls and alcohols as well.

Retention time data

A wide variety of compounds are used to study the behaviour of these synthetic diaromatic liquid substrates. They are divided into the following groups depending upon their nature. Since these compounds have different molities with different boiling or melting points, the retention times are recorded at different column temperatures. They are grouped as follows:

Group I comprises aliphatic ketones having low boiling points and their retention times are recorded at 65°C column temperature.

Group II contains the aliphatic alcohols, whose retention times are recorded at 100°C oven temperature.

Group III includes the aromatic hydrocarbons; retention time data of these compounds is recorded at 100°C column temperature.

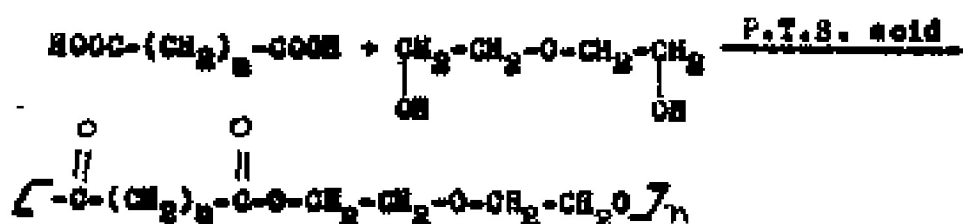
Cyclic ketones are chromatographed at 140°C temperature and are comprised under Group IV.

Aromatic substituted positional isomers are considered as Group V and are injected at 150°C column temperature. However to get better separation some of these were recorded at 170°C. Terpenoids which are chromatographed at 180°C column temperature are grouped in Group VI.

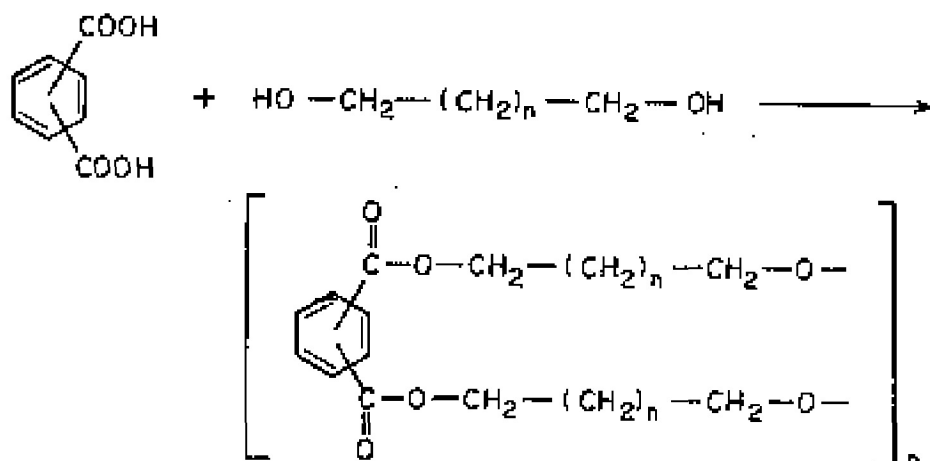
α and β -Pinene samples are included in Group III as they are recorded at 100°C column temperature. The groupwise results are presented in table Nos. II, III, IV, V, VI and VII respectively.

Nature of the polyester substrates

Most of the common aliphatic polyesters, used as substrates in GLC, are prepared from aliphatic dicarboxylic acids and diethylene glycol with or without crosslinking agents¹.

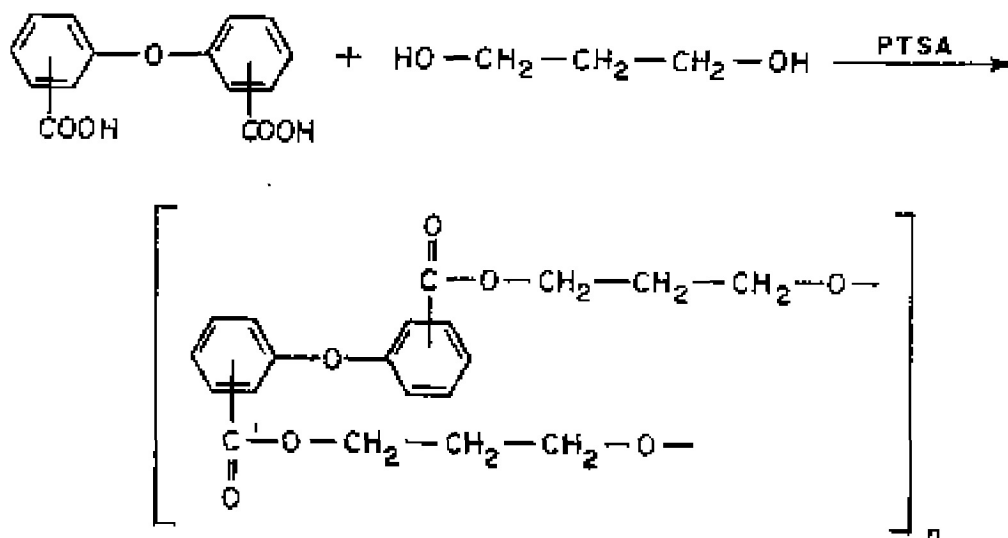


The ether linkage in diethylene glycol has some influence over polarity of the liquid phase increasing its versatility as GLC substrate. Earlier investigations in this laboratory, on polyester phases having aromatic ring, are found quite interesting to resolve substituted aromatic isomers. The phases were prepared from isomeric phthalic acids and aliphatic glycols².

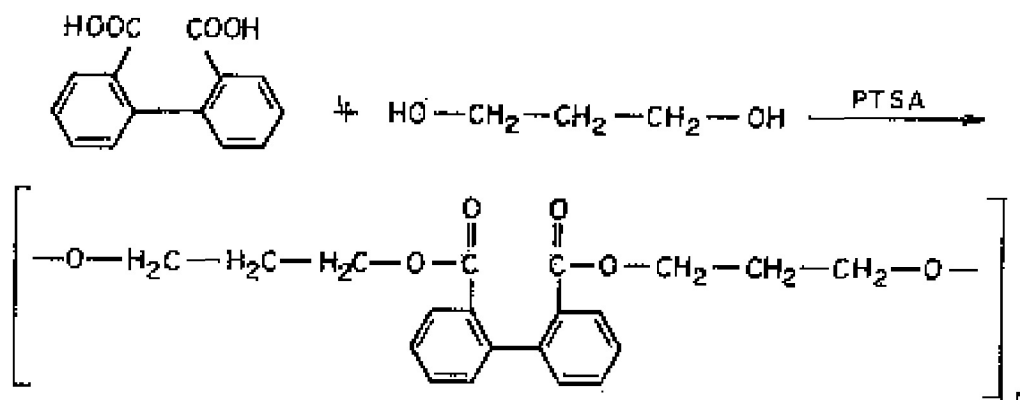


In this work it was observed that the aromatic phase mixed with small percentage of epoxy resin helped in getting better separation of some of the isomeric mixtures.

In continuation of the same studies it was decided to increase one aromatic ring along with the etheral oxygen directly on the phase which may help to give better solubilities for different isomers. Therefore a new series of polyesters was prepared from oxydibenzoic acids and 1,2-propanediol.

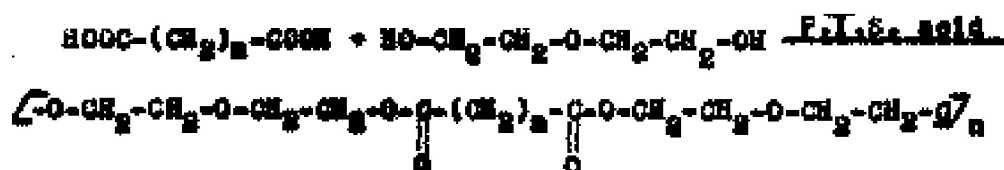


Simultaneously an additional phase was also prepared from diphenic acid and propane diol which was without etherial oxygen function.



The behaviour of this new series of diaromatic ether polyesters towards different classes of compounds is very interesting. Some of the homologous series of compounds showed arithmetical relationship on these polyester substrates.

The similar arithmetical relationship was observed earlier in this laboratory¹. In those series of polyesters, homologous aliphatic dicarboxylic acids and diethylene glycol and similar derivatives were used.



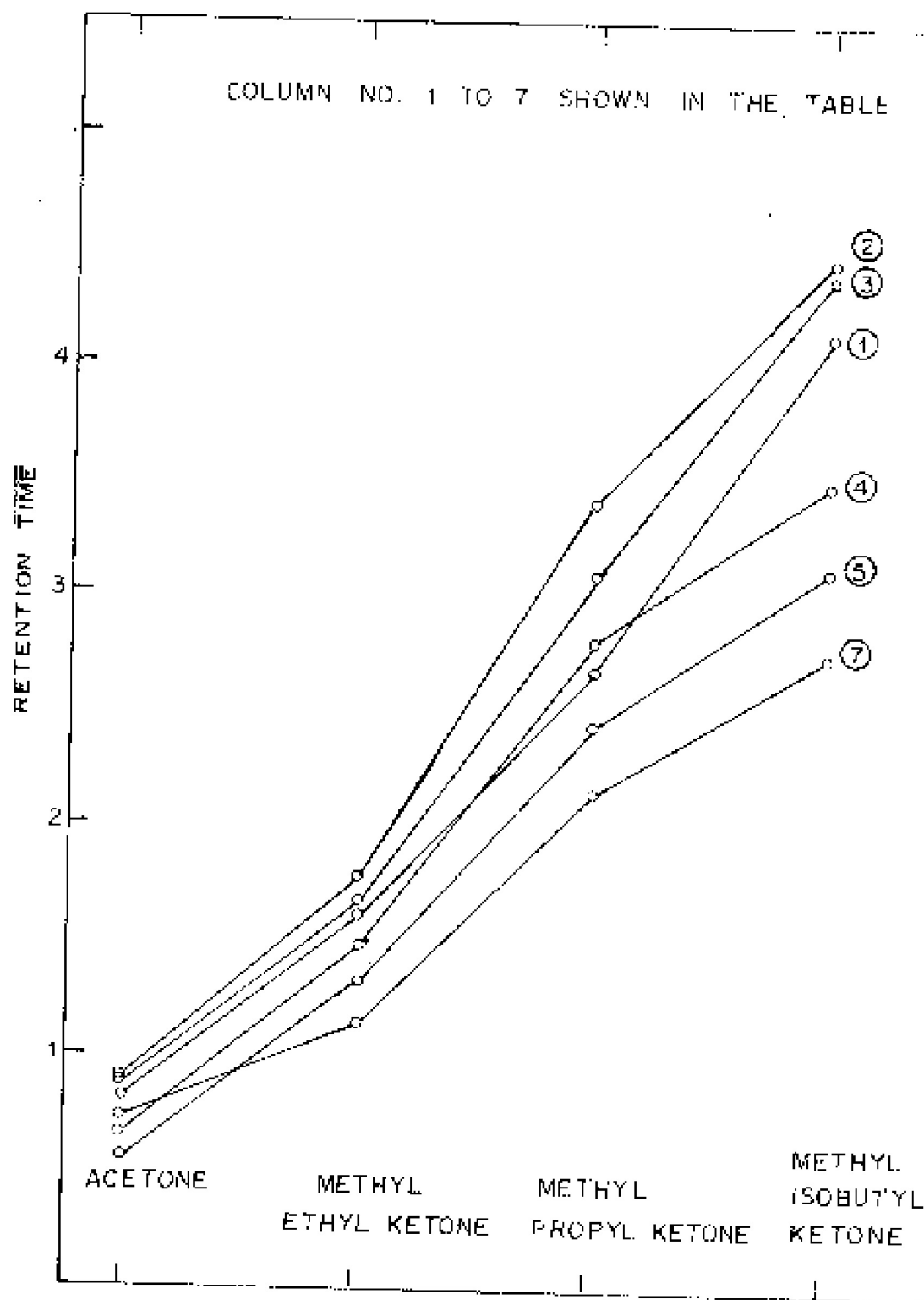
In above series the change in methylene group in acid fragment or glycol fragment could stretch individual

