STUDIES IN CHROMATOGRAPHY: GLC AND TLC - FID

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DEDICATED

TO

MY FATHER

CERTIFICATE

Certified that the work incorporated in the thesis "Studies in Chromatography: GLC and TLC-FID", submitted by Ms. Sujata S. Biswas, was carried out by the candidate under my supervision in NCL. Such material as has been obtained from other sources is duly acknowledged in the thesis.

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CHAPTER - 1

REVIEW: LIQUID STATIONARY PHASES IN GAS CHROMATOGRAPHY

1.1 Introduction

The choice of the proper stationary phase is one of the most important decisions in gas liquid chromatography (glc). The large number of stationary phase liquids described in the literature and offered for sale is bewildering to the gas chromatographer. For the time being, it can be said that the selection of the stationary phase for a new analytical problem is done on a trial-and-error basis, the success of a certain separation depending greatly on the experience accumulated previously by the analytical chemist. The requirements, which should be considered in selecting the appropriate stationary phase are as follows:

1) low vapour pressure at the desired operating temperature;

2) chemical stability at the operating temperature;

3) selectivity for the components to be separated;

4) sufficient dissolving power for the components;

5) low viscosity at the desired operating temperature;

6) adequate wetting of the support surface or the column wall;

7) reasonable solubility in some common volatile solvents.

For the majority of analytical problems, glc has proved to be an efficient technique. The use of liquid phases has the following advantages[1]:

1) The adsorption isotherm is linear under usual operating conditions so that symmetrical peaks can be obtained.

2) Liquid phases are available in great variety, thus adequately selective phases can be chosen for a particular separation.

3) The amount of liquid phase in the column can be varied easily; therefore analytical columns of high efficiency and preparative columns can be made with the same liquid phase.

4) High purity liquid phases are available due to which retention values are reproducible.

The drawback in gas liquid chromatography is the volatility of liquid phases. The liquid stationary phase always has a vapour pressure, the magnitude of which depends on the nature of the phase and on the working temperature. The continuously flowing carrier gas removes a certain amount of the liquid phase per unit gas volume which is known as bleeding. This bleeding leads to improper function of the detector, baseline shift in programmed temperature operation, contamination of the trapped solutes and changes in the separating capability of the column. The vapour pressure of the stationary phase in the column might be influenced by the support material and also by the loading. Recommended maximum temperatures for different stationary phases can be found in tables. The thermal stability of the liquid phase is also very important. The thermal behaviour of the liquid phase can be studied by thermogravimetry. The lower temperature limit for operating a column is generally the melting point of the liquid phase. The upper temperature limit for operating a column can be safely kept at 40-50°C lower than the evaporation point of the liquid phase. Any conformational changes, polymerization, cross-linking, condensation at high temperature can also be studied by thermogravimetry.

1.2 Types of Stationary Phases

Classification of stationary phases as suggested by Littlewood[2] is discussed here. The different stationary phases have been clubbed under four groups viz.,

I. non-polar (paraffinic) stationary phases;

II. dilute stationary phases;

III. concentrated stationary phases; and

IV. specific stationary phases.

1.2.1 I. *Non-polar stationary phases:*

The phases that contain neither polar nor polarizable groups fall in this group. They include all alkanes and mixtures of alkanes and alkyl silicones. The intermolecular forces in non-polar stationary phases consist of dispersion and induction forces. The hydrocarbons are much more soluble in these stationary phases than any polar solute with a similar boiling point. This property is used in the separation of these hydrocarbons from different mixtures of solutes. During isomers separation, the linear alkane has the greatest retention, and the general rule among branched alkanes is that the greater the branching the smaller the retention. According to Dyson and Littlewood[3] branching decreases retention because it causes the molecule to become more compact with a smaller surface-to-volume ratio. Polar solutes separate according to size because their polarity is irrelevant.

Because non-polar stationary phases do not interact specifically with the solutes, they can be used as reference stationary phases. The most important members of this class are:

Squalane: (2,6,10,15,19,23 - hexamethyltetracosane)

Squalane, is produced by hydrogenation of squalene and has the structure as shown in **FIG. 1.1.** It has maximum operating temperature T_{max} - 150°C.

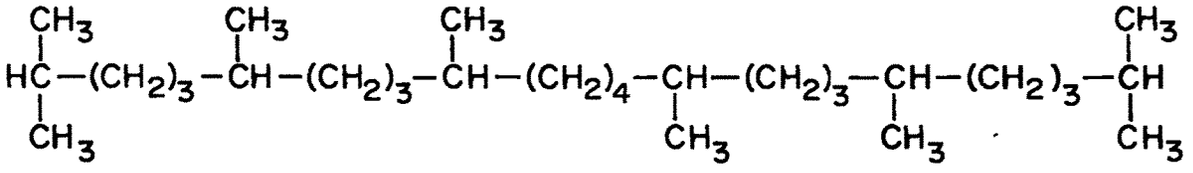
Applications: For separation of C_5 - C_8 hydrocarbons, some mercaptans[6] and anaesthetics[7].

Apiezon greases:

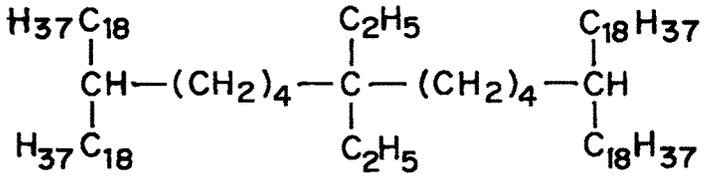
Apiezons are obtained by subjecting selected lubricating oils to a high temperature treatment. The most labile fraction is thus destroyed and the residue is purified by molecular distillation. According to the temperature of the heat treatment, the Apiezons are classified alphabetically: C, E, H, J, K, L, M, N, T.

As Apiezon L has a lower vapour pressure and allows higher operating temperatures (300°C) it has been most widely used. Commercial materials with a characteristic yellow-brown colour contain residual carbonyl and carboxylic acid groups which may cause tailing of polar solutes. Prior to use the greases should be passed over an alumina column. The structure of the Apiezon greases has not clearly been established. Although they are known to contain both olefinic and aromatic unsaturated groups. Hydrogenation removes any residual unsaturation and produces superior high temperature phases.

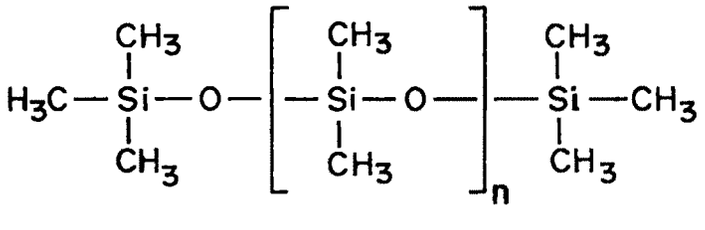
Applications: Separation of the methyl esters of high molecular weight fatty acids[8,9,10], fatty acid amides[11], fatty amines[12], phenothiazine and derivatives[13] lead alkyls[14] and pentaboranes and decarboranes[15].



SQUALANE



APOLANE-87



METHYL SILICONE

FIG. 1·1 : NON-POLAR STATIONARY PHASES

Apolane - 87: (24,24-diethyl - 19, 29-dioctadecylheptatetracontane)

Kovats[16] has prepared a synthetic, high temperature hydrocarbon phase (C_{87} H_{176}), see **FIG. 1.1**, with a molecular weight of 1222 and a maximum operating temperature of 280-300°C. Apolane-87 and hydrogenated Apiezon, Apiezon MH, are suggested as substitutes for squalane for study of McReynolds constants at 180° and above.

Methyl silicones:

This group of compounds is among the most widely used non-polar stationary phases. The general formula for methyl silicones can be seen in **FIG. 1.1.** Dimethyl silicone oils are liquids with low solubility parameter, high compressibility, low heats of fusion, and very little tendency for specific interaction, example SE-30, OV-1, OV-101.

Applications: Used for analysis of steroids, pesticides, terpenes and phenols.

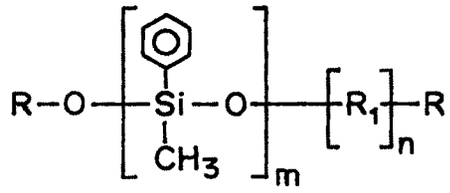
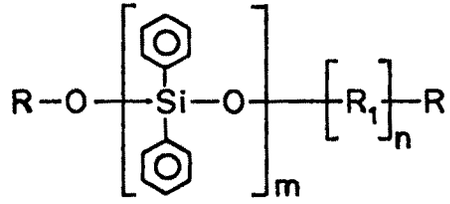
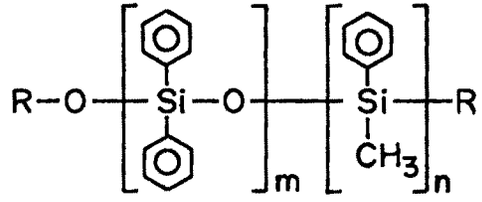
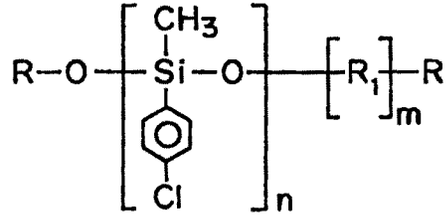
1.2.2 II. *Dilute stationary phases:*

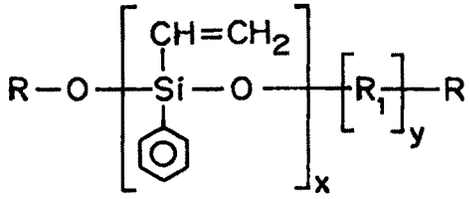
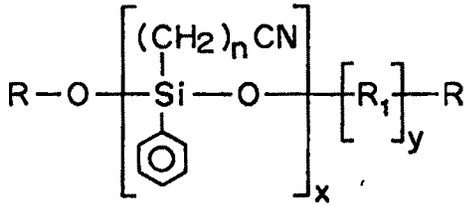
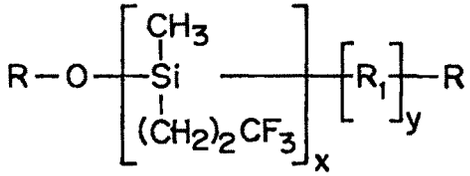
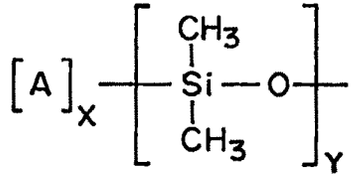
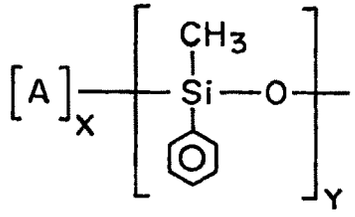
The majority of the molar volume of these phases is occupied by non-polar groups but in which there are also some polar groups. The polar and non-polar solutes are readily dissolved in these phases and hence they can be used as universal stationary phases in gas chromatography.

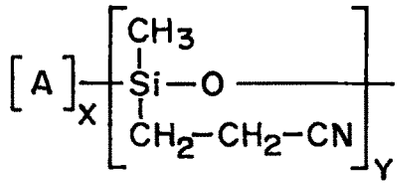
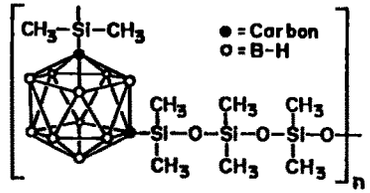
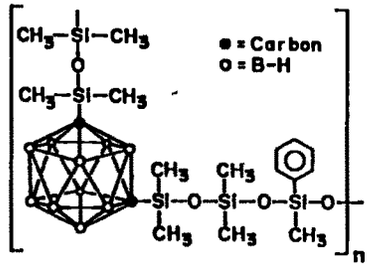
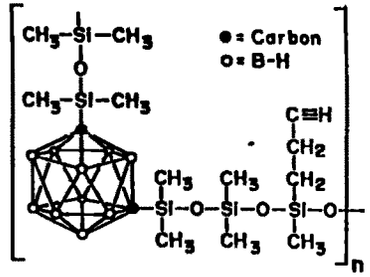
Their retention characteristics are determined by the fact that they have polar groups with which polar solutes can interact, but not so many as to make a strongly cross linked solvent lattice, the internal pressure of which is such as to exclude non-polar molecules. The members included in this class are tabulated in **TABLE 1.1.**

TABLE - 1.1

DILUTE STATIONARY PHASES : POLYSILOXANES

	Phase	Substituent	Structure	Commercial Name	Maximum working temperature (°C)
1.	**Methyl Phenyl Silocones**				
	(i) Small content of phenyl groups	5% phenyl		SE-52, OV-73	350, 325
		10% - 30% of phenyl		OV-3, OV-7, OV-11	350, 350, 350
	(ii) Medium content of phenyl groups	30% - 50% phenyl		OV-17, OV-61	375, 350
	(iii) High content of phenyl groups	60% - 75% phenyl		OV-25, OV-22	350, 350
2.	**Methyl Chloro-phenyl Silicones**	11% chlorophenyl group		DC 560, SP 400	250,350

3.	**Methyl Phenyl/ Vinyl Silicones**	1% vinyl		SE - 31	300
		1% vinyl, 5% phenyl		SE - 54	300
4.	**Methyl Cyanosilicones**	25% cyanoethyl		AN - 600	300
		25% cyanopropyl 25% phenyl		OV - 225	265
5.	**Methyl Trifluoropropyl Silicones**	50% trifluoropropyl 1% vinyl, 50% trifluoropropyl		OV - 210, SP 2401 OV - 202, OV - 215 LSX - 3 - 0295	275, 275 275, 275 275
6.	**Organosilicone Polymers** *Polyethylene succinate and methylsiloxane*	-		EGSS - X for Y/X < 1 EGSS - Y for Y/X > 1	225 230
	Polyethylene succinate and phenylmethyl siloxane	-		EGSP - A for Y/X < 1 EGSP - Z for Y/X > 1	220 230

	Polyethylene succinate and cyanomethylsiloxane	-		ECNSS - S for Y/X < 1 ECNSS - M for Y/X > 1	210 210
7.	**Carborane-Siloxane Polymers**	Polymethyl carborane methylsiloxane = 1 : 4		Dexsil 300 GC	400
		Polymethyl carborane methyl phenyl phenylsiloxane = 1 : 5		Dexsil 400 GC	400
		Polymethyl carborane methylcyanoethyl siloxane = 1 : 5		Dexsil 410 GC	400

R = $(CH_3)_3$ - Si

$R_1 = (CH_3)_2$ - Si - O

A = $-(CH_2)_2-CO_2-(CH_2)_2-CO_2-$

Polysiloxanes:

They cover nearly the entire range of chromatographic polarities by substituting various amounts of pendant phenyl, fluoroalkyl and cyanopropyl groups for methyl in the 'backbone' polymer structure. The different polysiloxanes are listed below.