hydrocarbons with arithmetical rise in retention times on various polyesters. The present series shows arithmetical rise in retention times of homologous series of not only in hydrocarbons but also in carbonyls and alcohols with the rise in methylene group in the solutes (probe compound). Along with this property etherial oxygen in the phase is also influencing the elution pattern of various compounds.

GROUP 1 Aliphatic Ketones

In the structures of aliphatic ketones ranging from acetone to methyl propyl ketone there is stepwise rise of one methylene group. Retention time data for these ketones on all the columns when carefully observed it could be seen that there is definite arithmetical rise in retention times with the rise of methylene groups.

Column Numbers 3 and 4 are showing clear multiple rise in retention time from acetone to methyl propyl ketone giving arithmetical pattern (graph No.1). Remaining columns also show more or less the same pattern. This behaviour of all these aliphatic ketones on polyester column is observed for the first time. In earlier communications from MCL^{1,2} though they have revealed arithmetical relationship between hydrocarbons they could not get similar stretching for other types of compounds. When retention times of individuals are considered on this present series of columns it was observed



GRAPH NO. 1.
ALIPHATIC KETONES

that column No.6 and column No.7 are showing lower retention times. This can be attributed to the fact that the molecule in both the cases must be in twisted condition because of the presence of both the bulky -COOH groupings at ortho positions. When the remaining columns are considered the solubility of these ketones is quite high which may be due to the possible planarity of the molecule of the polyester. In somewhat planar structure of molecule the effect of etherial oxygen and aromaticity of both the rings may be having better influence on polarity of the liquid phase. Column No.6, where both -COOH groups are at meta position, shows maximum retention time i.e. maximum solubility for all the ketones.

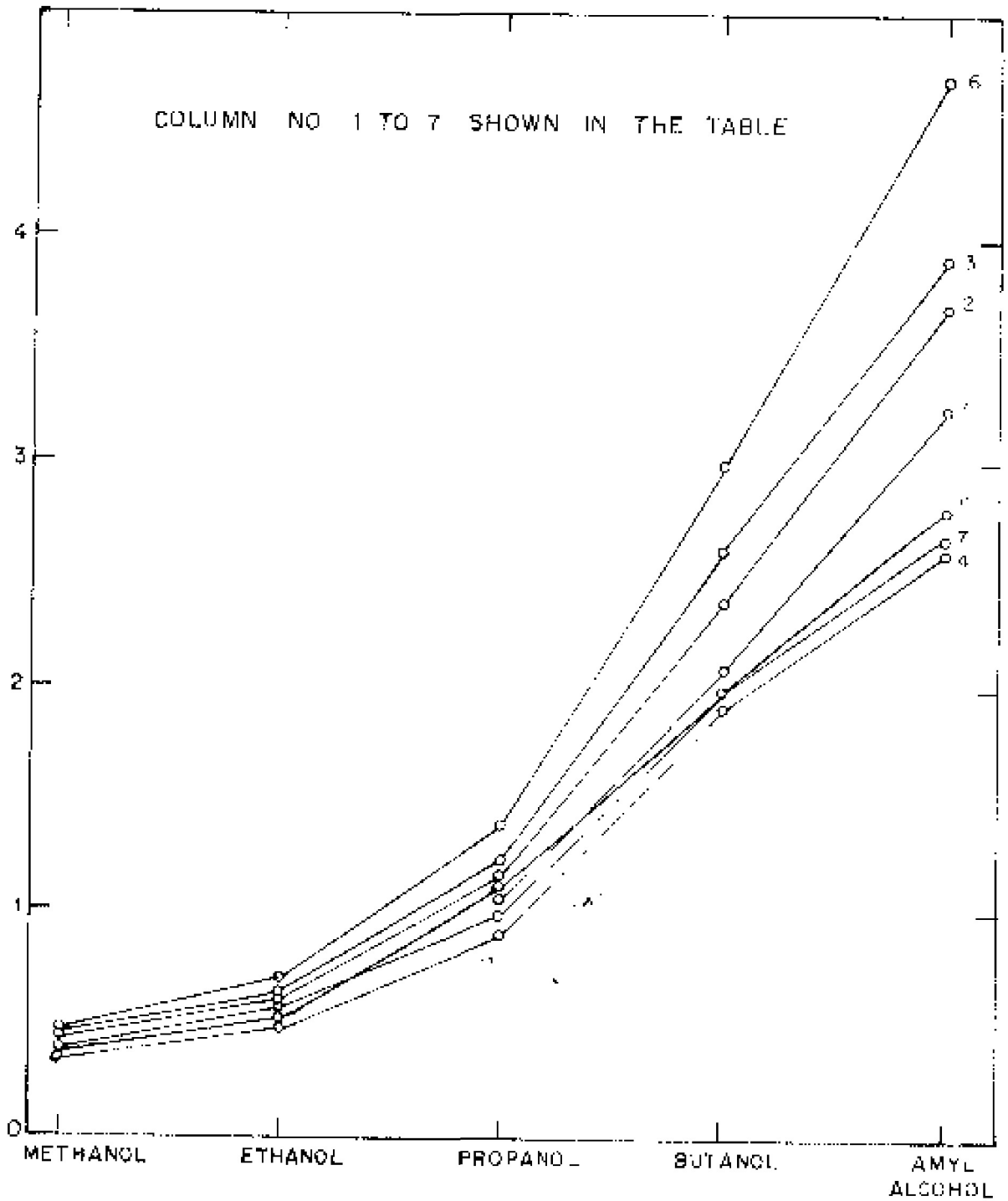
GROUP II Aliphatic Alcohols

Aliphatic alcohols are nicely stretched apart when chromatographed on all the columns. Retention time data is shown in Table No.III which reveals this fact.

There is stepwise 100% rise in retention time with the addition of one methylene group from methanol to n-butanol showing pure arithmetical relationship as in case of ketones shown earlier. From butanol to amylalcohol rise in retention time is 80% (Graph No.II) when individual isomeric alcohols are studied it is found that the secondary alcohol of the higher homolog shows almost same retention time as that of lower primary alcohol.

e.g. Retention time of ethanol and isopropanol

Retention time of propanol and secondary butanol



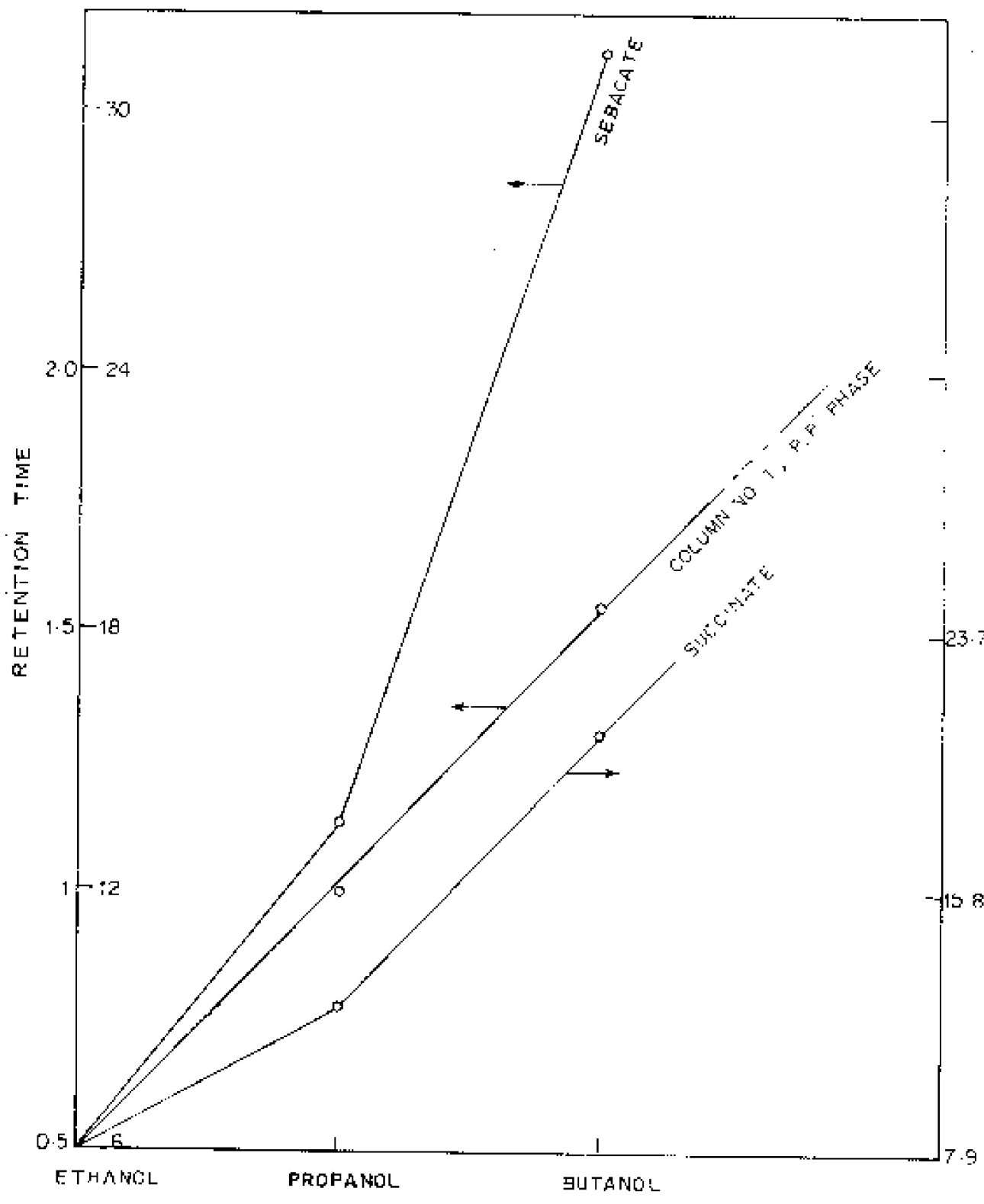
GRAPH NO. II.
ALIPHATIC ALCOHOLS

Stretching of individual alcohols from each other is found exactly in multiplication with rise of methylene groups, while in earlier work of straight chain polyesters⁴ there is abrupt jump in retention times (Graph No.III). Similar to the observation of aliphatic ketones the solubilities of these alcohols are maximum in *m,m'*-oxy-dibenzic phase (column No.6).

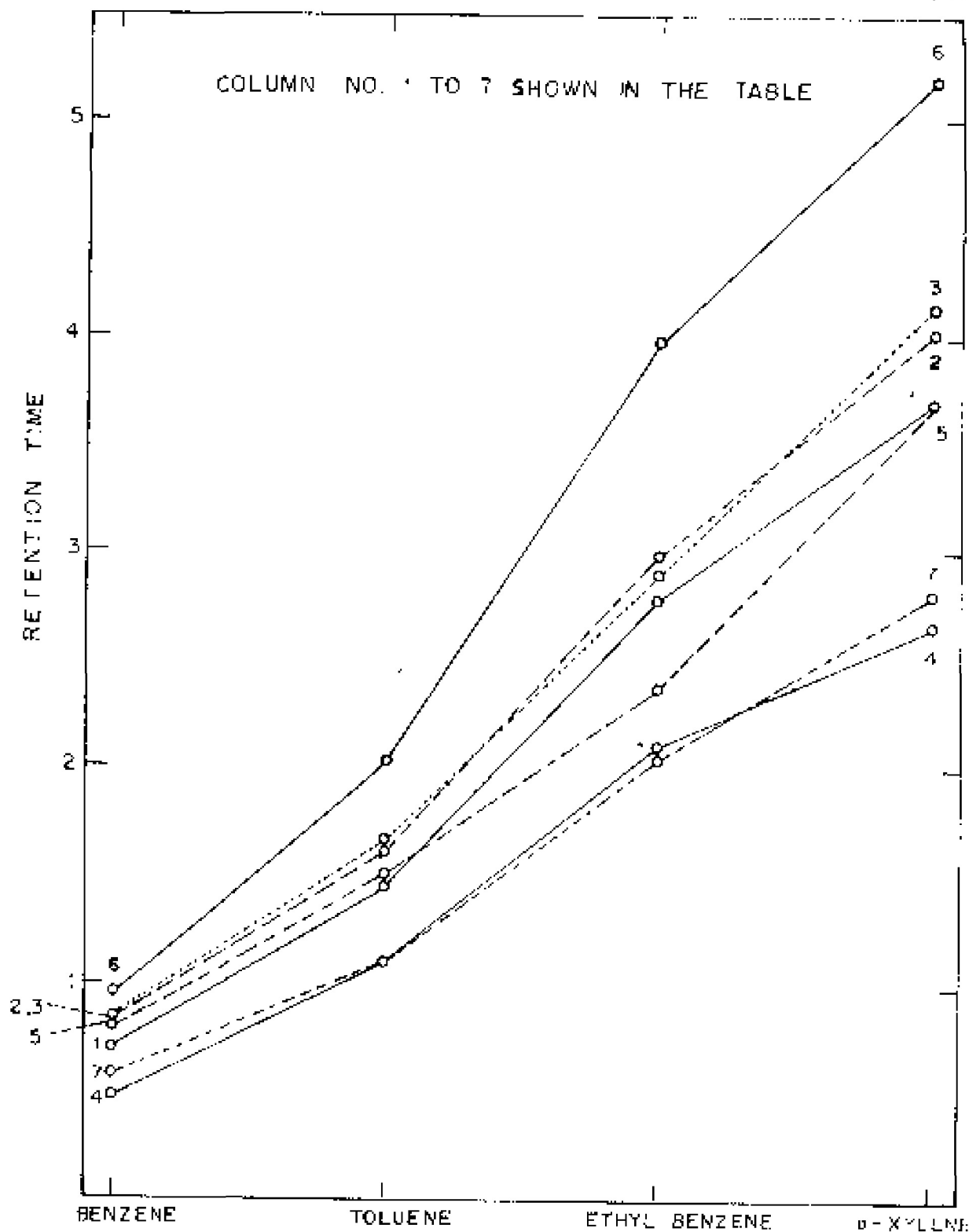
GROUP III aromatic hydrocarbons

When retention times of benzene, toluene and ethylbenzene or *p*-xylene are considered for all the columns, there is a perfect i.e. 100% stretching with the increase of methyl group on benzene ring; graphical representation of the retention times of all these hydrocarbons shows nearly a straight line for all the columns (Graph No.IV).

Column No.6 i.e. *m,m'*-oxydibenzic acid polyester again shows maximum solubilities of all the aromatic hydrocarbons. Column No.7 shows minimum solubilities for all the aromatic hydrocarbons. Column No.7 shows minimum solubilities for all the aromatic hydrocarbons except benzene. The separation of ethyl benzene from *p*-xylene is observed on column No.8 and 9 while on other columns they are eluting out at almost same retention times. All the three xylene isomers could not be separated. When retention time data is compared with the earlier results published from NCI^{1,2,3},



GRAPH NO. III.
ALIPHATIC ALCOHOLS



GRAPH NO. IV.

AROMATIC HYDROCARBONS

present results are showing better stretching of the aromatic hydrocarbons. The retention time data for the above three groups indicates the possibility of separation of the mixture comprising of the following compounds. Actual separation was tried.

(1) Acetone (2) Methanol (3) Ethanol (4) Benzene (5) n-Propanol (6) Toluene (7) n-Butanol (8) Ethyl benzene and (9) o-Xylene.

All these compounds can be nicely resolved. The chromatogram on m,m'-oxydibenzic polyester i.e. column No.6 is shown in chromatogram No.1

GROUP IV - Cyclic Ketones

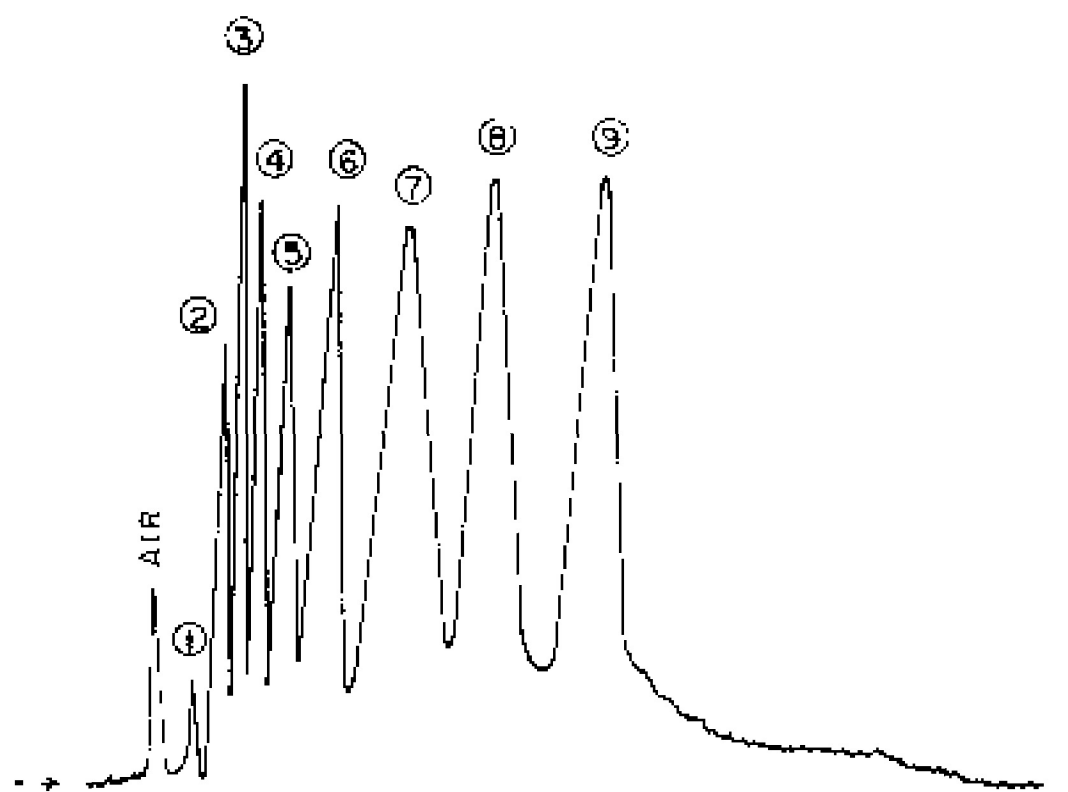
Behaviour of the cyclic ketones on all these columns is somewhat peculiar. Going from cyclopentanone to cyclohexanone the retention time rise is about 50% on all the columns, while cycloheptanone retention times are almost double to that of cyclohexanone. Cyclooctanone retention times show 90% rise from cycloheptanone retention times.

In general with the increase of one member in the ring structure there is nearabout 100% rise in retention time. When retention times are plotted the graph shows nearly straight lines (Graph No.V). All these columns form two groups showing similarity in behaviour. Column Nos 4,6 and 7 show lower retention times as compared to column No.1,2,3 and 5.