1. Phenyl Silicone

Phenyl silicones are used for the analysis of drugs and pesticides[17,18], some higher aromatic hydrocarbons[19], trifluoroacetyl acetonates of Al, Be and Ur[20,21], iodofluoroalkanes[22].

2. Methyl Chlorophenyl Silicones

The introduction of a chlorophenyl group in methyl silicones yields a high temperature stationary phase with solvent properties resembling those of chlorobenzene. The chlorphenyl groups improve lubrication properties and reduce some dipole interactions between chains.

3. Methyl Phenyl Vinyl Silicones

The presence of vinyl groups aids crosslinking.

4. Methyl Cyanosilicones

The presence of a nitrile group increases the retention of aromatics of the same carbon number over aliphatics, aromatics over olefins, alcohols over ethers, ketones over ethers, and increases the retention of ketones over primary alcohols but to a smaller extent.

5. Methyl Trifluoropropyl Silicones

The methylene group adjacent to trifluoropropyl methyl silicone molecule might be polarized somewhat by the strong electronegative trifluoromethyl group so that some positive charge is induced there. Trifluoropropyl methyl silicones have an unusual affinity for the carbonyl group.

6. Organosilicone Polymers

These stationary phases have a greater polarity because of the presence of ester groups. The donor oxygen atoms interact with the solutes and separation takes place due to hydrogen bonding.

7. Carborane Siloxane Polymers

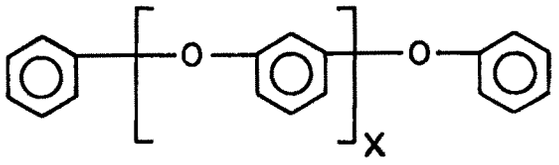
By polymerization of carboranes with polysiloxanes, a thermally stable compound is obtained.[23] Dexsil 300 GC contains one carborane group attached in the *meta* position as part of a chain with dimethyl siloxane groups repeating the unit. Dexsil 400 GC and 410 GC have as the repeating unit one carborane group and five dimethyl siloxane units, the central Si atom has a phenyl and a 2-cyanoethyl group respectively.

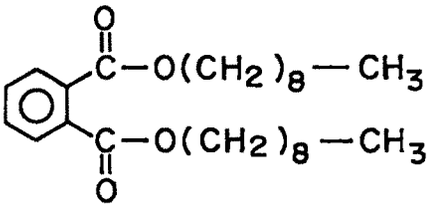
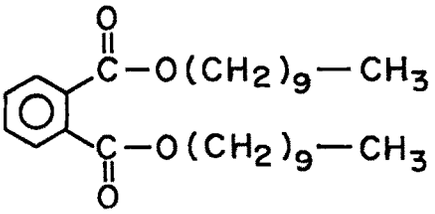
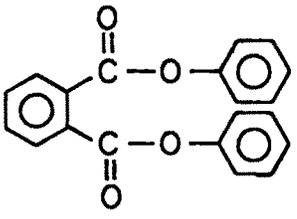
Ethers:

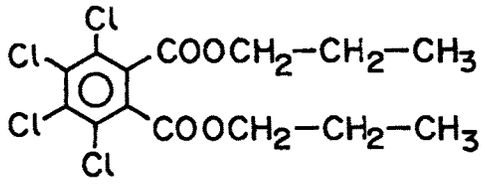
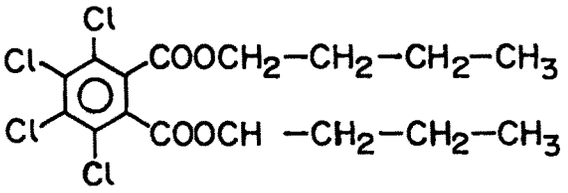
The *meta* linked poly (phenyl ethers) as can be seen in **TABLE 1.2** are useful moderately polar liquid phases. The OS-124 (5 rings) and OS-138 (6 rings) are stable upto 200°C and 250°C respectively. The flexibility imparted to the chain by the ether linkage is responsible for their low viscosity. A high molecular weight poly (phenyl ether) containing an average of 20 rings shows an excellent thermal stability up to 400°C.

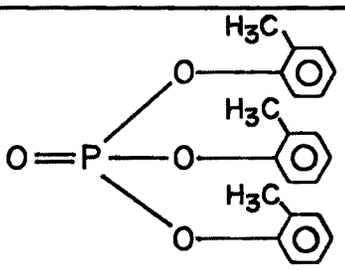
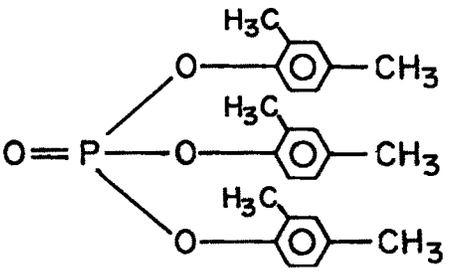
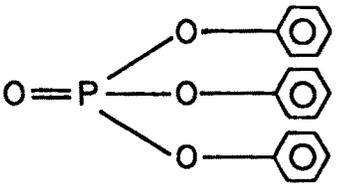
TABLE 1.2

DILUTE STATIONARY PHASES : ETHERS AND ESTERS

Ethers		
Name	**Structure**	**Maximum working temperature°C**
Poly (phenyl ether)		
	X = 5	200
	X = 6	250
	X = 20	400

Esters		
Phthalates	**Structure**	**Maximum working temperature°C**
Dinonyl phthalate		150
Didecyl phthalate		150
Diphenyl phthalate		150

Tetrachlorophthalates	Structure	Maximum working Temperature °C
Dipropyl tetrachlorophthalate		150
Dibutyl tetrachlorophthalate		150

Phosphates	Structure	Maximum working Temperature °C
Tricresyl phosphate		125
Tri-(2,4-xylenyl) phosphate		150
Triphenyl phosphate		150

Poly (phenyl ethers) have been modified in several ways. Mathews *et al.*[25] synthesised sulfonated polyphenyl ethers such as Poly S-179 (200 - 400°C) and Poly S-176 (150 - 400°C) by reacting dinuclear aromatic sulfonyl chlorides with five or six ring polyphenyl ethers using Friedel-Craft catalysts.

Esters:

Their general characteristic is the presence of donor oxygen atoms that may form hydrogen bonds, thus resulting in high relative retentions for solutes having protonated functional groups. Some of the well known phthalates, tetrachlorophthalates and phosphates are listed in **TABLE 1.2.** The affinity of tetrachlorophthalate stationary phases for electron donor solutes is increased due to the presence of the four chlorine atoms. The esters of tetrachlorphthalic acid are used for the separation of xylene isomers,[26,27] and for a mixture of n-C_7-C_{10} mono-olefins[28,29]. Phosphates are used for the separation of hydrocarbons, ketones, alcohols[30,31] some chlorinated derivatives of hydrocarbons[32], cresols[33] xylenols, fluorophenols and chlorophenols[34]

1.2.3 III. *Concentrated stationary phases:*

This class includes those stationary phases in which a large proportion of the molar volume is occupied by polar groups. The polar part may be strongly electronegative atoms F,O,N, or electron attracting groups, such as - NO_2, -CN, and -CF_3, or electron repelling groups such as -NMe_2, - CMe_3, and -OMe. The polarity of the molecule is determined by the type and number of polar grops present relative to the size of the neutral part or by the distribution of electrons on the different parts of the molecule. If a double bond is present in the molecule, the π electrons allow electron attracting or repelling groups to transmit their effect further through the hydrocarbon part of the molecule than is possible with saturated hydrocarbon chains.

Most of the stationary phases in this group act as electron donors or acceptors or both, but usually one effect is predominant. The donor/acceptor property of the stationary phases is governed by concentration of electrons or the electron cloud density. Many of these phases interact with the solutes to be separated, forming hydrogen bonds. Non polar solutes like alkanes and other hydrocarbons have very less retentions. Polar solutes have considerable retentions and these phases are mainly used for them.

The stationary phases most frequently used in this group are the poly ethylene glycols. They are prepared for gas chromatography purposes by polymerization of ethylene oxide. The product obtained are separated into series of fractions having nominal average molecular weights of 200, 300, 400 etc. (calculated from the chemically determined hydroxyl concentration). The principal factors determining the retention characteristics of these phases are the presence of moisture, concentration of the hydroxyl (-OH) end groups and to a very small extent the molecular weight distribution of the liquid phase. The presence of water changes the retention of the solutes to a great extent. This is because the moisture causes the formation of a hydrogen bonded cross-linked network between the water molecules and the poly ethylene glycol molecules, which excludes all but the most strongly hydrophilic solutes.

The retention characteristic of the poly oxyethylene glycol liquid phases depend on the concentration of hydroxyl groups available for partitioning purposes. The molecular weight distribution has very little effect on the retention. Elution of alkanes on these phases is determined mainly by variations in the inductive forces between the solvent and the solute, the methylene groups of the phase contribute only a minimum influence.

The simultaneous presence of acceptor atoms (hydroxyl and ether oxygen) and donor atoms (hydroxyl hydrogen) in poly glycols enables interaction of the stationary phases with the

compounds having not only hydroxyl groups and primary amino group but also carbonyl and secondary or tertiary amino groups. The lower the molecular weight of the stationary phase, the stronger the interaction with the polar solutes. As an example, the retention of butanol-1 decreases in the sequence polyethylene glycol PEG 400 > PEG 1000 > PEG 4000. As hydrogen bonding is the main attractive force, the aldehydes, ketones, and ethers are eluted in the order of their boiling points. When few hydroxylic hydrogen atoms are present as in large molecules of polyglycols, the hydrogen bonds are formed when the hydrogen atoms are "supplied" by the analyte. Thus poly ethylene oxide selectively separates primary, secondary and tertiary amines with similar b.p.'s, the order of elution being tertiary, secondary, primary. The structures and the commercial names of the stationary phases are listed in **TABLE 1.3.** By significant reaction of Carbowax 20M with 2-nitroterephthalic acid one obtains FFAP (free fatty acid phase), which is useful as a stationary phase for analysis of a wide variety of organic compounds. The Supelco phase SP-1000 is similar to FFAP and shows greater stability.

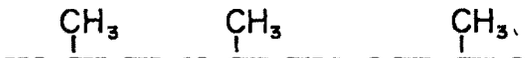
Polyesters:

The polyesters also fall in the class of concentrated stationary phases. A polyester is a macromolecular compound formed by a multitude of carboxylic ester linkages, derived from the reaction of a poly basic acid and a polyhydric alcohol. They are highly polar materials whose structures are somewhat varied depending on the particular acids and glycols from which they are prepared. Since these esters possess carboxyl oxygen atoms with donor properties which can enter into hydrogen bonds, the retention of solutes with protonated functional groups is high on such stationary phases.

In the use of polyesters attention should be paid to the following:

TABLE 1.3

CONCENTRATED STATIONARY PHASES : POLYGLYCOLS

Name	Structure	Commercial Name
Polyethylene glycol	$HO\text{-}(CH_2)_2\text{-}(O\text{-}CH_2\text{-}CH_2)_n\text{-}O\text{-}(CH_2)_2\text{-}OH$	Carbowax 1540 M.W. = 1300-1600 Carbowax 400 M.W. = 3000 Carbowax 1500 M.W. = 500-600
Polystyrene oxide		Dow Polyglycol 174 upto 500
Polypropylene glycol		Ucon fluids
Polyethylene oxide	$HO\text{-}(CH_2\text{-}CH_2\text{-}O\text{-})_n\text{-}H$	Carbowax 20M, Carbowax 6000

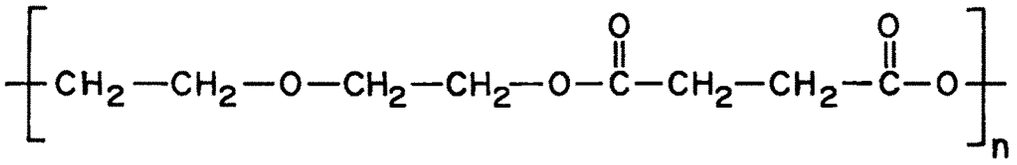
1. Polyesters get degraded when they come in contact with water and compounds which are strongly acidic or basic. The more hydrophobic the glycols and dicarboxylic acids, the higher the stability of polyesters towards hydrolysis.
2. Compounds capable of reacting with residual carboxyl or hydroxyl groups e.g. epoxides, isocyanates, anhydrides, halo acids and acyl acids should not be analyzed on these phases.
3. At high temperatures, polyesters are oxidised by atmospheric oxygen. Oxidation leads to the formation of hydroperoxides, which decompose the polymer chain by means of free redicals.

Some of the important and frequently used polyester phases are illustrated in **FIG. 1.2.** Polyesters are generally recommended as stationary phases for the separation of fatty acid methyl esters, essential oils, steroids, amino acid derivatives etc. The polyesters, because of their limitations have been replaced by polar polysiloxanes which show better batch-to-batch reproducibility and film-forming characteristics.

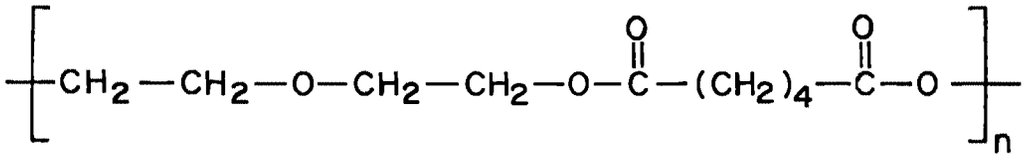
Amines and Amides:

Aliphatic amines capable of forming hydrogen bonds provide high selectivity towards alcohols, pyridines, glycols and mercaptans. Aromatic amines with π-electron systems may interact with aromatic hydrocarbons. Thus they show a high selectivity in separation of xylene isomers. The commercially available amine stationary phases are shown in **FIG. 1.3.**

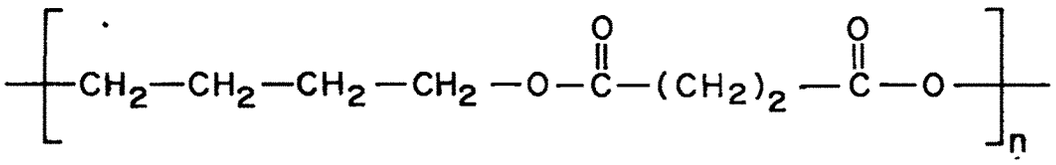
Among the amides, dimethyl formamide and formamide were used in the analysis of low-boiling hydrocarbons, previously. Nowadays these phases have been replaced by long carbon chain stationary phases e.g. Hallcomid. By condensation of piperidines with dicarboxylic acids different polyamides were prepared that were stable upto 275°C.[25]



DEGS



DEGA



BDS

FIG. 1·2: CONCENTRATED STATIONARY PHASES: POLYESTERS

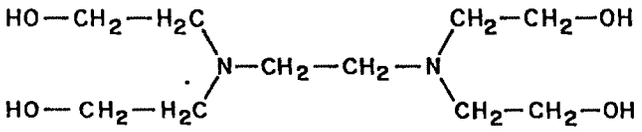
Nitriles and Nitrile Ethers:

Nitriles, and especially nitrile-ethers have weak interactions with saturated or non-polar solutes, but interact strongly with polar and non-saturated solutes and also with those compounds containing a hydrogen atom capable of hydrogen bonding. The interaction with the polar solutes is because of the strongly polar nitrile groups. (The dipole moment is 3.60 D for the alkyl-CN bond and 4.05 D for the phenyl-CN bond). The nitriles act as electron acceptors, they can induce an electric field in the molecules of unsaturated and polarizable compounds and form hydrogen bonds. Because of the electronegativity of the nitrile group, the compounds with easily inonizable π-electrons get selectively retained in the column. The nitrile phases used frequently are illustrated in **FIG. 1.3.**

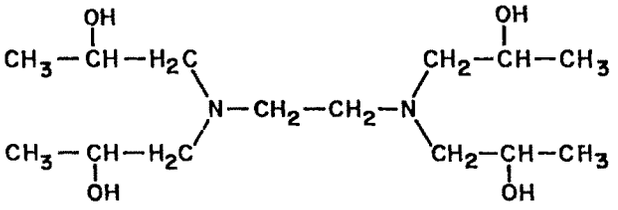
Among the nitrilic stationary phases the most utilised is 1,2,3-tris (2-cyanoethoxy) propane. Stationary phases that contain nitro groups behave similarly to those containing nitrile groups (from g.c. point of view). Such phases may interact through orientation and inductive forces (nitrobenzene has a dipole moment of 4.01 D), while its oxygen atoms are donor atoms and thus they are able to form hydrogen bonds with compounds with protonated functional groups. The nitro groups are generally attached to an aromatic nucleus, and because of the presence of phenyl groups the aromatic hydrocarbons have greater solubility in these phases. These phases have a good selectivity for separation of aromatic hydrocarbons from alkanes, substituted aromatic hydrocarbons, and low boiling halogenated hydrocarbons[35,36]. Only a few of the stationary phases with nitro groups are known. At higher temperatures and hydrogen as carrier gas nitro groups may get reduced.

1.2.4 IV. *Specific stationary phases:*

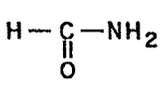
When designing specific stationary phases, the resulting interactions between the stationary phase and the solute should be strong enough to ensure a selective retention and also be



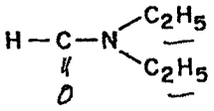
THEED



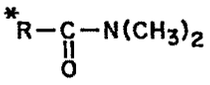
QUADROL



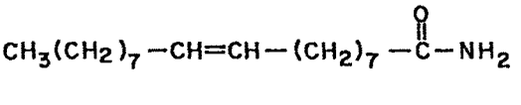
FORMAMIDE



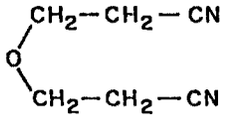
DIMETHYL? FORAMIDE



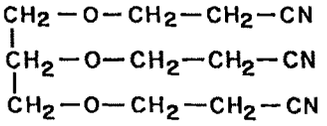
HALLCOMID M 18



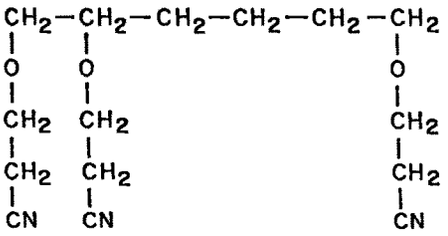
HALLCOMID M 180L



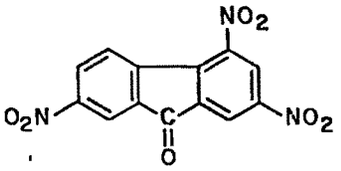
β,β,OXIDIPROPIONITRILE



1,2,3-TRIS(2-CYANOETHOXY) PROPANE



1,2,6-TRIS(2-CYANOETHOXY) HEXANE



2,4,7-TRINITRO-9-FLUORENONE

*R = $CH_3(CH_2)_{16}$

FIG. 1·3 CONCENTRATED STATIONARY PHASES. AMINES, AMIDES, NITRILES AND NITRILE ETHERS.

sufficiently labile to remove the components to be analysed from the stationary phase. Highly specific interactions can occur when transition metal complexes, enantioselective compounds, liquid crystals, and molten salts are used as stationary phases.

Transition Metal Complexes:

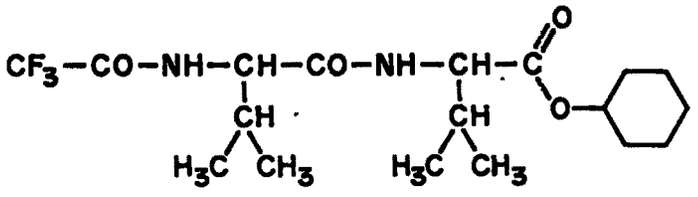
Transition metals possess d,p, and s orbitals interacting with ligand orbitals in a defined spatial orientation. The central metal ion and the ligand influence the chromatographic ability of the transition metal complex. The ligand is capable of controlling the interaction between the stationary phase and solutes through steric or inductive factors (e.g. the trans effect for square-planar complexes) and may play a role in stabilization of certain oxidation state of the metal. In turn, the separation ability of analytes is governed by electronic, steric and polarizibility factors. Thus substances differing only slightly in their structure such as *cis* and *trans* are resolved by means of chemical complexations[37,38]. The selectivity of silver-nitrate containing phases in general results from the relatively small structural changes in olefins on the stability constants of the complexes. The same holds true for other organic molecules containing π-bonds or free electron pairs.

Cartoni *et al.*[39] prepared N-dodecyl salicylaldimines of Ni, Pd, Pt and Cu and methyl-n-octyl glyoximes of Ni, Pd and Pt. They succeeded in separating substances that possess ligands (amines, ketones, alcohols, molecules containing double bonds). Barber *et al.*[40] used stearates of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) as liquid phases. When a mixture of amines was injected the primary amine decomposed the stearate column. The basicity of secondary and tertiary amines decided the retardation factor on Mn(II), Co(II) and Zn(II) stearates. This special interaction with metal stearates could be applied to difficult separations e.g. a 20% Mn stearate column on Celite at 156°C separated β-picoline, α-picoline and 2,6-lutidine, all these compounds have the same b.p. 143°C. Schurig[41] examined the use of enatioselective transition metal complexes to separate

enantiomers. Because of their extraordinary separation ability transition metal complexes are very often termed "superselective" packings. Crystal hydrates also rank as "superselective" packings[42,43]. A molecular sieve packing based on a polymer schiff base-chromium complex has been introduced for GC separations[44]. The introduction of a metal into a siloxane chain notably influences separation characteristics. Polymetalloorganopoly siloxanes are capable of interacting coordinatively with compounds having π bonds or lone electron pairs[45]. Recently, porous polymers containing various metal chelates bonded to nitrogen functionalities on the surface of the polymers have been synthesized and found to bind oxygen reversibly[46].

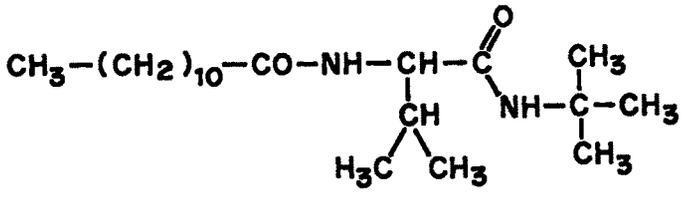
Optically active stationary phases:

There is a vast number of references concerned with the subject of optically active stationary phases and the same has been reviewed by stalwarts of gas chromatography[47, 48-51]. Recently, the separation of optical isomers was treated by Souter[52]. There are two fundamental approaches to separating enantiomers: (i) the conversion of enantiomers into diastereomeric derivatives by reaction with absolutely optically pure chiral reagents and then separation of the derivative on chiral or commonly used phases. **FIG. 1.4** shows an example of the separation of some diastereomeric N(O,S)-trifluoroacetyl-L-menthyl-amino acid esters on SE-54. (ii) Direct separation of enantiomers on optically active or enantioselective phases. Gil-Av *et al.*[53-54] were the first to prepare an optically active phase namely N-trifluoroacetyl-L-isoleucine lauryl ester which could separate highly volatile amino acid derivatives (alanine, valine). The upper temperature limit of this phase was only 100°C. The dipeptide phases such as N-trifluoroacetyl-L-valyl-L-valine-cyclohexyl ester or N-trifluoroacetyl-L-phenylalanine-L-leucine-cyclolhexyl ester having upper temperature limit of 110°C and 140°C respectively were also prepared by Gil-Av and co-workers. Both the above phases have two asymmetrical centres as can be seen by the structure *a*.



(a)

The substitution of tripeptide derivatives for dipeptides did not improve temperature stability and enantioselectivity. The tetraamide stationary phases derived from L-phenyl-alanine were recently prepared by Palla *et al.*[55]. Some more diamides were prepared which had a higher upper temperature limit, example N-dodecanoyl-L-valine-tert-butylamide with T_{max} of 180°C. Its structure is as shown in *b*.



(b)

The diamide phase N-stearoyl-L-valine-tert-butylamide exhibits satisfactory enantioselectivity but has poor temperature stability. Commercially available phases are N-lauroyl-L-valine-tert-butylamide- supplied by Supelco and N-behenoyl-L-valine-tert-butylamide supplied by Serva.

The introduction of polymeric chiral phases has been a major breakthrough in terms of temperature stability and upper temperature operating limit. These phases are prepared in two ways.

(a) Frank *et al.* synthesized polysiloxanes with L-valine-tert-butylamide covalently bound in the side chain. The chiral moieties, which are very enantioselective, are separated from each other by seven dimethylsiloxanes units. This arrangement gives enantioselectivity and thermal stability to the polymer phase which is known commercially as Chirasil-Val.

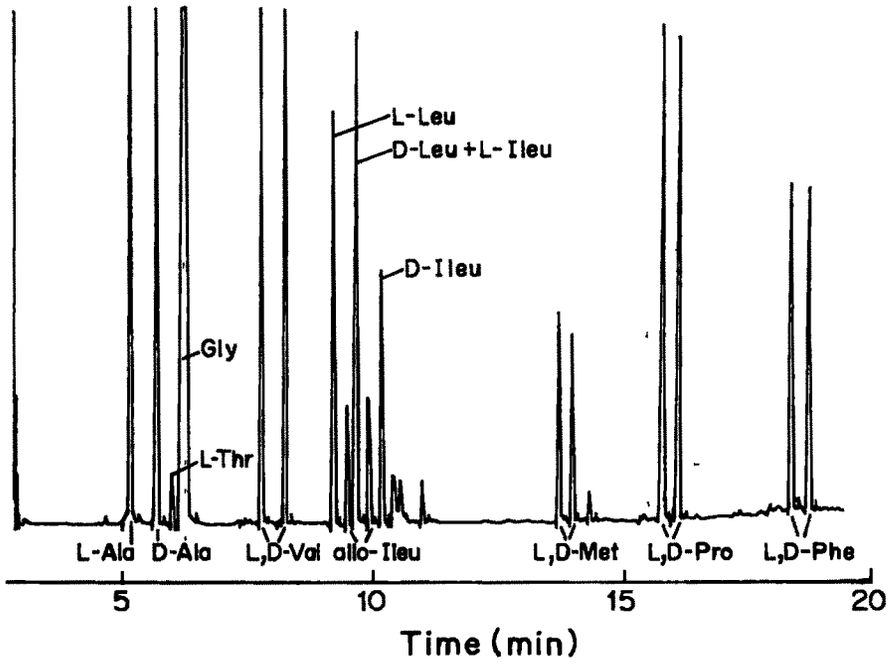


FIG. 1·4 : DIASTEREOISOMERS SEPARATION ON SE-54 GLASS CAPILLARY COLUMN (20m×0·25mm i.d., 0·32μm)

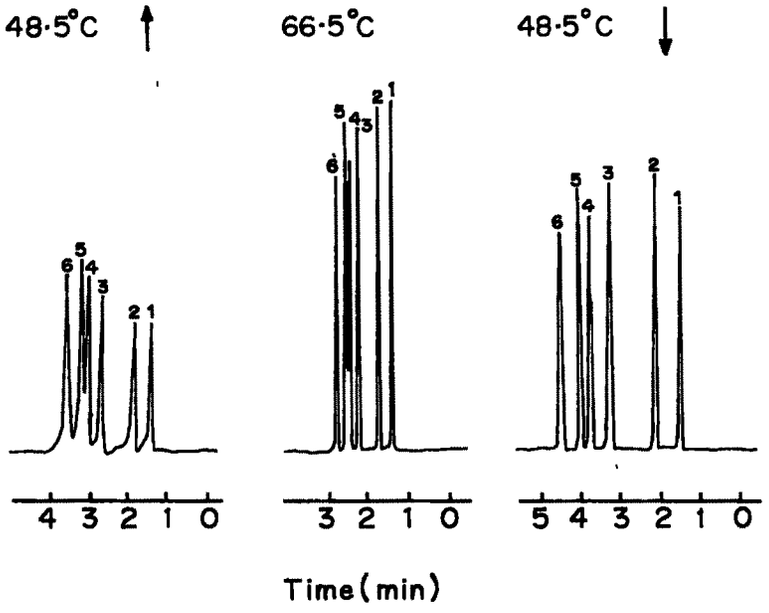


FIG. 1·5 .SEPARATION OF XYLENES ON LIQUID CRYSTALLINE COLUMN. CRYSTALLINE RANGE (48·5°C), NEMATIC RANGE (66·5°C) AND SUPERCOOLED RANGE (48·5°C). PEAKS. 1) BENZENE ; 2) TOLUENE ; 3) ETHYLBENZENE ; 4) m-XYLENE ; 5) p-XYLENE ; 6) o-XYLENE

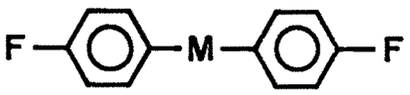
This phase is stable at 220°C and can separate high and low - volatile amino acid derivatives in a single chromatographic run. The polarity of Chirasil-Val is relatively low. It is comparable to the polarity of Dexsil 400. The phase shows high value for n-butanol (($\Delta I = 264$) which indicates formation of hydrogen bonds.

(b) The second method modifies the commercially available cyanopolysiloxanes e.g. OV-225, XE-60 or Silar 10C by hydrolyzing the cyanoalkyl side chains and coupling L-valine-tert-butylamide via an amide linkage. The chiral moiety L-valine-(S)-α-phenylethyl - amide coupled to the hydrolyzed phase shows very good enantioselectivity. Pioneering work in this field was carried out by Verzele[56] and Konig[48].