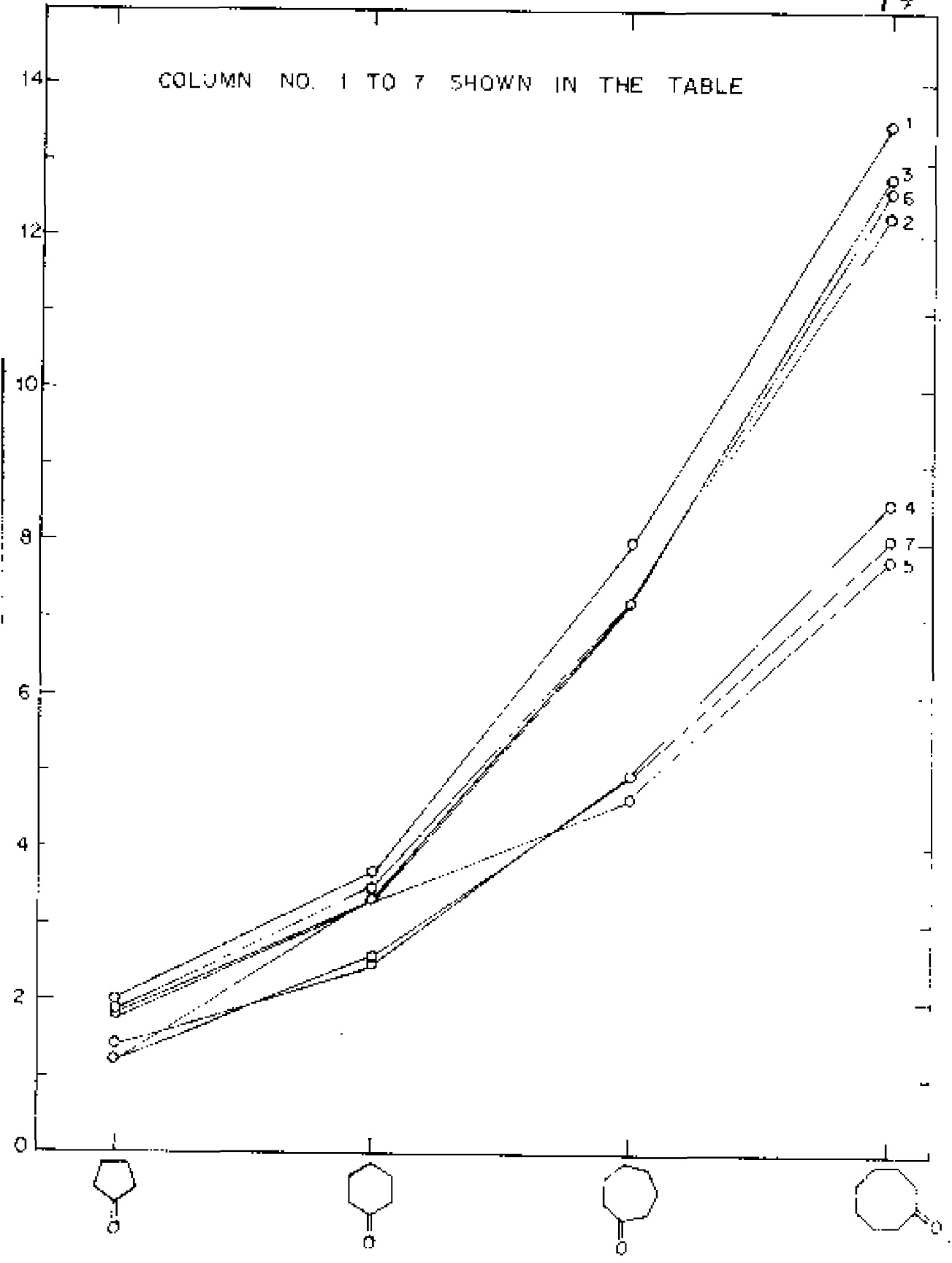
- 1 ACETONE
- 2 METHANOL
- 3 ETHANOL
- 4 BENZENE
- 5 n-PROPANOL
- 6 TOLUENE
- 7 n-BUTANOL
- 8 ETHYL BENZENE
- 9 o-XYLENE

TEMP. 100° C

CHART SPEED - 600 mm / hr



COLUMN NO. 1 TO 7 SHOWN IN THE TABLE



GRAPH NO. V
CYCLIC KETONES

6-Methyl heptanone which was chromatographed simultaneously along with these cyclic ketones has shown considerably low retention times.

When the retention times of cyclopentanone and cyclohexanone on the column No. 2 were compared with the retention times on normal polyester column prepared from diethylene glycol + adipic acid¹, it can be observed that the present series have better separation factor i.e. 1.93 as against 1.88.

GROUP V - Aromatic Positional Isomers

Earlier in this laboratory isomeric phthalic acid, polyesters and substituted aromatic polyesters liquid phases were prepared and were used for separation of some of the positional aromatic isomers².

In continuation of the same work the present series of polyesters was prepared and studied as substrates for separation of all these positional isomers.

General observation of the retention time data of all these aromatic positional isomers reveals that these diaromatic polyester phases are showing more solubility for all the components when compared with the retention time data on earlier aromatic columns². This fact can be explained on the basis of the nature of present phases. They are having two aromatic rings and one etherial oxygen function in them.

Even the liquid phase with only two aromatic rings shows higher retention times. This clearly indicates that these new polyester phases have definite advantage over the earlier liquid polyester phases in separation of these intricate positional isomers.

The behaviour of the individual polyesters towards actual separation of different isomers is discussed below.

Picolines - Except column No.1 β and α are separated on every column while β and γ are having separation factor 1.1.

On column No.5 β and γ -picolines are having the separation factor of 1.2 (Chromatogram No.2).

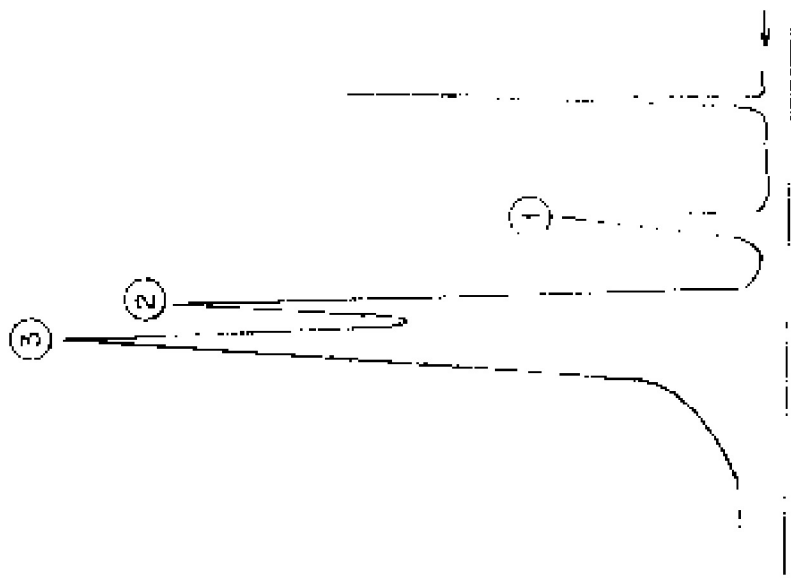
Nitrotoluenes - It was pointed out by Fense et al.² that introduction of oxygen function in the substrate reduces the separation possibility of positional isomers of picolines as well as of nitrotoluenes. Our observation supports this fact as only column No.7 shows effective separation of ortho and para as well as meta and para isomers (Chromatogram No.3). All of remaining columns are not showing the separation of meta and para components.

Toluidines - These isomers when chromatographed on all these columns follow more or less the same pattern. They are not influenced by the different structures of all these liquid phases. The literature survey indicates that under normal conditions and on normal phases these ortho, meta and para isomers are not resolved from each other. In present work

- 2 β -PICOLINE
- 3 γ -PICOLINE

TEMP. 140°C

CHART SPEED ~ 600 mm / hr

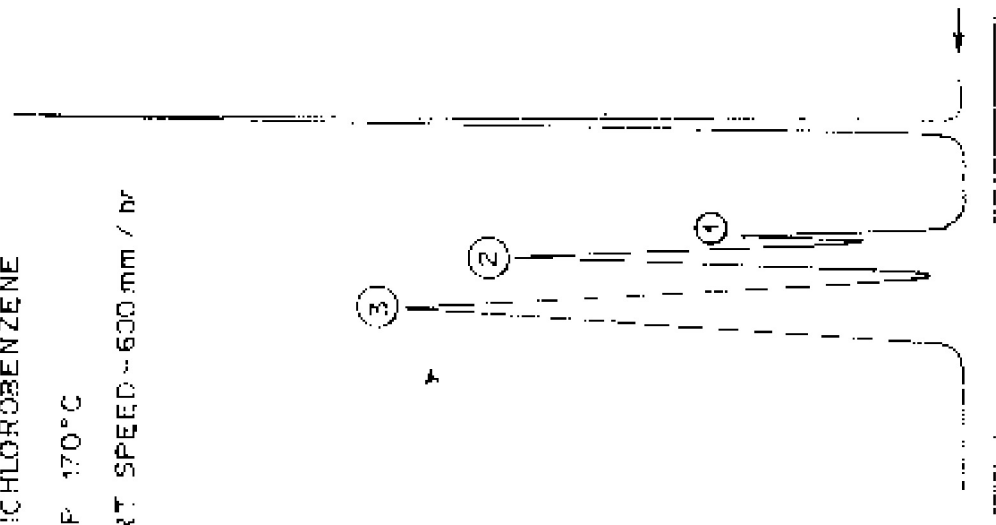


COLUMN NO. - 5.