This stationary phase is useful for separation of amino alcohols, hydroxy acids, halogenocarboxylic acids, trifluoroacylated carbohydrates, sulfur-containing compounds, ketones etc.[57-61]. Several attempts have been made to explain the separation mechanism. Separation depends on the formation of diastereomeric complexes between chiral phase and chiral analyte termed as selectand-selector interactions e.g. hydrogen bonding are mainly responsible for the resolution of amino acids via their N-acyl esters. The separation takes place when the complexes are formed rapidly and reversibly. By using Chirasil-L-Val the L enantiomer of amino acid derivatives is eluted last owing to its more stable complex with the stationary phase and vice-versa when using Chirasil-D-Val column. Some enantioselective phases containing β-ketoenolates have been introduced by Schurig *et al.*[62]. They have been used for separation of underivatized oxygen containing insect pheromones. Cyclodextrins are also used for separation of optical isomeric compounds.[63]

Liquid Crystals:

The term liquid crystal was first suggested by Lehmann[64] for those substances, which, within definite temperature intervals, are liquid in mobility and crystalline in optical properties. These substances do not pass directly from the solid to normal or isotropic liquid phase but go through one or more discreet phase transformations involving liquid crystal intermediate phases (mesophases). There are two main types of mesophases the smectic and the nematic. The smectic phases are more effective for GC. These phases are characterized by a parallel orientation of molecules in layers, whereas the nematic phases are less ordered and the molecules are free to move within the limits of parallel configurations; layered structures are not observed. The first investigation in which a liquid crystal phase was used was described by Kelker[65]. The compound studied as stationary phase was p,p′-azoxyphenetole, which has a nematic phase between 138-168°C. This phase could separate *m*- and *p*-xylenes. The chemical structure of liquid crystals is commonly as follows:



F =	M =
$C_n H_{2n+1}$	- N = N -
$C_n H_{2n+1}$ O	- CH = N -
$C_n H_{2n+1}$ OCO	- C ≡ C -
$C_n H_{2n+1}$ OCOO	- CO - O -
	- N = N - ↓ O

Several kinds of interactions between the stationary phase and the solute such as dispersive, orientation, and inductive forces contribute to retention. But the main criteria for separation on these types of phases is the high degree of compatibility of the dimension of the solute molecules and the structure of the stationary phase itself. Thus geometrical isomers will elute according to their length-to-breadth ratio and planarity of the solute molecules. Rod-like molecules enter the orientated phase more readily than broad ones e.g. the *p*-isomer is more elongated than *m*-isomer and fits between the layers of the mesophase more readily. Hence, the *p*-isomer is eluted later.

Liquid crystals, in their supercooled temperature range show very good separation. Kraus[66] compared the selectivity of the crystalline, nematic and supercooled ranges with respect to the separation of isomeric xylenes. **FIG. 1.5** shows the superior separation of the xylene in the supercooled state which is a result of a higher degree of order, while the separation at the same temperature in the solid state is because of the parallel orientation of the surface molecules. Recent progress in polymer chemistry has shown that polysiloxanes with liquid crystalline properties in the side chains can be prepared.[67] The mesomorphic side chains can have nematic or smectic features. These Mepsil phases (mesomorphic polysiloxanes) have good column efficiencies and temperature stabilities upto 300°C. They are suitable for preparing capillary columns due to their sufficient film-forming capabilities.

Molten Salts as Stationary Phases:

Among the ionic liquids there are three types: inorganic molten salts, inorganic hydrated melts, and organic molten salts. GC columns using molten inorganic salts as stationary liquid phase apparently behave in the same manner as columns with conventional organic liquid phases. Metal halides may be separated by partition gas chromatography using inorganic molten salts as stationary

phases. A mixture of tin (IV) chloride, bromide and iodide at 150°C was separated using aluminium bromide as liquid phase and the chlorides of iron (III) and mercury (II) were separated on a column containing Bi(III) chloride at 290°C.[68]

The hydrated molten salts such as a eutectic mixture of anhydrous bismuth (III) chloride and lead (II) chloride (89 mole % $BiCl_3$) separated a mixture of titanium (IV) chloride saturated at room temperature with antimony (III) chloride.[69] On the $InCl_3$-TlCl eutectic mixture, when a mixture of $TaCl_5$-$NbCl_5$ was injected at 329°C, $TaCl_5$ was selectively retained by the liquid phase; apparently a thallium tantalum chloro-complex is formed that is more stable than the corresponding indium complex. The use of inorganic molten salts raises some instrumental problems resulting from high temperature and corrosive influence of the molten salts. The last type is only of interest because the organic salts are capable of sufficiently dissolving organic molecules. Typical members of liquid organic molten salts phases used in GC are quaternary ammonium salts with various anions such as tetra-n-butyl ammonium picrate, tetra-n-butylammonium tetrafluroborate or tetra-n-hexylammonium benzoate etc. The interactions between the stationary phase and the solute are strong orientation, moderate to strong proton acceptor forces and weak dispersive interactions.

Gas chromatography is basically and primarily a separation process and the number of possible combinations of solutes to be separated is infinite. As a rule of thumb nearly 75% can be analysed on nonpolar columns of high thermal stability. To distinguish finer structural features of solute molecules some polar phases are essential. Special separation problems call for speciality phases such as liquid crystals, optically active phases etc.

Some novel semi-polar to polar stationary phases have been explored and characterized in Chapter 2.

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CHAPTER - 2

N-ALKYL DERIVATIVES OF 4-AMINO-3-NITROBENZOPHENONE AND 2-AMINO-5-NITROBENZOPHENONE AS STATIONARY PHASES IN GAS-LIQUID CHROMATOGRAPHY

CHAPTER - 2

N-ALKYL DERIVATIVES OF 4-AMINO-3-NITROBENZOPHENONE AND 2-AMINO-5-NITROBENZOPHENONE AS STATIONARY PHASES IN GAS-LIQUID CHROMATOGRAPHY

2.1 Evaluation of N-alkyl derivatives of 4-amino-3-nitrobenzophenone and 2-amino-5-nitrobenzophenone as stationary phases.

2.1.1 Introduction

Mebendazole a valuable anthelmintic drug is obtained from 3,4-diaminobenzophenone, which in turn involves the reduction of 4-amino-3-nitrobenzophenone. The conventional route for nitrazepam, a popular tranquiliser, employs 2-amino-5-nitrobenzophenone as a starting material. A new and convenient synthesis of 4-amino-3-nitrobenzophenone, 2-amino-5-nitrobenzophenone and their N-alkyl derivatives was carried out in our laboratory. All the compounds synthesised were characterised by spectral and elemental analysis and has been published[1]. We explored the possibility of using some of the N-alkyl derivatives as stationary phases in gas liquid chromatography with respect to their selectivities and evaluation of their McReynolds constants[2].

The following N-alkyl derivatives of aminonitrobenzophenones were studied:

1. 4-Ethylamino-3-nitrobenzophenone (4-ETHA-3-NB)
2. 4-Isopropylamino-3-nitrobenzophenone (4-IPA-3-NB)
3. 4-Butylamino-3-nitrobenzophenone (4-BUTA-3-NB)
4. 4-Dimethylamino-3-nitrobenzophenone (4-DMA-3-NB)

5. 4-Cyclohexylamino-3-nitrobenzophenone (4-CYHA-3-NB)

6. 2-Cyclohexylamino-5-nitrobenzophenone (2-CYHA-5-NB)

2.1.2 General Synthesis

Preparation of 4-amino-3-nitrobenzophenone and its N-alkyl Derivatives

A mixture of 4-methoxy-3-nitrobenzophenone (25.7g, 0.1 mole) and excess of either aqueous ammonia (25%) or alkylamine (0.6 mole) was heated in a closed pressure vessel at 120°C for 5 hours. The reaction mixture was cooled to 30°C and diluted with water (500 ml). The product which separated out was filtered and recrystallised to give yellow needles.

Preparation of 2-Amino-5-nitrobenzophenone and its N-alkyl Derivatives

Procedure was same as above only 2-methoxy-5-nitrobenzophenone was used.

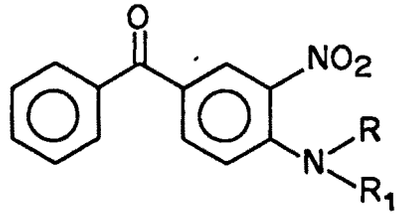
2.1.3 Experimental Methods

The structures, physical properties and the maximum operating temperatures of the aminonitrobenzophenone derivatives used as stationary phases are given in **TABLE 2.1.** The derivatives were recrystallised, their melting points checked and their purities were ascertained by GC. Thermogravimetric analysis was recorded on STA 409 apparatus from Netzsch Geratbau, Selb, F.R.G. and from these thermographs maximum operating temperatures (MAOT) were decided. The aminonitrobenzophenone derivatives were used as substrates at concentrations of 5 and 10% (w/w). Solvent used for dispersing the stationary phases on the solid support was dichloromethane. The solid support was chromosorb W AW DMCS (80-100 mesh) which has a temperature limit of 375°C and can accept loadings from 1-15% .

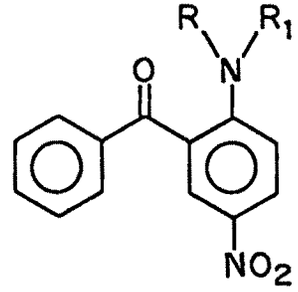
TABLE 2.1

AMINONITROBENZOPHENONE DERIVATIVES USED AS STATIONARY PHASES. THEIR MELTING POINTS, DECOMPOSITON TEMPERATURES AND MAXIMUM OPERATING TEMPERATURES (MAOT)

General formula



4-amino-3-nitrobenzophenone



2-amino-5-nitrobenzophenone

Column Number	Aminonitrobezophenone derivatives	R	R_1	Melting point°C	Decompositon Temp. °C *	MAOT °C
I	4-Ethylamino-3-nitrobenzophenone (4-ETHA-3-NB)	H	CH_2-CH_3	99	192.7	180
II	4-Isopropylamino-3-nitrobenzophenone (4-IPA-3-NB)	H	CH-$(CH_3)_2$	88	202.3	180

III	4-n-Butylamino-3-nitrobenzophenone (4-BUTA-3-NB)	H	$(CH_2)_3$-CH_3	65	223.5	190
IV	4-Dimethylamino-3-nitrobenzophenone (4-DMA-3-NB)	CH_3	CH_3	116	248.5	180
V	4-Cyclohexylamino-3-nitrobenzophenone (4-CYHA-3-NB)	H	c - C_6H_{11}	170	257	240
VI	2-Cyclohexylamino-5-nitrobenzophenone (2-CYHA-5-NB)	H	c - C_6H_{11}	95	209	200

* Determined by thermogravimetry

Preparation of Packed Columns[3]

Appropriate weight of the liquid phase was taken and dissolved in solvent dichloromethane. To this solution was added aliquots of the previously weighed solid support. Some more solvent was added to submerge the solid support. After keeping for sufficient time the excess of solvent was removed on rotary evaporator. Rapid rotation of the flask or violent bumping of the damp solid was avoided. After coating, the support was oven dried. Thus, to prepare 10g of a 10% w/w phase loaded column, 9 g of solid support and 1 g of liquid phase should be used.

The dried and coated support was then packed into stainless steel columns of 3 mm outer diameter and 1.8 meter length. One end of the column was plugged with glass wool while the other end was attached to a funnel. Small aliquots of the packing material was added accompanied by gentle tapping of the column sides with the aid of an electric vibrator. When sufficient packing was added the funnel was removed and the end was plugged with glass wool.

Conditioning of the Columns

The packed columns were conditioned before use. During conditioning the column was disconnected from the detector end to avoid contamination of the detector. Each column was conditioned in incremental steps as shown below. During conditioning there was a constant flow of nitrogen.

$$50°C\ (5\ min) \xrightarrow{4°min^{-1}} 100°C\ (2\ hr) \xrightarrow{4°min^{-1}} 150°C\ (4\ hr) \xrightarrow{4°min^{-1}} MAOT$$

Col. I	4 - ETHA-3-NB	180°C (12 hr)
Col. II	4 - IPA-3-NB	180°C (16 hr)
Col. III	4 - BUTA-3-NB	190°C (16 hr)

Col. IV	4 - DMA-3-NB	180°C (16 hr)
Col. V	4 - CYHA-3-NB	230°C (12 hr)
Col. VI	2 - CYHA-5-NB	200°C (18 hr)

Apparatus and Chemicals:

A Hewlett Packard Model 5880 A Gas Chromatograph equipped with a level 4 integrator and computing system with flame ionization detector was used. The selectivities of the N-alkyl derivatives of aminonitrobenzophenone were characterized by injecting various aromatic positional isomers. **TABLE 2.2** give the m.p.'s and b.p.'s of these isomers. The individual compounds which were used as standard samples were 97-99% pure. The details of the GC conditions are given with the **FIGS. (2.2 - 2.12).**

The McReynolds solute probes used were benzene, n-butanol, 2-pentanone, nitropropane and pyridine at 120°C, and 1-butylbenzene, benzyl alcohol, acetophenone, nitrobenzene and aniline at 180°C. The reference aliphatic alkanes were taken from a standard hydrocarbon kit (Analabs). The column dead time was determined by a graphical method[4].

2.1.4 Results and Discussion

All the columns except Col. V were checked for general polarity and selectivity by the method of McReynolds at 120°C. Column V was checked for polarity and selectivity at 180°C. Graphs of the logarithm of corrected retention time *versus* carbon number for n-alkanes plotted for the five phases were found to be linear **(FIG. 2.1).** The McReynolds constants (ΔI), retention indices (I), capacity factors (k'), dead time values (t_m) and average polarities of the various aminonitrobenzophenone derivatives are given in **TABLE 2.3.** The absolute values of the retention indices on squalane and other standard stationary phases were taken from the original reference[2].