- 2 p-DICHLOROBENZENE
- 3 o-DICHLOROBENZENE

TEMP. 170°C

CHART SPEED ~ 600 mm / hr

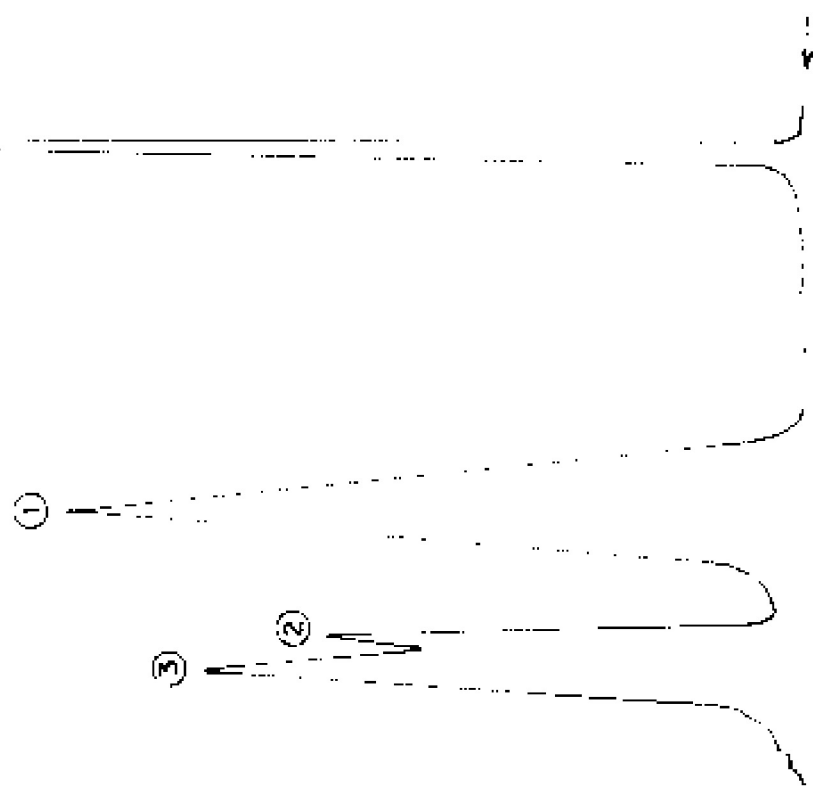


COLUMN NO. 3.

CHROMATOGRAM NO. 5

- 1 o-NITROTOLUENE
- 2 m-NITROTOLUENE
- 3 p-NITROTOLUENE

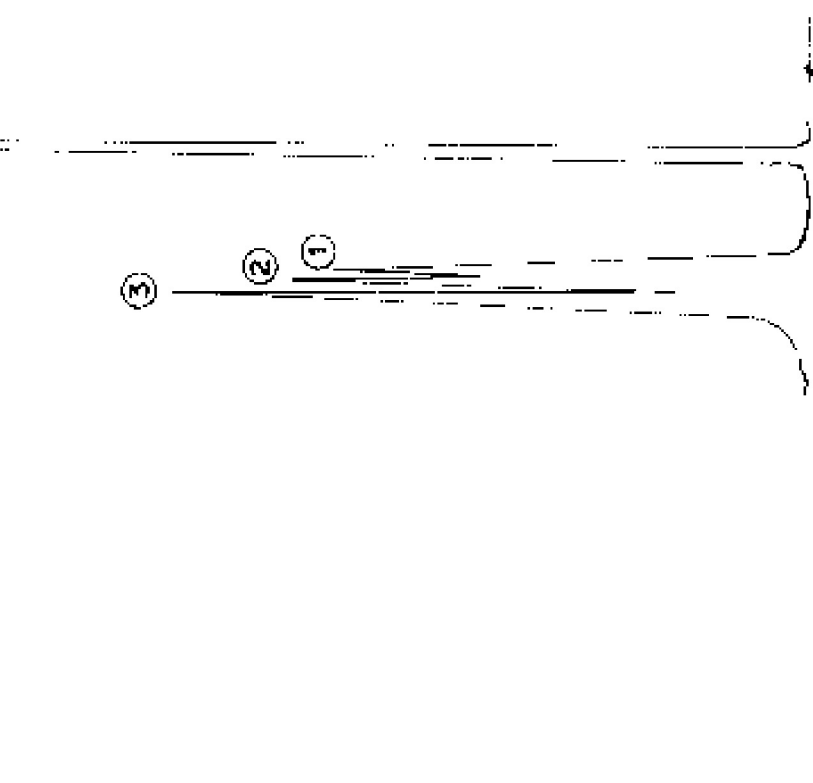
TEMP 180°
CHART SPEED 600 mm / hr



COLUMN NO. 7

- 1 neo-MENTHOL
- 2 L-MENTHOL
- 3 ISO-MENTHOL

TEMP 180°
CHART SPEED 600 mm / hr



COLUMN NO. 7

the separation factor between ortho and para isomers is 1.92 on column No.3 and 6 while remaining columns show this same factor between 1.7 to 1.8. Separation factor between meta and para isomer is in the range of 1.07 to 1.08 for all the columns.

Conclusions - Separation of all these three isomers is unsolved problem yet. Under normal conditions clearcut separation is difficult to be obtained on common liquid substrates. They are partially resolved on 4 aminophthalic acid ester-1,3-propene diol polyester column prepared earlier³.

Present series of polyester could give the separation of ortho and para isomers on each and every column showing the separation factor between 1.29 to 1.39 but the meta and para isomers are mixing with each other proved by the very low i.e. 1.08 separation factor.

Discussion - The present liquid phases are effective for the separation of all the three isomers. Column number 2,3,4 and 7 show clearcut separation of all the isomers (Chromatogram No.4). The meta and para isomers are not separated on symmetrical dicarboxylic acid polyesters i.e. column number 1,5,6.

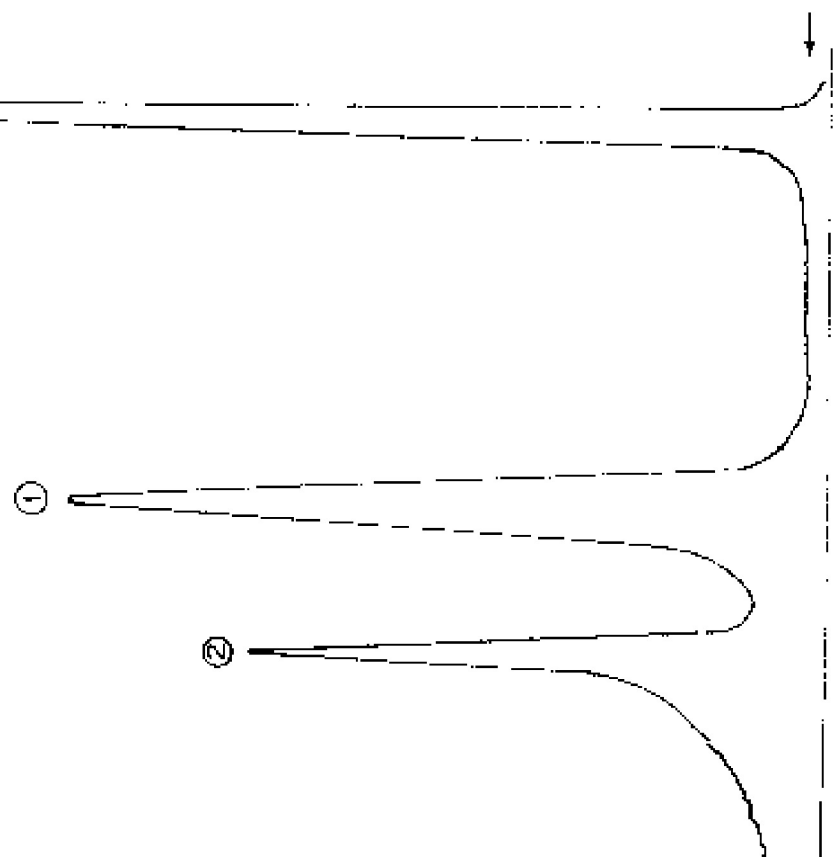
However, column No.7 inspite of having symmetrical structure separates the meta and para isomers from each other along with ortho and para isomers. This can be attributed to the fact that there is no etherial oxygen function in the

polyester phase. Above observation is in agreement with the earlier conclusions.

Chloronitrobenzene - All the three ^{isomers} could not be directly resolved on one single column. Column No.4 resolves meta isomer from para but fails to separate ortho and para from each other whereas column No.5 separates the para and ortho isomer mixture completely and meta and para isomers are eluted out almost at the same time (Chromatogram No.5). This property of these two columns can be jointly used to resolve all the three isomers from each other.

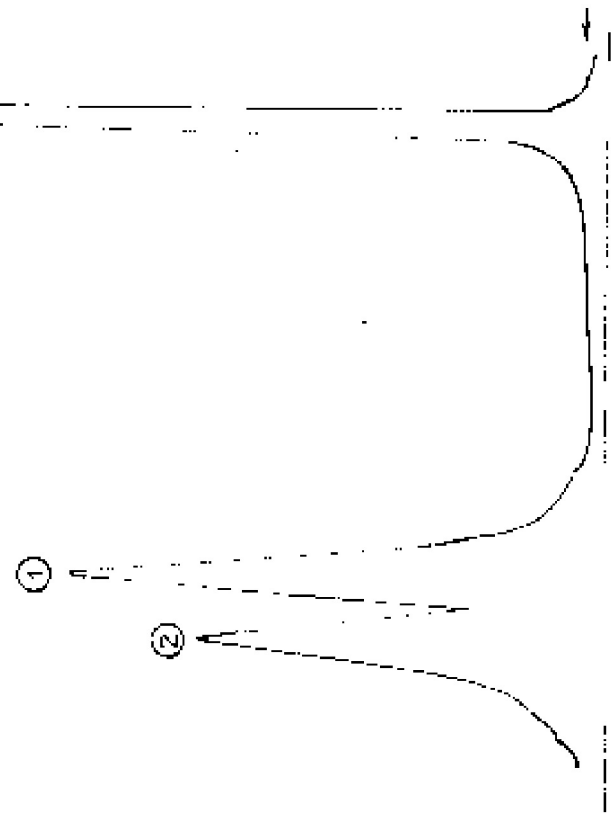
General behaviour of the isomers is also very interesting. The order of emergence is first meta, second para and third is ortho on column No.2,2,4,5 and 7 while on remaining columns the order is meta first, then ortho and last is para isomer. This change in order of emergence is likely to be due to symmetrical structure of p,p'-oxydibenzic acid as well as m,m'-oxydibenzic acid polyester phase. Column No.5 i.e. o,o'-oxydibenzic polyester phase which is also having symmetrical structure does not show the order change effect as above. It is likely that o,o' structure may not be remaining in plane because of steric hindrance and this may be the cause of variation in the behaviour as compared to symmetrical p,p'- and m,m'-oxydibenzic acid polyester phases. Further investigation of this point will be taken up in the future.

1 m, p-CHLORONITROBENZENE
2 o-CHLORONITROBENZENE
TEMP. 180°C
CHART SPEED - 600 mm / hr



COLUMN NO. - 5

1 m-CHLORONITROBENZENE
2 o, p-CHLORONITROBENZENE
TEMP. 180°C
CHART SPEED - 600 mm / hr



COLUMN NO. - 4

Chloranilings - Elution pattern of the isomers is not same on all the columns. In general it has been observed that in most of these aromatic isomeric mixtures ortho isomer emerges last. In present series ortho isomer elutes out first showing the least retention time. Column No.1 resolves all the three isomers nicely. (Chromatogram No.6).

All the other columns could separate the ortho isomer from para isomer (separation factor 2.1) while meta and para isomer are emerging out at the same time. The order of emergence of these isomers on column No.1,3 and 5 is ortho, meta and para which is according to the boiling point range while on rest of the columns it is in the order of ortho, para and meta. This inversion of meta and para isomers seems to be due to solubility differences in respective liquid phases.

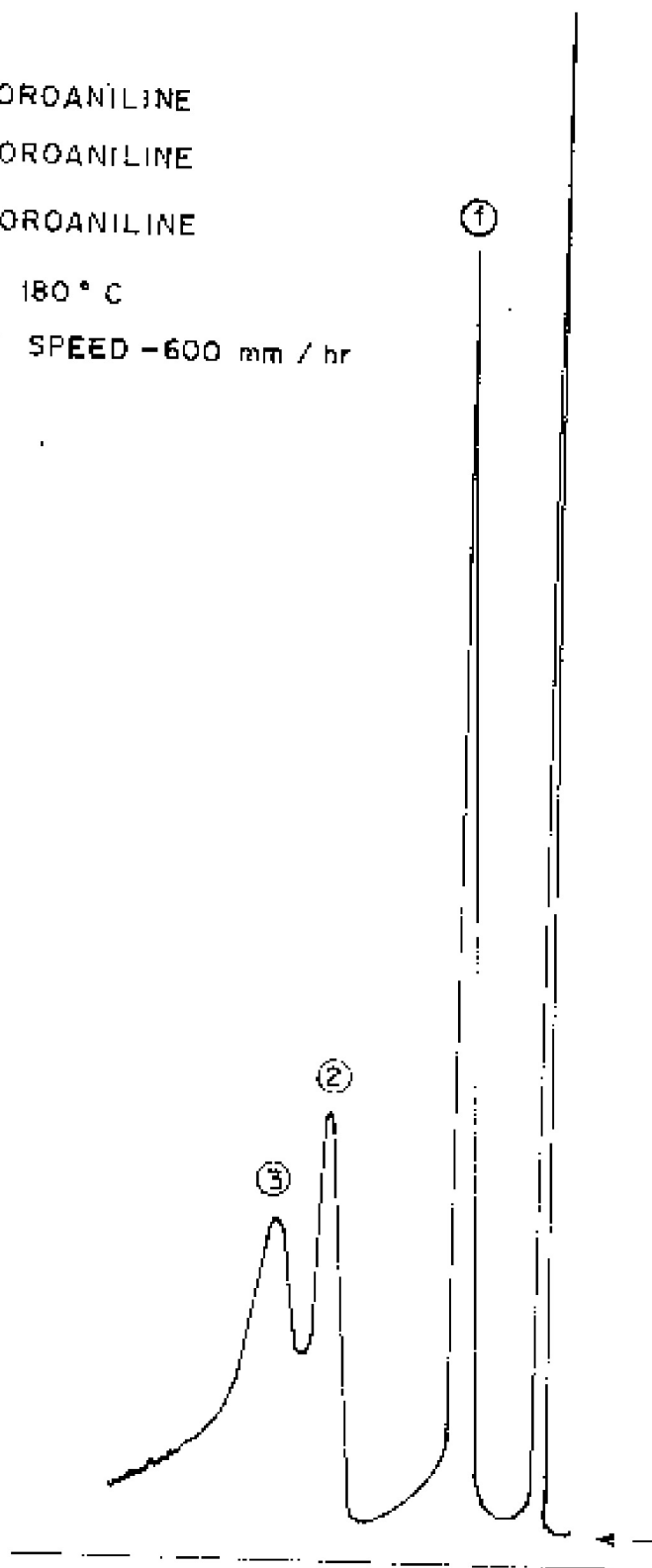
Methyl anisole - Methyl anisole isomers are separated from each other on boiling point basis thereby showing no special affinity by these liquid phases. Meta and para methyl anisoles are not showing any difference in retention time. Ortho isomer is eluted out first among all. The separation factor in between ortho and meta or para is of the order of 1.2. The column No.7 i.e. diphenic acid polyester shows little effective solubility of these isomers. This column shows uniform stretching for all these three isomers.

CHROMATOGRAM NO. 6.

- 1 o-CHLOROANILINE
- 2 m-CHLOROANILINE
- 3 p-CHLOROANILINE

TEMP. 180° C

CHART SPEED - 600 mm / hr



COLUMN NO. 1.

Hydrocarbons - These isomers are not showing any effective separation on any of these polyester phases. Ortho isomer is eluted out first according to boiling point category. Column No.1 and Column No.7 show the separation of ortho and para isomers which is evident from the separation factors 1.44 and 1.35 respectively. The meta and para isomers are showing almost same retention times.

The elution pattern of different positional isomers on this series showed mixed behaviour. On some columns they are eluted on boiling point basis thereby showing no special affinity for liquid substrates. Some of the columns are definitely showing varied polarity and solubility for these isomers. This fact can only be attributed to the structural differences of these polyesters. Further work will be continued to find out possible structural pattern in these polyesters.

GROUP VI Terpenoids

Terpenoids are subdivided in the following groups

- | | |
|-------------------|-------------------------------------------------|
| 1. Hydrocarbons - | I) Longifolene, Longicyclene,
Isolongifolene |
| 2. Alcohols | I) L-menthol, Neomenthol,
Isomenthol |
| | II) Citronellol, Geraniol,
Nerol |

One more alcohol i.e. α -terpineol has been recorded only on some columns.

- | | |
|------------|------------------------------------------------------|
| 3. Ionones | I) α -Ionone, β -Ionone, ψ -Ionone |
| 4. Pinenes | I) α -Pinene, β -Pinene |

1. Experiments

Mixture of longicyclene, isolongifolene and longifolene is completely resolved on Column No.4 (Chromatogram No.7). Column No.3 and Column No.6 show almost same retention times for all these isomers. Column Nos.1, 2 and 5 could separate the isolongifolene from longifolene (separation factor 1.25) effectively but failed to resolve longicyclene and isolongifolene (separation factor 1.1). Column No.7 i.e. diphenic acid polyester column shows better resolution for the all the three isomers compared to column Nos.1,2 and 5. In earlier results⁵ it was observed that polyesters prepared from succinic acid and adipic acid with 1,3 propane diol do not show complete separation of these three isomers. Present series could get some success in separating all the three isomers. Communication by Sapet et al.³ shows the separation factor between longifolene and longicyclene as 1.2 on adipic acid and 1,3 propane diol. They have also tried the columns of silicone and eploxon which show same retention times for longicyclene and longifolene. These diaromatic polyesters could stretch these compounds apart showing the separation factor of 1.25. Column No.3 and 6 are showing more solubilities of these compounds compared to rest of the columns which is clear by higher retention time on these two columns.

Alcohols

2a) Menthols - Separation of menthols is usually found very difficult on commonly used columns. In present series of polyesters all the three menthols are completely separated on Column No.7 (Chromatogram No.8) and are partially separated on Column No.8. On other columns neomenthol is comfortably separated from l-menthol while isomenthol is mixed up with l-menthol.

Our interest was to resolve all the four isomers i.e. l-menthol, neomenthol, neo-isomenthol and isomenthol of menthol since they are normally obtained together in the menthol producing oils. As Neo-isomenthol was not available we could not try for this separation.

2b) Alcohols - Geraniol, citronellol and nerol are showing comparable retention times on all the columns and can be very easily separated on Column No.1,4,5 and 6 (Chromatogram No.9). This property is quite in agreement with the solubilities of these compounds in general polyester phases.

Isomers

All the three isomers are eluted out separately on most of these columns (Chromatogram No.10). The solubility of these compounds on all these polyester phases may help in practical application of the phases for the separation of such compounds on chromatography. Careful study of the

CHROMATOGRAM NO. 9.

1. CITRONELLOL
2. NEROL
3. GERANIOL

TEMP. 180°

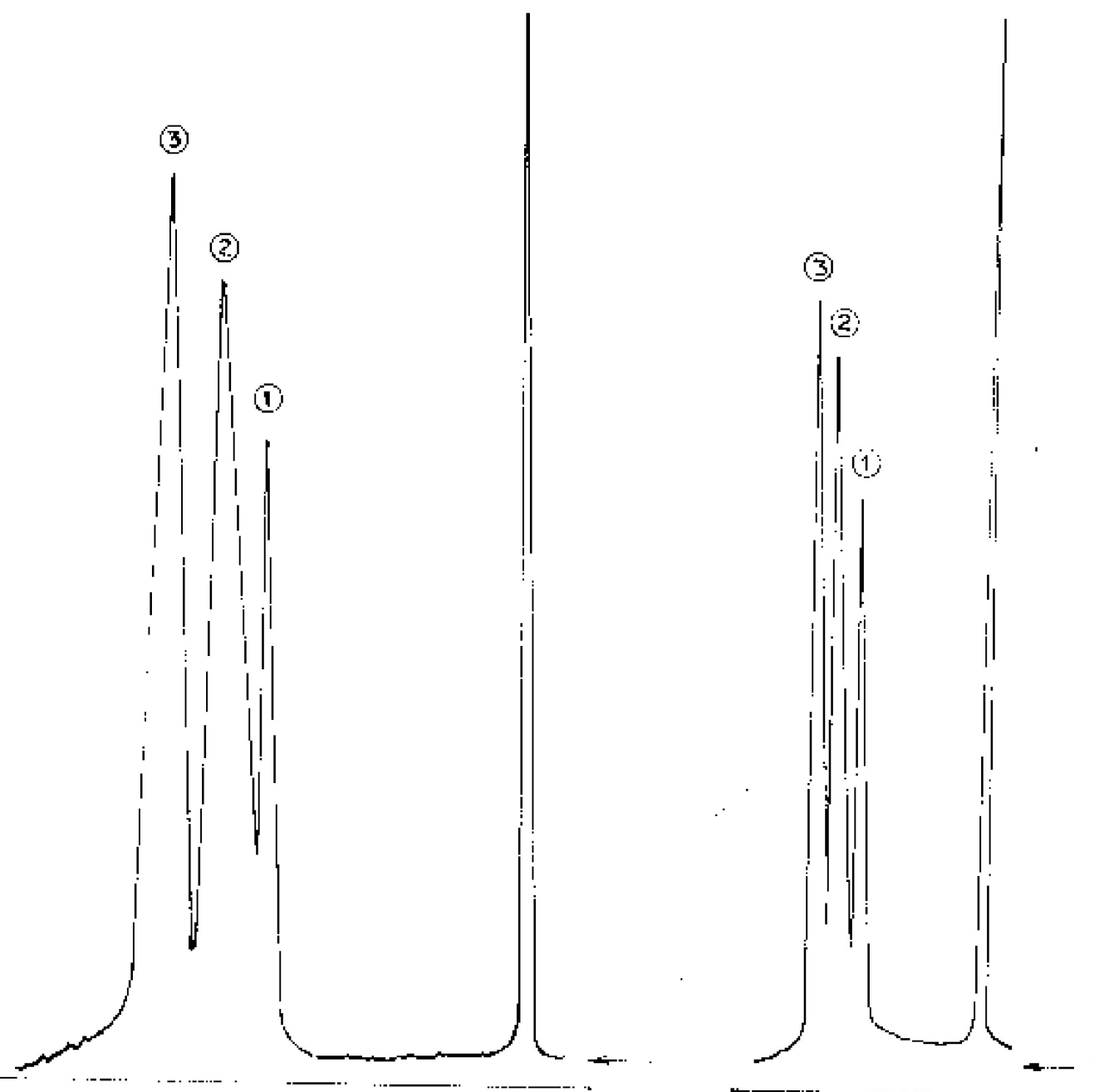
CHART SPEED - 600 mm / hr

CHROMATOGRAM NO. 7.