TABLE 2.2

POSITIONAL ISOMERS STUDIED

	Name of the compounds	Melting point	Boiling point
1.	o-Dichlorobenzene	-18 to -15°C	179-80°C
2.	m-Dichlorobenzene	-24°C	172-173°C
3.	p-Dichlorobenzene	54-56°C	173°C
4.	o-Xylene	-25 to -23°C	143-145°C
5.	m-Xylene	-	138-139°C
6.	p-Xylene	12-13°C	138°C
7.	1-Picoline	-70°C	128-129°C
8.	2-Picoline	-	144°C
9.	3-Picoline	-	145°C
10.	1-Chloro-2-nitrobenzene	33-36°C	246°C
11.	1-Chloro-3-nitrobenzene	42-44°C	236°C
12.	1-Chloro-4-nitrobenzene	83-84°C	242°C
13.	o-Toluidine	-28°C	200°C
14.	m-Toluidine	-	203-204°C
15.	p-Toluidine	41-46°C	200°C
16.	o-Nitrotoluene	-3°C	225°C
17.	m-Nitrotoluene	16°C	231°C
18.	p-Nitrotoluene	52-54°C	238°C
19.	o-Cresol	32-34°C	191°C
20.	m-Cresol	8-10°C	203°C
21.	p-Cresol	32-34°C	202°C
22.	o-Chloroaniline	-1 to 1°C	208-210°C
23.	m-Chloroaniline	-11 to -9°C	230°C
24.	p-Chloroaniline	68-71°C	232°C

25.	o-Anisidine	5-6°C	225°C
26.	m-Anisidine	-1 to 1°C	251°C
27.	p-Anisidine	57-60°C	240-243°C
28.	3,4-Dichloronitrobenzene	41-44°C	255-256°C
29.	2,3-Dichloronitrobenzene	61-62°C	257-258°C
30.	2,5-Dichloronitrobenzene	29-32°C	258°C
31.	2,6-Dimethyl phenol	46-48°C	203°C
32.	2,5-Dimethyl phenol	75-77°C	212°C
33.	2,4-Dimethyl phenol	22-23°C	211°C
34.	2,3-Dimethyl phenol	73-75°C	217°C
35.	3,5-Dimethyl phenol	65-66°C	222°C
36.	3,4-Dimethyl phenol	65-66°C	227°C
37.	2,6-Dichloro phenol	65-68°C	220°C
38.	2,5-Dichloro phenol	56-58°C	211°C
39.	2,4-Dichloro phenol	42-43°C	210°C
40.	2,3-Dichloro phenol	58-60°C	206°C
41.	o-Chlorophenol	8°C	175-176°C
42.	p-Chlorophenol	43-45°C	220°C
43.	Tetralin	-35°C	207°C
44.	Naphthalene	80-82°C	217°C
45.	1-Tetralone	5-6°C	250°C
46.	o-Aminophenol	174-177°C	-
47.	m-Aminophenol	124-126°C	250°C
48.	p-Aminophenol	188-190°C	284°C
49.	o-Nitroanisole	9.5°C	273°C
50.	m-Nitroanisole	36-38°C	260°C
51.	p-Nitroanisole	54°C	260°C
52.	1-Naphthol	95-96°C	278-280°C

53.	2-Naphthol	122-123°C	285-286°C
54.	o-Nitrophenol	44-45°C	214-216°C
55.	m-Nitrophenol	96-98°C	270°C
56.	p-Nitrophenol	113-115°C	279°C
57.	o-Nitroaniline	73-76°C	284°C
58.	m-Nitroaniline	112-114°C	306°C
59.	p-Nitroaniline	149-150°C	340°C
60.	o-Phenylenediamine	103-105°C	256-258°C
61.	m-Phenylenediamine	64-66°C	282-284°C
62.	p-Phenylenediamine	143-145°C	267°C

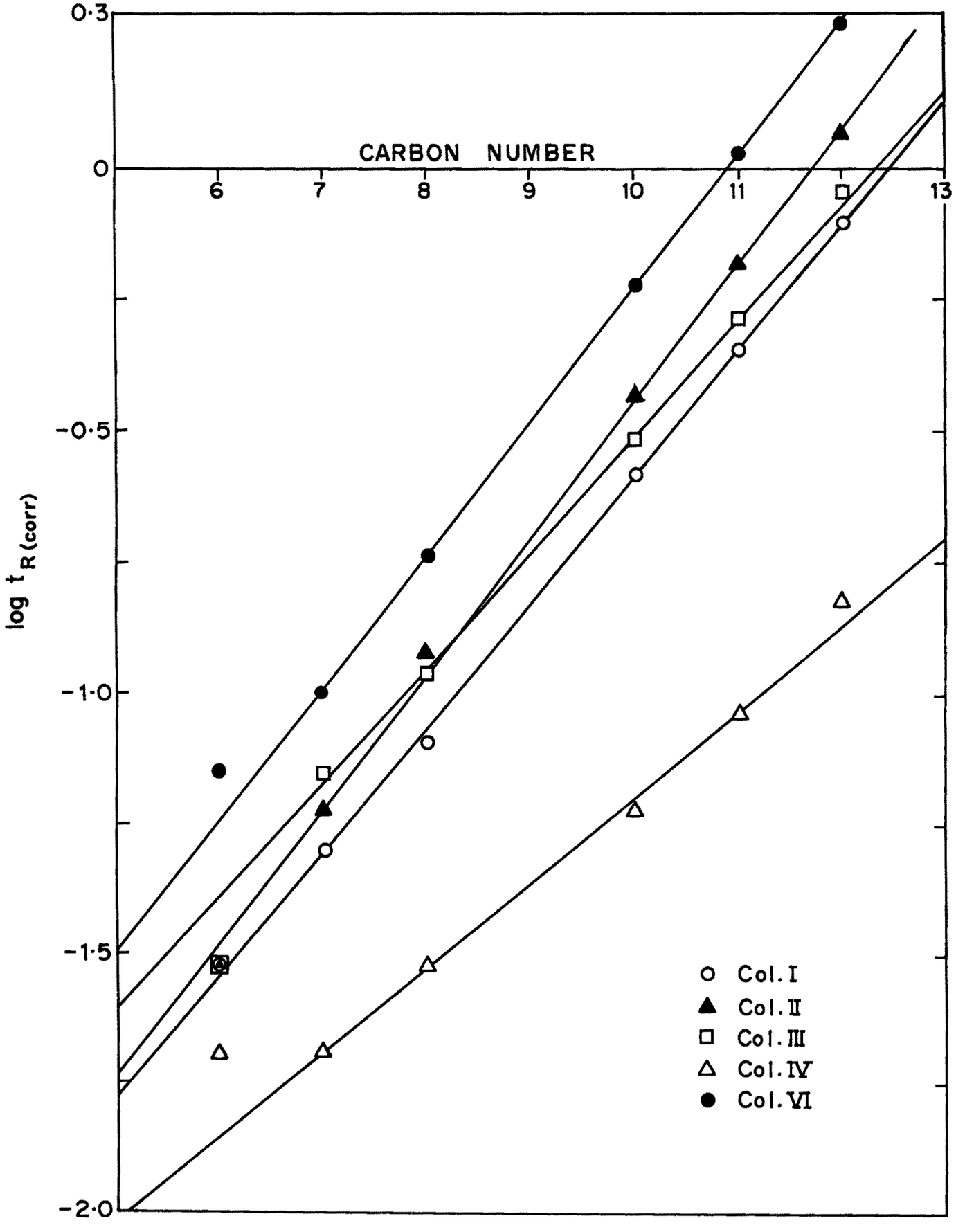


FIG. 2·1: PLOT OF log $t_{R(corr)}$ *versus* NUMBER OF CARBON ATOMS

All the aromatic compounds being studied as stationary phases have both π electrons of the ring and the substituent groups acting as donor or acceptor sites. The nitro and benzoyl groups act as electron acceptors while the N-alkyl group acts as the electron repelling group. Hence, the general characteristics of these compounds will be to selectively retain aromatic hydrocarbons over aliphatic hydrocarbons, aromatic alcohols and ketones will be better retained depending on their hydrogen bond formation and solubility in the phase. Nitro compounds should have strong retention on these phases because of donor-acceptor interactions.

TABLE 2.3 shows the average polarity listed in the descending order of polarity. The polarity of Col. IV is comparable to that of Carbowax 1540. Ketones (C) and aromatic bases (E) are better retained on Col. IV than on Carbowax 1540. Col. I exhibits a very high selectivity for ketones (C) as compared to the (C) value of Carbowax 20M. The values for (B) probe is quite comparable but (A), (D) and (E) values are higher for Carbowax 20M. This suggests that hydrogen bonding capacity is similar but interactions such as dispersion, dipole orientation and proton accepting capability are higher for Carbowax 20M. The average polarity of Col. II is very near the average polarity value of OV-225. The phase in Col. II has higher values for probes (A), (B) and (E) as compared to OV-225, suggesting better retention of hydrocarbons, alcohols and aromatic bases. Almost similar value for probe (C) suggests equivalent capacity to retain ketones. The phase OV-225 has higher (D) values indicating better retention of nitro compounds. Compared to Diethoxyethyl phtalate, Col. III has better retention for hydrocarbons, nitrocompounds and aromatic bases. The former retains alcohols better than Col. III while ketone retention is similar in both. The phase in Col. VI and the phase Ornite NIW differ only slightly for all other test probes except (A) and (B). Aromatics get retained better on Col. VI while alcohols are better retained on Orinite NIW.

TABLE 2.3

McREYNOLDS CONSTANTS (ΔI), CAPACITY FACTORS (k'), RETENTION INDICES (I), DEAD TIME VALUES (t_m) AND AVERAGE POLARITY FOR VARIOUS STATIONARY PHASES AT 120°C

Stationary phase (10%)	McReynolds probes																		
	Benzene (A)			1-Butanol (B)			2-Pentanone (C)			Nitropropane (D)			Pyridine (E)			t_m min.	Average polarity	b[a]	r[b]
	I	ΔI	k'	I	ΔI	k'	I	ΔI	k'	I	ΔI	k'	I	ΔI	k'				
4-DMA-3-NB (Col. IV)	1021	368	0.23	1235	645	0.69	1100	473	0.34	1299	647	0.96	1354	655	1.27	0.26	559	0.1657	1.581
Carbowax 1540		371			639			453			666			641			554	0.2137	1.635
4-ETHA-3-NB (Col. I)	927	274	0.74	1111	521	1.08	1111	521	1.08	1165	513	2.83	1163	464	2.78	0.23	459	0.2355	1.743
Carbowax 20-M		322			536			368			572			510			462	0.2235	1.673
4-IPA-3-NB (Col. II)	900	247	0.76	977	387	1.19	963	336	1.09	1107	455	2.52	1116	417	2.67	0.21	368	0.2264	1.742
OV-225		228			369			338			492			386			362	0.2275	1.688
4-BUTA-3-NB (Col. III)	873	220	0.69	931	341	0.96	937	310	1.00	1103	451	2.58	1086	387	2.35	0.26	342	0.2509	1.778
Diethoxyethyl phthalate		214			375			305			446			364			340	0.2504	1.780
2-CYHA-5-NB (Col. VI)	861	208	0.89	880	290	1.00	897	270	1.11	1041	389	2 64	1038	339	2.61	0.28	299	0.2571	1.813
Omite NIW		185			370			242			370			327			298	0.2576	1.809

a. The constant *b* is the slope of the curve obtained from the plot of log t_R versus carbon number.

b. The constant *r* is calculated from the square root of the ratio of corrected retention time for dodecane to corrected retention time for decane.

$\Delta I = I_{st.ph} - I_{squalane}$. Absolute values of the retention indices for squalane are benzene = 653; 1-butanol = 590; 2-pentanone = 627; 1-nitropropane = 652; pyridine = 699.

As can be seen from **TABLE 2.3** the polarity of Col. II, Col. III and Col. VI show them to be of intermediate polarity while Col. I and Col. IV are polar. As might be expected, the polarity of the phase declines as the (R_1) group increases in size. Therefore, we can say that polarity decreases in this sequence Col. IV > Col. I > Col. II > Col. III > Col VI. **TABLE 2.3** also gives *b* and *r* values for the new stationary phases. The constant *b* is the slope of curve obtained from the plot of log of corrected retention time versus the carbon number. The constant *r* is calculated from the square root of ratio of the corrected retention time for dodecane and the corrected retention time for decane. The *b* and *r* values for standard phases are taken from the original reference[2]. Improved separations are expected for any series of homologous compounds using the phase with the higher *r* value even though both the phases may have identical McReynolds constants. The phase in Col. I, has higher *b* and *r* values than Carbowax 20M, while the phases in Col. II, III and VI have nearly similar *b* and *r* values as compared to the respective standard phases. The *b* and *r* values for the phase in Col. IV are lower than Carbowax 1540 but they are closer to the values for Cyanoethyl Sucrose phase, ($b = 0.1653$, $r = 1.463$).

TABLES 2.4, 2.5 and **2.6** list the retention times of the different positional isomers studied on these new stationary phases, at 100°C, 150°C and 170°C respectively. The isomers injected at 100°C were dichlorobenzenes, xylenes and picolines. There was either no separation or partial separation between *meta* and *para* isomers of these low boiling compounds. **FIG. 2.2** shows the separation of nitrotoluenes on Col. I, Col. II, Col. III and Col. VI at 150°C. The order of elution was *ortho, meta* and *para*. The separation of five out of the six dimethylphenol isomers is shown in **FIG. 2.3.** Unfortunately none of the columns could separate 2,4- and 2,5-dimethyl-phenols. The other isomers injected at 150°C were dichlorophenols, chloroanilines, cresols, nitrochlorobenzenes, toluidines, anisidines and some polyaromatic hydrocarbons. The monochloronitrobenzenes elute as partially resolved peaks on all columns at 150°C. Their retention

TABLE 2.4

RETENTION TIMES (min) FOR POSITIONAL ISOMERS AT 100°C ON AMINONITROBENZOPHENONE PHASES

	Stationary Phase 10%			
Solute	**Col. I**	**Col. II**	**Col. III**	**Col. VI**
o-Xylene	1.88	2.05	1.43	2.55
m-Xylene	1.51	1.66	1.17	2.10
p-Xylene	1.53	1.68	1.21	2.13
1-Picoline	3.22	2.35	1.50	3.55
2-Picoline	3.28	2.49	1.53	3.56
3-Picoline	3.41	2.57	2.50	3.75
o-Dichlorobenzene	7.73	12.01	5.31	10.05
m-Dichlorobenzene	5.44	4.33	3.48	7.30
p-Dichlorobenzene	5.82	4.65	3.85	7.71

TABLE 2.5

RETENTION TIMES (min) FOR POSITIONAL ISOMERS AT 150°C ON AMINONITROBENZOPHENONE PHASES

Solute	Stationary Phase 10%			
	Col. I*	Col. II	Col. III	Col. VI*
1-Chloro-2-nitrobenzene	12.72	7.57	8.34	13.54
1-Chloro-3-nitrobenzene	9.95	5.90	6.71	10.61
1-Chloro-4-nitrobenzene	11.08	6.34	7.22	11.37
3,4-Dichloronitrobenzene	11.38	13.94	15.54	10.49
2,3-Dichloronitrobenzene	13.19	16.14	17.99	12.14
2,5-Dichloronitrobenzene	19.94	24.60	27.35	18.40
o-Toluidine	7.34	6.87	5.12	7.39
m-Toluidine	4.27	4.10	2.97	4.29
p-Toluidine	3.96	3.78	2.76	3.97
o-Nitrotoluene	5.82	5.69	4.13	6.25
m-Nitrotoluene	7.93	7.73	5.60	8.39
p-Nitrotoluene	9.44	9.21	6.60	9.81
o-Cresol	4.55	4.37	3.55	4.39
m-Cresol	6.08	5.87	4.85	5.58
p-Cresol	5.88	5.69	4.71	5.38
o-Chloroaniline	5.84	5.60	3.95	6.03
m-Chloroaniline	12.43	11.60	8.13	11.63
p-Chloroaniline	12.58	11.73	8.26	11.75

o-Anisidine	7.26	6.84	4.87	7.28
m-Anisidine	15.84	14.43	10.36	13.66
p-Anisidine	14.37	13.88	9.96	13.26
2,6-Dimethylphenol	4.45	4.52	3.30	4.74
2,5-Dimethylphenol	6.82	6.88	5.23	6.79
2,4-Dimethylphenol	6.70	6.72	5.20	6.71
2,3-Dimethylphenol	8.60	8.44	6.60	8.47
3,5-Dimethylphenol	9.55	9.28	7.39	8.88
3,4-Dimethylphenol	11.44	10.98	8.82	10.43
2,6-Dichlorophenol	9.04	8.63	7.05	9.60
2,5-Dichlorophenol	9.20	8.77	7.87	9.94
2,4-Dichlorophenol	9.16	8.72	7.69	9.89
2,3-Dichlorophenol	9.70	9.18	8.13	9.97
o-Chlorophenol	2.81	2.68	2.24	2.91
p-Chlorophenol	18.65	16.88	15.05	17.10
2,4-Dichlorophenol	9.16	8.72	7.69	9.89
Tetralin	2.23	1.15	1.76	3.31
Napthalene	4.83	2.02	3.47	5.97
1-Tetralone	19.99	7.48	14.01	21.91

* The dichloronitrobenzene isomers were injected on 5% loading on these two columns.

TABLE 2.6

RETENTION TIMES (min) FOR POSITIONAL ISOMERS AT 170°C ON AMINONITROBENZOPHENONE PHASES

	Stationary Phase 5%				
Solute	**Col. I**	**Col. II**	**Col. III**	**Col. V***	**Col. VI**
o-Aminophenol	5.86	2.34	-	1.67	4.28
m-Aminophenol	12.74	3.85	-	3.46	8.23
p-Aminophenol	10.05	3.01	-	2.74	6.35
1-Naphthol	16.39	16.03	12.95	8.60	18.92
2-Naphthol	19.62	18.89	14.95	9.71	21.96
o-Nitroanisole	6.23	3.88	2.94	0.83	5.95
m-Nitroanisole	4.30	2.60	2.10	0.65	4.25
p-Nitroanisole	7.75	4.65	3.63	0.94	7.38
o-Nitrophenol	1.33	0.56	0.90	0.51	1.61
m-Nitrophenol	-	7.39	-	13.02	-
p-Nitrophenol	-	12.80	-	29.22	-
o-Nitroaniline	11.03	2.98	-	2.78	11.22
m-Nitroaniline	-	5.73	-	5.38	-
p-Nitroaniline	-	16.36	-	16.85	-

* Nitrophenols and nitroanilines were injected at 180°on Col. V.

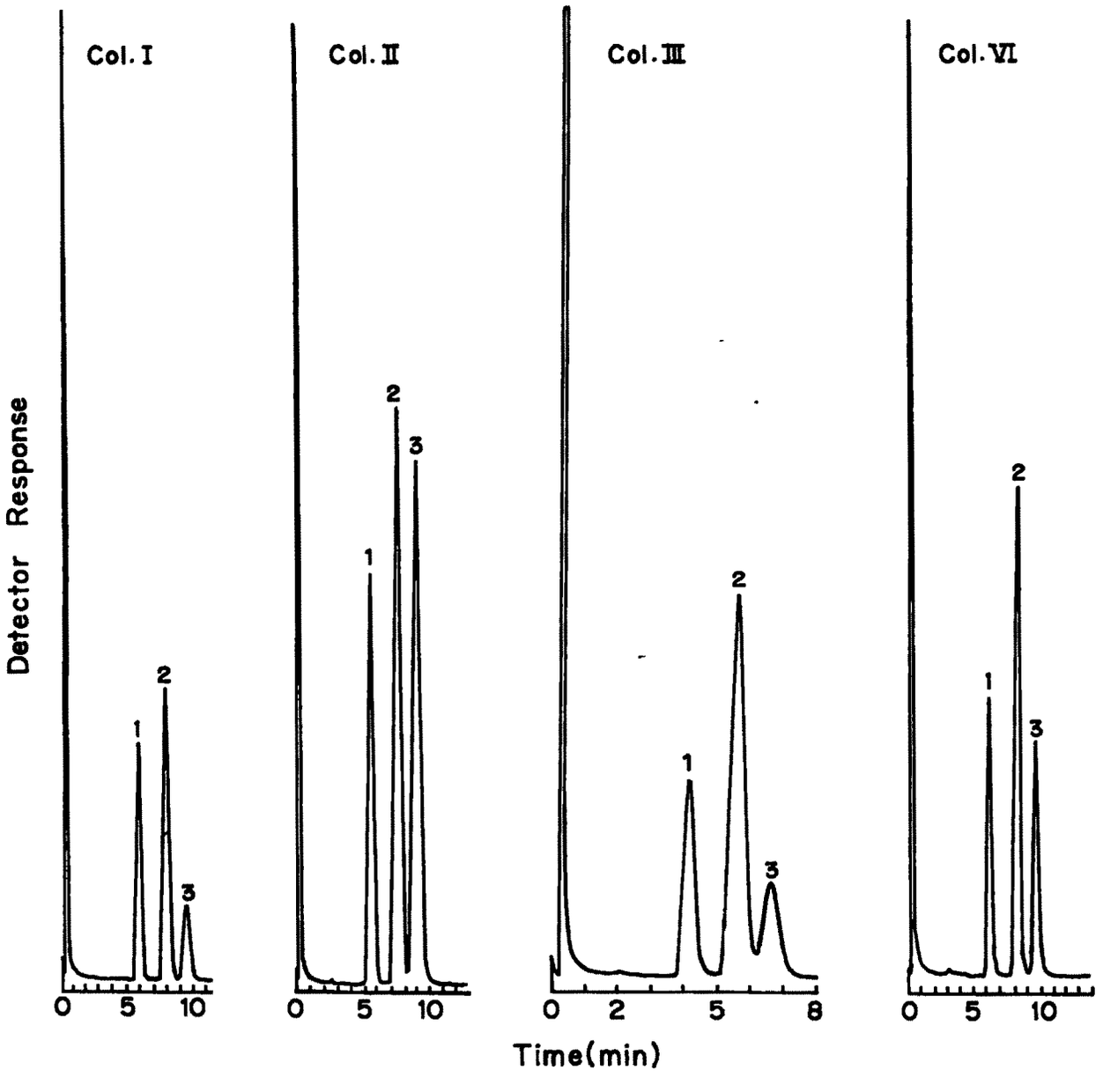


FIG.2·2 : SEPARATION OF NITROTOLUENE ISOMERS AT 150°C ON 10% Col. I , Col.II , Col·III AND Col.VI. NITROGEN FLOW RATE : 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C.
PEAKS : 1) o-NITROTOLUENE; 2) m-NITROTOLUENE; 3) p-NITROTOLUENE

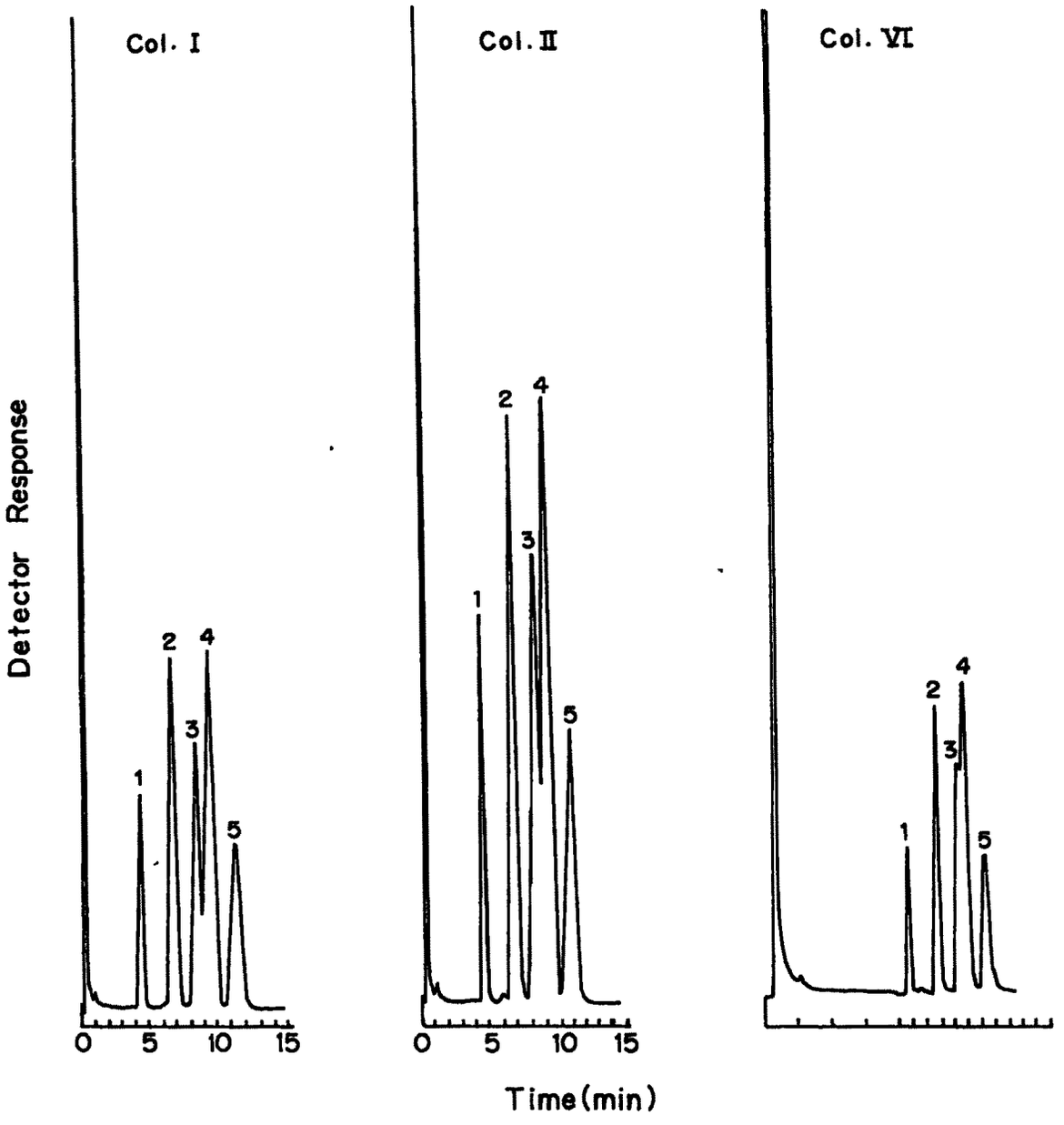


FIG. 2·3 : SEPARATION OF DIMETHYLPHENOL ISOMERS AT 150°C ON 10% Col. I, Col. II AND Col. VI. NITROGEN FLOW RATE : 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C.
PEAKS : 1) 2,6 DMP; 2) 2,4 + 2,5 DMP ; 3) 2,3 DMP; 4) 3,5 DMP; 6) 3,4 DMP.

times are given in **TABLE 2.5.** On the other hand the dichloronitrobenzenes such as 3,4-, 2,3-, and 2,5- separate without any difficulty on all columns. Best separation is shown in **FIG. 2.4.** The 3,4-Dichloronitrobenzene isomer is used as starting material for production of a number of valuable herbicides (propanil, linuron, diuron etc.)[6]. **FIG. 2.5** shows the separation between Napthalene, Tetralin and 1-Tetralone at 150°C. Baseline separation of chlorinated phenols such as *ortho* chlorophenol, *para* chlorophenol and 2,4-dichlorophenol can be seen in **FIG. 2.6.** These phenols are industrially important as insecticides, fungicides and herbicides[7].

Some high boiling nitro compounds were injected on columns I, II, III and VI at 170°C. A clear separation of nitroanisole isomers can be seen in **FIG. 2.7.** The order of elution was *meta, ortho, para.* Even though *meta* and *para* isomers have identical boiling points (260°C) they separate from each other because of their difference in molar volume. Two substances with identical boiling points differ in their "polarity" if their molar volumes are different[5]. Nitroanilines and nitrophenols were very well separated on 5% loading of Col. II. Except for the *ortho-* isomer of nitroanilines and nitrophenols none of the other isomers eluted on Cols. I and III indicating a high degree of solubility and hydrogen bonding. Order of elution for both sets of nitro compounds on Col. II was *ortho-, meta-, para-,* as can be seen in **FIGS. 2.8** and **2.9.** A baseline separation was obtained on Col.I 5% (See **FIG. 2.10**) for underivatised aminophenols. Aminophenols could also be separated on 5% Col. VI and II but the separation between *para* and *meta* was not baseline. The aminophenols were totally adsorbed on Col. III and did not show elution of any isomer. The order of elution on the other columns was *ortho-, para-,* and *meta-.* Retention of the *meta-* isomer suggests that it must be strongly hydrogen bonding with the substrate than the *para-* isomer. The columns I, II and VI show very good separation of 1- and 2-naphthols and can be seen in **FIG. 2.11.** The order of elution of the naphthol isomers on these columns is Col. II < Col. I < Col. VI.

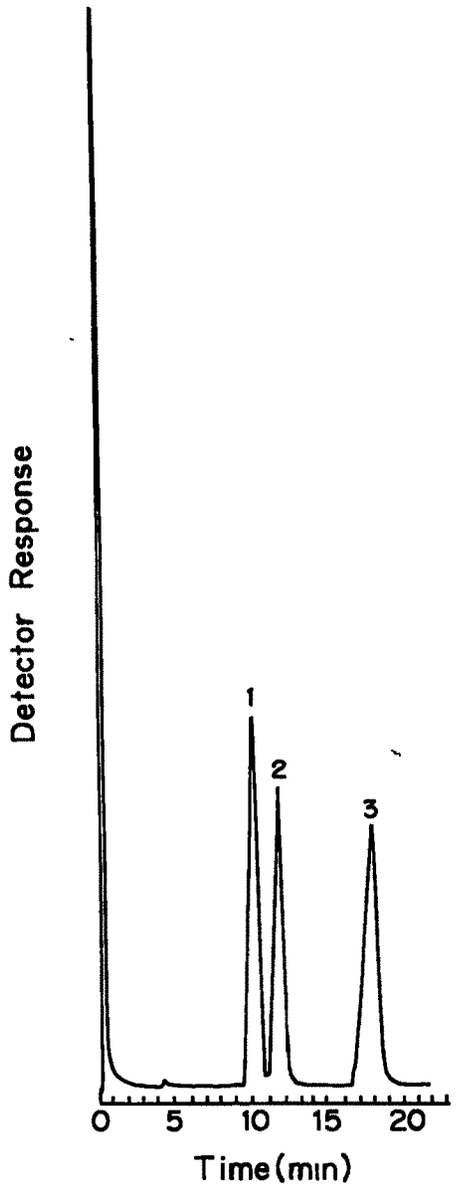


FIG.2·4 : SEPARATION OF DICHLORONITROBENZENE ISOMERS AT 150°C ON 5% Col. VI. NITROGEN FLOW RATE: 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C. PEAKS: 1) 3,4 DCNB; 2) 2,3 DCNB; 3) 2,5 DCNB.