1. LONGICYCLENE
2. ISO-LONGIFOLENE
3. LONGIFOLENE

TEMP 180°

CHART SPEED - 600 mm / hr



COLUMN NO. 6.

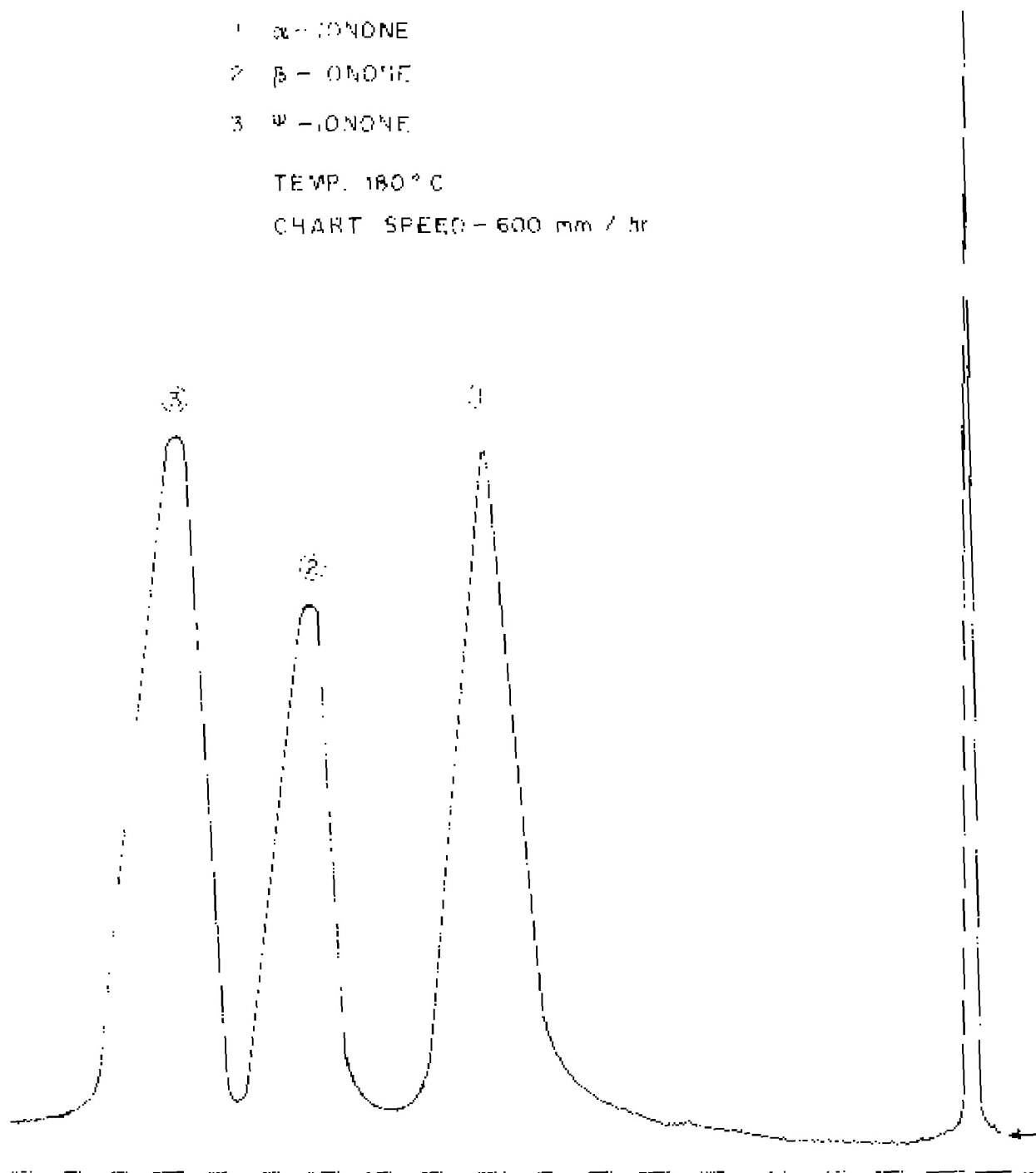
COLUMN NO. 4.

CHROMATOGRAM NO. 10.

- 1 α -IONONE
- 2 β -IONONE
- 3 ψ -IONONE

TEMP. 180° C

CHART SPEED - 600 mm / hr



COLUMN NO. 5.

retention time data of all these terpenoids reveals certain peculiar behaviour of these liquid phases. Except Column No.7 i.e. diphenic acid polyester liquid phase, all other substrates have aromatic rings having dicarboxy substituents joined by etherial oxygen. Different positions of acid groups have profound influence on emergence of the various terpenoids. Column No.2 shows the elution pattern for all the terpenoids according to their boiling points (Chart VI) while all other columns give indication that elution is partly on the boiling point basis and partly on polarity basis. If retention times of citronellol, geraniol, nerol and longifolene are considered (Table No.VII) the order of emergence is varied. Column No.2 elutes longifolene at the last while it appears in between nerol and geraniol on Column No.5. Retention time of longifolene is further reduced on Column No.1 and it appears in between citronellol and nerol (Table No.I). Longifolene emerges out first on Column No.3,4,5 and 7. This can be due to different polarities of different liquid substrates caused by the different positions of dicarboxylic acid groups. General observation suggests that meta and ortho positions of dicarboxylic groups are influencing the polarity of the liquid phases.

Table IColumn Numbers

1	2	3	4	5	6	7
Citronellol	Citronellol	Longifolene	Longifolene	Longifolene	Citronellol	Longifolene
Longifolene	Nerol	Citronellol	Citronellol	Citronellol	Nerol	Citronellol
Nerol	Geraniol	Nerol	Nerol	Nerol	Longifolene	Nerol
Geraniol	Longifolene	Geraniol	Geraniol	Geraniol	Geraniol	Geraniol

Pinenes

These samples are chromatographed at 100°C temperature and that is why they are included in group III. The present series of polyesters could effectively separate the α -pinene from β -pinene.

The earlier workers⁵ could achieve the separation factor of about 1.45 magnitude on phthalic acid and 1,2-propane diol polyester liquid phase. Diethylene glycol and succinate polyester¹ has shown the separation factor of the order of 1.7. The present series of Column Nos. 5 and 6 show the separation factor of 1.65 magnitude thereby indicating some definite contribution from aromatic rings, along with the etherial oxygen in resolution of these compounds.

Volatility of some of these liquid phases

There are several communications in the literature in which separation of these aromatic isomers had been tried and results were recorded.

Present investigations have revealed that some of the new phases described in this thesis have shown some advantages. Comparative record of relative volatility (α) of some of the separations of isomers has been described in Table No.IX.

It can be concluded that *m,m'*-oxydibenzoic acid polyester and diphenic acid polyester phases can be useful for separation of different types of compounds and will be further investigated for their applications.

Table No. II

Sample	Column No.						
	1	2	3	4	5	6	7
Acetone	0.76	0.9	0.87	0.72	0.58	0.8	0.7
Methyl ethyl ketone	1.58	1.68	1.77	1.50	1.33	1.63	1.18
Methyl propyl ketone	2.7	3.1	3.42	2.80	2.43	3.06	2.15
Methyl isobutyl ketone	4.13	4.36	4.43	3.50	3.195	5.12	3.75

Table No.III

Sample	Column No.						
	1	2	3	4	5	6	7
Methanol	0.22	0.5	0.47	0.26	0.26	0.48	0.40
Ethanol	0.61	0.59	0.63	0.50	0.55	0.70	0.5
n-Propanol	1.05	1.12	1.23	0.90	1.07	1.40	1.00
Isopropanol	0.54	0.55	0.62	0.55	0.55	0.75	0.5
n-Butanol	2.11	2.27	2.62	1.96	2.02	2.02	2.0
Iso-butanol	1.24	1.52	1.72	1.25	1.42	2.02	1.47
Sec-butanol	1.02	1.24	1.20	0.97	1.07	1.22	1.02
tertiary butanol	0.42	0.52	0.54	0.32	0.50	0.2	0.55
Amylalcohol	2.22	2.52	2.91	2.52	2.2	4.72	2.57

Table No. II

Sample	Column No.						
	1	2	3	4	5	6	7
Benzene	0.69	0.83	0.83	0.48	0.8	0.95	0.57
Toluene	1.45	1.62	1.64	1.1	1.5	2.025	1.1
Ethylbenzene	2.2	3.03	3.93	2.1	2.38	4.00	2.06
o-Xylene	3.69	4.22	4.15	2.62	3.68	5.21	2.6
m-Xylene	2.93	3.4	3.12	2.125	2.5	4.12	2.2
p-Xylene	2.87	3.4	3.12	2.05	2.44	4.00	2.1
α -Pinene	-	-	-	-	1.42	3.25	1.37
β -Pinene	-	-	-	-	2.42	5.36	2.15