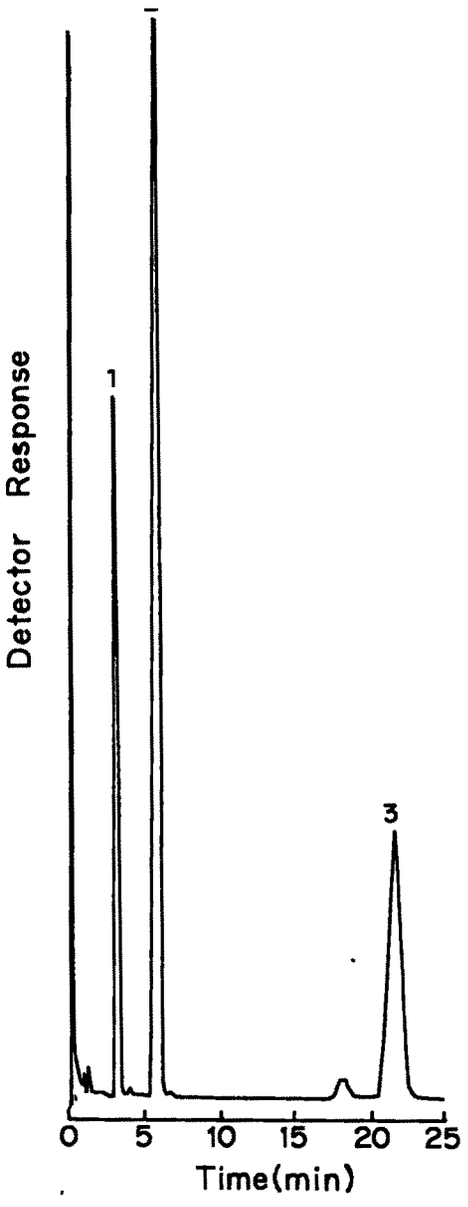


FIG.2·5 : SEPARATION OF SOME POLYAROMATIC HYDROCARBONS ON 10 % Col. VI AT 150°C. NITROGEN FLOW RATE· 30 ml min^{-1} INJECTION TEMP. 200° C, DETECTOR TEMP. 250°C PEAKS: 1) TETRALIN , 2) NAPHTHALENE , 3) 1-TETRALONE

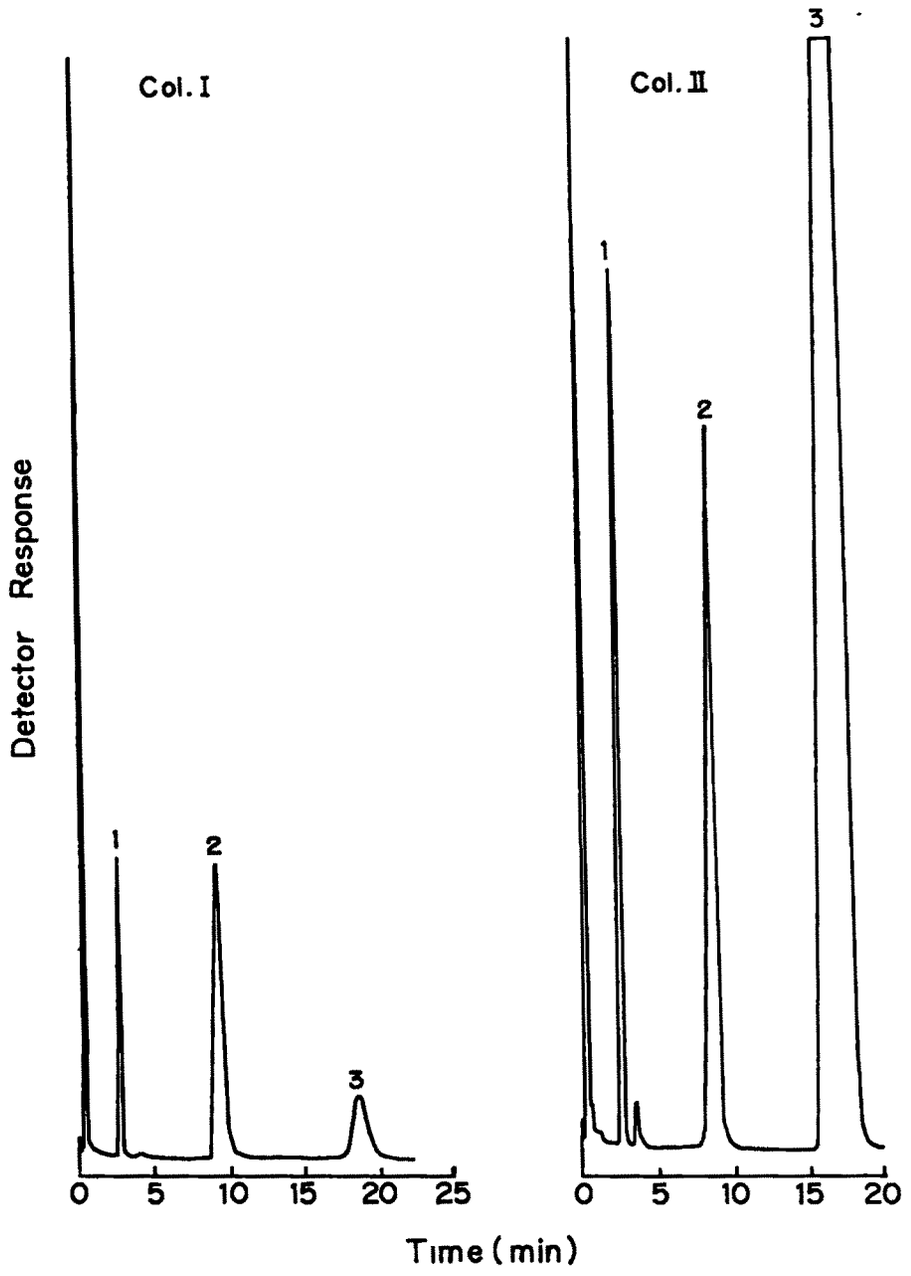


FIG.2·6 : SEPARATION OF CHLOROPHENOL ISOMERS ON 10% Col.I AND Col. II AT 150°C. NITROGEN FLOW RATE : 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C. PEAKS : 1) o-CP ; 2) 2,4-DCP ; 3) p-CP

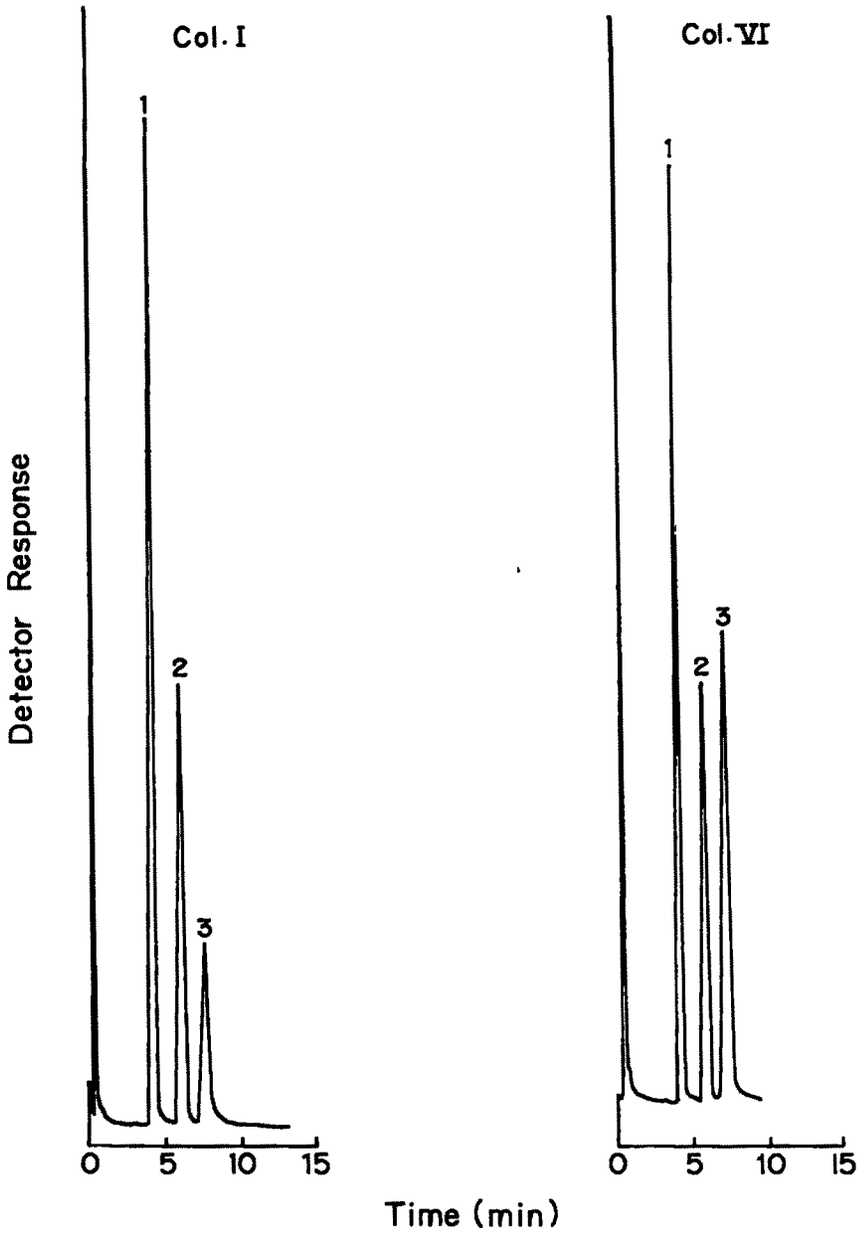


FIG.2·7 : SEPARATION OF NITROANISOLES AT 170°C ON 5% Col.I AND Col.VI . NITROGEN FLOW RATE : 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C . PEAKS . 1) m-NITROANISOLE ; 2) o-NITROANISOLE ; 3) p-NITROANISOLE

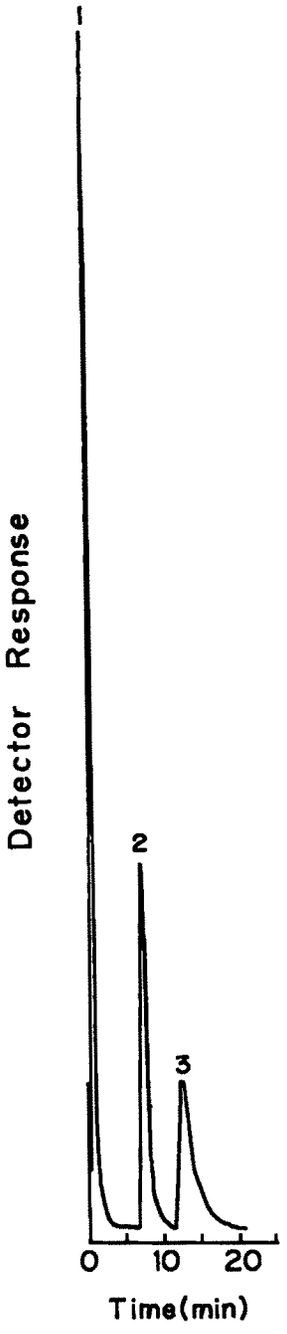


FIG.2·8

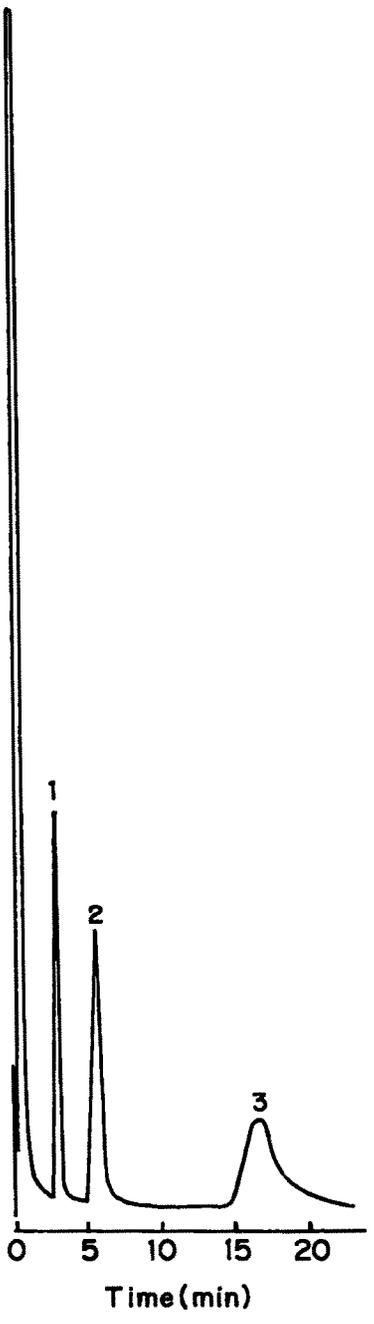


FIG.2·9

FIG.2·8: SEPARATION OF NITROPHENOL ISOMERS.
PEAKS: 1) o-NITROPHENOL; 2) m-NITROPHENOL; 3) p-NITROPHENOL

FIG.2·9: SEPARATION OF NITROANILINE ISOMERS.
PEAKS. 1) o-NITROANILINE; 2) m-NITROANILINE; 3) p-NITROANILINE

Col. II 5% AT 170°C. NITROGEN FLOW RATE : 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C.