Table No. V

Column No.							
Sample	1	2	3	4	5	6	7
Cyclo- pentanone	2.00	1.8	1.95	1.40	1.30	2.00	1.5
Cyclo- hexanone	3.71	3.48	3.62	2.52	2.33	3.47	2.53
Cyclo- heptanone	8.01	7.275	7.25	5.10	4.67	7.16	5.03
Cyclo- octanone	13.91	12.25	12.63	8.5	7.75	12.67	8.03
6-Methyl- heptanone	3.92	3.75	3.62	2.35	2.30	3.82	2.62

Table No. VII

Sample	Column No.						
	1	2	3	4	5	6	7
o-dichlorobenzene	1.88	2.77	2.84	1.4	1.93	2.55	1.88
m-dichlorobenzene	1.45	1.49	1.47	1.0	1.49	2.00	1.88
p-dichlorobenzene	1.6	2.4	2.35	1.13	1.5	2.16	1.82
o-chloroaniline	7.06	5.1	7.67	6.05	6.38	7.05	5.79
m-chloroaniline	10.83	10.32	15.5	12.4	13.12	14.32	12.45
p-chloroaniline	14.88	10.30	15.92	12.17	12.73	14.25	12.37
o-chloronitrobenzene	12.25	11.67	11.12	10.67	10.92	12.6	8.78
m-chloronitrobenzene	11.25	10.73	10.12	8.50	7.82	12.3	6.72
p-chloronitrobenzene	12.40	10.75	10.52	10.25	6.72	14.00	6.05
α -picoline	4.44	2.28	2.47	1.32	1.77	2.03	1.7
β -picoline	4.06	2.64	3.75	2.71	2.00	3.20	2.6
γ -picoline	4.425	2.92	4.08	2.00	2.67	3.32	2.87
o-bromotoluene	1.21	2.52	2.64	1.17	1.65	2.29	1.82
m-bromotoluene	1.75	2.69	2.63	1.25	1.73	2.32	1.68
p-bromotoluene	1.74	2.65	2.69	1.22	1.72	2.45	1.6

Table No. VIIb

Sample	Column No.						
	1	2	3	4	5	6	7
o-methyl- anisole	1.31	1.32	1.97	1.60	1.30	1.75	1.5
m-methyl- anisole	1.55	2.35	2.37	1.87	1.58	2.08	1.77
p-methyl- anisole	1.52	2.35	2.33	1.78	1.65	2.1	1.62
o-nitro- toluene	6.16	8.8	6.5	8.03	5.00	6.65	4.58
m-nitro- toluene	9.72	13.48	10.00	7.78	7.4	10.88	6.43
p-nitro- toluene	10.00	13.2	10.18	8.20	7.4	10.3	7.27
o-cresol	6.85	6.45	9.1	5.80	6.57	6.22	6.77
m-cresol	12.17	8.67	12.85	7.67	8.91	8.12	9.23
p-cresol	11.78	8.55	12.65	7.43	8.77	8.00	8.7
o-toluidine	11.00	6.92	12.00	7.61	7.97	9.50	7.62
m-toluidine	6.42	9.9	5.25	4.43	4.89	5.36	4.57
p-toluidine	6.00	3.5	5.25	4.13	4.55	5	4.35

Table No. VII

Sample	Column No.						
	1	2	3	4	5	6	7
Longi- cycloene	2.8	5.08	3.68	2.30	2.60	4.7	2.08
Longi- folene	4.9	6.47	3.82	3.04	2.28	6.03	2.72
Isolongi- folene	4.1	5.23	2.6	2.62	2.27	5.00	2.33
Citracellol	4.82	3.52	5.02	3.5	4.00	4.87	4.23
Geraniol	6	4.75	5.24	4.5	5.55	6.5	5.37
Nerol	5.25			4.0	4.67	5.58	5.23
Isomenthol		3.07	3.42	2.83	3.43	4.37	3.03
Neo-menthol		2.47	2.27	2.00	2.53	3.45	2.13
l-Menthol		2.83	3.32	2.58	3.05	4.12	2.67
α -terpineol				3.5	3.82	5.00	4
α -ionone			9.5	8.55	9.00	12.25	8.27
β -ionone			12.5	11.5	12.22	17.68	11.4
γ -ionone					15.02	20.87	14.08

Table No. VIII
Data on Relative Volatility (α)

Compound pair	Calculated No.						
	1	2	3	4	5	6	7
p-bromo- toluene to o-bromo- toluene	1.44	1.06	1.06	1.09	1.07	1.07	1.125
m-bromo- toluene to p-bromotoluene	1.01	1.003	1.02	1	1.02	1.02	1.07
p-methyl anisole to o-methyl anisole	1.21	1.2	1.12	1.16	1.27	1.2	1.06
p-methyl anisole to m-methyl anisole	1.02	1.00	1.02	1.04	1.04	1.01	1.09
p-nitro- toluene to o-nitro- toluene	-	1.57	1.56	1.62	1.42	1.55	1.71
p-nitro- toluene to m-nitrotoluene	-	1.02	1.01	1.06	1.00	1.00	1.13
p-cresol to o-cresol	1.23	1.22	1.29	1.26	1.33	1.29	1.29
m-cresol to p-cresol	1.02	1.01	1.02	1.03	1.01	1.01	1.06
o-toluidine to p-toluidine	1.4	1.75	1.92	1.84	1.75	1.9	1.73
m-toluidine to p-toluidine	1.02	1.02	1.07	1.07	1.07	1.02	1.05

Table No. VIIIa

Data on Relative Volatility (α)

Compound pair	Column No.						
	1	2	3	4	5	6	7
Longifolene to iso-longifolene	1.19	1.24	1.005	1.18	1.003	1.21	1.17
Isolongicyclene to longicyclene	1.1	1.09	1.06	1.14	1.1	1.06	1.12
Nerol to citrenal	1.15	-	-	1.42	1.7	1.145	1.21
Geraniol to nerol	1.13			1.125	1.2	1.165	1.03
L-Menthel to Neomenthol		1.145	1.27	1.29	1.7	1.2	1.25
iso-Menthel to l-menthol	-	1.025	1.09	1.097	1.2	1.061	1.19
β -ionone to α -ionone			1.316	1.22	1.27	1.23	1.322
Ylonone to β -ionone					1.22	1.19	1.222
β -pinene to α -pinene					1.64	1.66	1.57
o-dichlorobenzene to p-dichlorobenzene	1.25	1.15	1.21	1.23	1.29	1.19	1.16
p-dichlorobenzene to m-dichlorobenzene	1.03	1.6	1.6	1.13	1.06	1.07	1.27
p-chloroaniline to o-chloroaniline	2.06	2.01	2.07	2.01	2.15	2.03	2.14
m-chloroaniline to p-chloroaniline	1.24	1.001	1.01	1.02	1.05	1.00	1.005

Table No. VIIIa (continued)

Data on Relative Volatility (α)

	1	2	3	4	5	6	7
o-chloro-nitrobenzene to p-chloro-nitrobenzene	1.01	1.07	1.05	1.06	1.25	1.04	1.09
p-chloronitrobenzene to m-chloronitrobenzene	1.1	1	1.05	1.2	1.05	1.14	1.19
o-xylene to p-xylene	1.226	1.26	1.326	1.31	1.51	1.3	1.33
m-xylene to p-xylene	1.081	1	1.016	1.037	1.02	1.03	1.05
β-picoline to α-picoline	1.09	1.6	1.5	1.2	1.7	1.6	1.52
γ-picoline to β-picoline	1.104	1.08	1.09	1.1	1.2	1.04	1.1

Table No.IX

Relevant examples from the literature for the attempted separation of positional isomers

S.No. References		Liquid phase	Column length	Column temp. ^o C	Total time of analysis (in mins)	α -Value	Relative volatility
1	2	3	4	5	6	7	8
Dichlorobenzenes							
1.	Nortimere et al. ⁶	dextrose-24 +silicous MS 550	18 ft	100	35	2.32	1.32
2.	Habboush et al. ⁷	polyethylene glycol succinate	6 ft	80	-	1.23	1.14
3.	Fitzgerald ²	Diglycerol	12 ft	100	-	1.71	1.16
4.	Ghatge et al. ⁵	1,3-propane-diol 4-aminophthalic acid polyester	6 ft	170	3.23	1.26	1.16
5.	Pense et al. ³	phthalyl alcohol +adipic acid	6 ft	175	6.0	1.2	1.128
6.	Author	m,p'-oxydi-benzoic acid+ 1,3-propane-diol polyester	6 ft	170	2.84	1.21	1.6
Pinolines						α, β	β, γ
1.	Kristen et al. ⁶	Iveon-20	6 ft	70	76	1.62	1.11
2.	Ghatge et al. ⁵	1-3-propane-diol + 4-amino-phthalic acid polyester	6 ft	170	5.5	1.96	1.29
3.	Pense et al. ³	phthalyl alcohol+ adipic acid	6 ft	175	7	2.05	1.218

Table No. IX (continued)

1	2	3	4	5	6	7	8	
4. Author		<i>o,o'</i> -oxydibenzic acid + 1,3-propanediol polyester	6 ft	140	3.67	1.7	1.2	
Chlorocollins							<i>o,p</i>	<i>m,p</i>
1. Ghatge et al. ⁵		1,5-pentanediol-3,6-dichlorophthalic acid polyester	6 ft	170	16	1.64	1.25	
4. Panse et al. ³		3-amino-4-methoxy phthalic acid ester + diethylene glycol - 10% epoxy resin	6 ft	175	9.5	4.1	1.46	
3. Author		<i>p,p'</i> -oxydibenzic acid + 1,3-propanediol polyester	6 ft	180	14.58	2.06	1.24	
Chloronitrocollins								
1. Habboush et al. ¹⁰		Tritolyl phosphate	6 ft	145	79	1.16	1.15	
2. Vazaron et al. ¹¹		Igepal CO-860	18 ft	140	32	1.21	1.12	
3. Habboush et al. ⁷		polyethylene glycol succinate	6 ft	160	-	1.2	1.15	
4. Ghatge et al. ⁵		1,3-propanediol + 4-aminophthalic acid polyester	6 ft	170	20	1.15	1.22	
5. Panse et al. ³		3-amino-4-methoxy phthalic acid ester + diethylene glycol	6 ft	175	7	1.19	1.127	
6. Author		<i>o,o'</i> -oxydibenzic acid + 1,3-propanediol polyester	6 ft	180	10.25	-	1.2	
		<i>o,o'</i> -oxydibenzic acid + 1,3-propanediol polyester	6 ft	180	10.87	1.25	-	

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