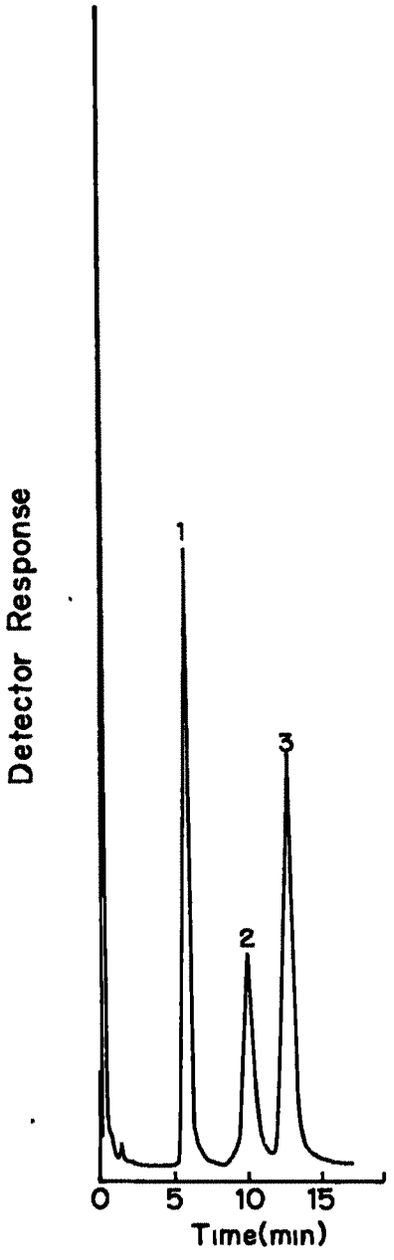


FIG.2·10: SEPARATION OF AMINOPHENOL ISOMERS ON 5% Col. I AT 170°C. NITROGEN FLOW RATE: 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C.
PEAKS: 1) o-AMINOPHENOL; 2) p-AMINOPHENOL; 3) m-AMINOPHENOL

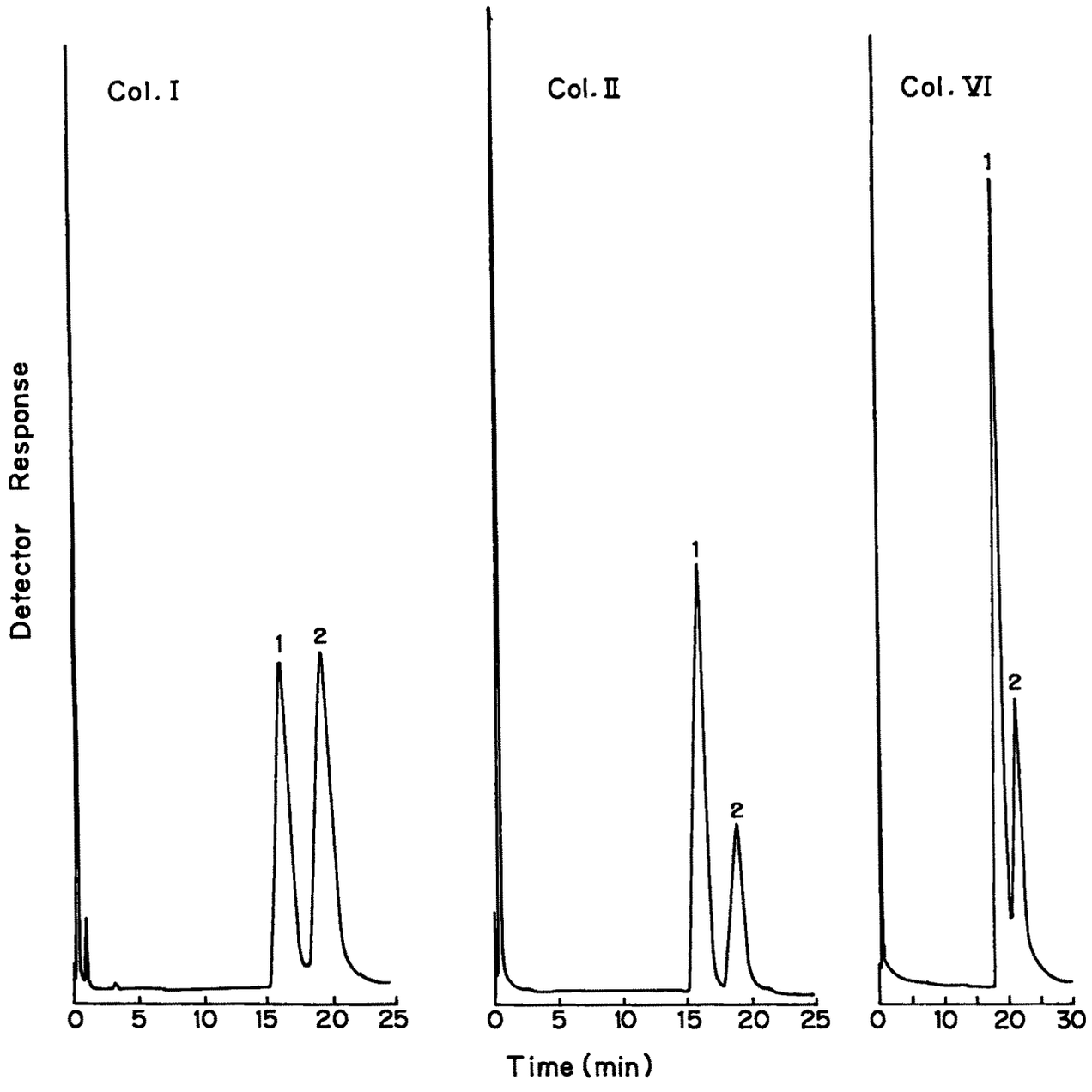


FIG. 2·11: SEPARATION OF NAPHTHOLS ON 5% Col. I, Col. II AND Col. VI AT 170°C. NITROGEN FLOW RATE: 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C.
PEAKS: 1) 1-NAPHTHOL, 2) 2-NAPHTHOL.

Even though TGA showed decomposition temperature of the phase in Col. IV to be 248.5°C, the column was thermally unstable above 150°C. Solutes injected on this column showed broad peaks with post tailing. The column was found chromatographically unfit for any other study except for McReynolds polarity determination at 120°C. Hence, the most polar, thermally stable and showing highest selectivity in the series was Col. I.

2.2 Comparision of the Chromatographic Properties of 4-Cyclohexylamino-3-nitrobenzophenone and 2-Cyclohexylamino- 5- nitrobenzophenone

2.2.1 Polarity determination

The McReynolds constants were determined at 180°C for Col. V and Col. VI. For comparision a non polar phase SE-30 and a polar phase Carbowax-20M were also evaluated at 180°C. **TABLE 2.7** gives the retention indices (I), McReynolds constants (ΔI), dead time (t_m), capacity factor (k') and average polarity for these phases. The retention indices on Apiezon MH at 180°C were taken from the original reference[8].

As can be seen from **TABLE 2.7** Col.V has very good retention for nitro compounds, compounds containing hydroxyl groups, aromatic bases and ketones. Col. VI shows better retention of ketones at 180°C than at 120°C as seen by the (C) value. Dispersive forces are diminished at 180°C for Col. VI as seen by the low retention index value for probe (A). Average polarity values show Col. V to be a very polar column even more polar than Carbowax 20M while Col. VI is of moderate polarity.

Since Col. V is a solid below 170°C all the solutes were injected on this column at or above 170°C. Selectivities of Col. V and Col. VI were compared to each other. The two phases differ from each other only in the position of their functional groups as can be seen in **TABLE 2.1.** Nitro compounds such as nitroanisoles, nitrophenols and nitroanilines were studied on both these

TABLE 2.7

McREYNOLDS CONSTANTS (ΔI), RETENTION INDICES (I), CAPACITY FACTORS (k'), DEAD TIME VALUES (t_m) AND AVERAGE POLARITY OF COL. V, COL. VI, CARBOWAX-20M AND SE-30 AT 180°C

Stationary phase (10%)	Probes																
	n-Butyl Benzene (A)			Benzylalcohol (B)			Acetophenone (C)			Nitrobenzene (D)			Aniline (E)			t_m min.	Average polarity
	I	ΔI	k'	I	ΔI	k'	I	ΔI	k'	I	ΔI	k'	I	ΔI	k'		
4-CYHA-3-NB (Col. V)	1679	596	1.15	2193	1161	4.19	2163	1110	3.88	2300	1195	5 50	2069	1083	4.07	0.26	1029
2-CYHA-5-NB (Col VI)	1238	155	1.39	1443	411	3.51	1464	411·	3.76	1526	421	5.12	1403	417	2.76	0 33	363
Carbowax 20M	1417	334		2100	1068		1865	812		1988	883		1953	967		0.28	813
SE-30	1133	50		1146	114		1165	112		1187	82		1115	129		0.14	97

$\Delta I = I_{st.\,ph.} - I_{Apiezon\,MH\cdot}$

Absolute retention indices for Apiezon MH at 180°C are: n-butyl benzene = 1083; benzyl alcohol = 1032; acetophenone = 1053; nitrobenzene = 1105; and aniline = 986.

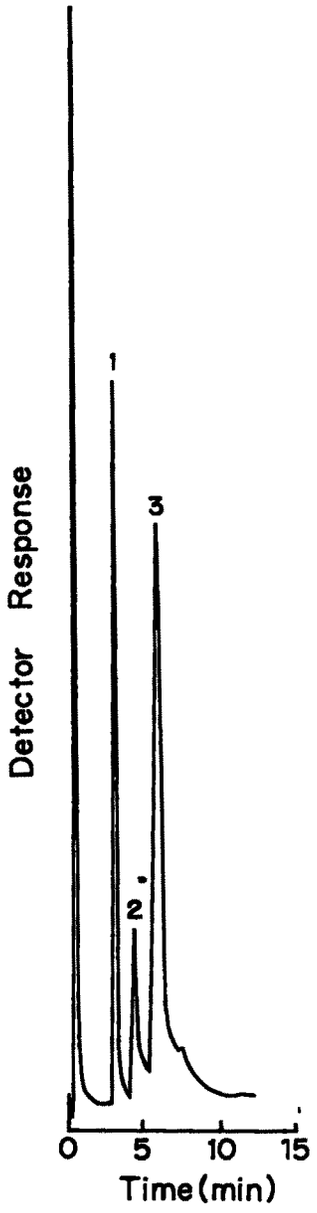


FIG.2·12: SEPARATION OF PHENYLENEDIAMINES AT 170°C ON 5% Col. VI. NITROGEN FLOW RATE: 30 ml min^{-1}, INJECTION TEMP. 200°C, DETECTOR TEMP. 250°C.
PEAKS: 1) o-PHENYLENEDIAMINE ; 2) p-PHENYLENEDIAMINE; 3) m-PHENYLENEDIAMINE.

columns. Nitroanisoles showed good retention and separation on Col. VI (*ca* **FIG. 2.7**) but they eluted very fast and without any separation on Col. V at 170°C. Nitrophenols and nitroanilines separated on 5% Col. V at a moderately reasonable time at 180°C but except for the *ortho* isomer of nitrophenols and nitroanilines there was no elution of these compounds on 5% Col. VI at 170°C. Probably a lower loading would elute these nitro compounds.

The Naphthol isomers were injected at 170°C on both the columns. Col. VI showed a good separation of 1-Naphthol from 2-Naphthol (*ca.* **FIG. 2.11**) Col. V, however showed only a hump like separation. The high boiling isomers of phenylenediamines separated well on Col. VI at 170°C but Col. V could not retain the isomers. The pattern of elution was *ortho*, *para* and *meta* as can be seen in **FIG. 2.12.**

2.2.2 Conclusion:

Although both Col. V and Col. VI show good selectivity for nitro compounds, Col. V could not separate the Naphthol isomers. The reason may be due to its high viscosity at 170°C. Again, the failure to retain and resolve the phenylendiamines isomers also may be attributed to this fact. The operating temperature of Col. VI ranges from 94°C to 200°C while that of Col. V is from 170°C to 240°C. The latter column would be more suitable for high boiling compounds while Col. VI could be used as a moderately polar column capable of separating all classes of compounds.

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CHAPTER - 3

GAS CHROMATOGRAPHIC SEPARATIONS OF PERMETHRIN; HYDROXYLATION PRODUCTS OF PHENOL AND AMMOXIMATION PRODUCTS OF CYCLOHEXANONE ON CONVENTIONAL GLC COLUMNS

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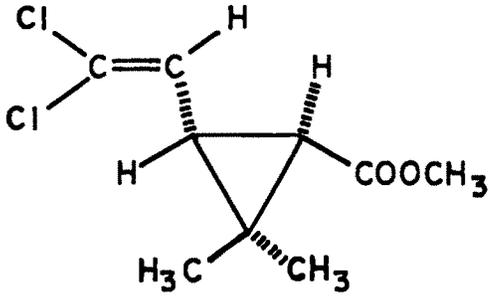
GAS CHROMATOGRAPHIC SEPARATIONS OF PERMETHRIN; HYDROXYLATION PRODUCTS OF PHENOL AND AMMOXIMATION PRODUCTS OF CYCLOHEXANONE ON CONVENTIONAL GLC COLUMNS

3.1 Study of the Percentage Composition of cis-trans Isomers of Permethrin Intermediate and Permethrin by GLC and ^{1}H-NMR

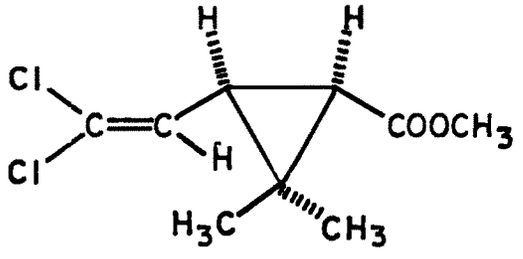
3.1.1 Introduction

One of the valuable intermediates for the manufacture of synthetic pyrethroid insecticides such as permethrin and cypermethrin is 3(2,2-dichlorovinyl) 2,2'-dimethyl carbomethoxycyclopropane (MDV). Elliot et al.[1] developed this insecticide in the early seventies. It is photostable and possesses high activity against household insects, agricultural and woody insects. The chemical nomenclature for permethrin is 3-phenoxybenzyl -(±)-*cis-trans*-3- (2,2-dichlorovinyl)- 2,2-dimethylcyclopropane carboxylate.

Workers on GLC have tried a variety of columns ranging from polar to non-polar stationary phases for permethrin analysis. Some have used a mixture of stationary phases and in the recent past some have used capillary columns[2]. Conditions for analysis also varied from isothermal to temperature programming using electron capture, flame photometric or dual flame ionization detectors. In the present investigation our aim was to separate *cis-trans* isomers of permethrin and its intermediate MDV and to compare percentage composition of *cis-trans* isomers obtained by GLC with the percentage composition obtained by ^{1}H-NMR. Structures of MDV and permethrin

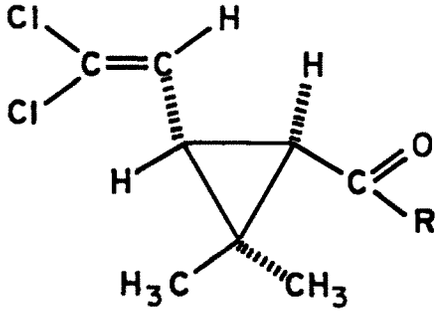


trans

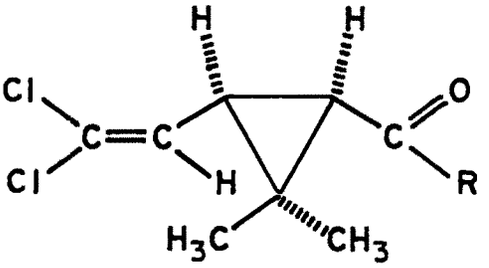


cis

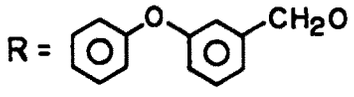
MDV



trans



cis



PERMETHRIN

FIG.3·1: STRUCTURES OF *cis-trans* MDV AND *cis-trans* PERMETHRIN

are given in FIG. 3.1.

3.1.2 Experimental

Chemicals, Apparatus and Conditions:

Permethrin and MDV were used as such.

A Hewlett-Packard (HP) Model 700 gas chromatograph equipped with a dual flame ionization detector was used together with a HP 3380 A integrator and a HP 240 temperature programmer. An aluminium column (1.8m x 4 mm i.d.) was filled with XE-60 at a concentration of 5% (w/w) as stationary phase and chromosorb W AW DMCS (60-80) mesh was used as the solid support.

The following conditions were used; detector temperature : 300°C; injection port temperature: 40°C above oven temperature; oven temperature: 115°C and 210°C. Nitrogen flow rate : 40 ml min^{-1} and chart speed was 0.5 cm min^{-1}.

^{1}H-NMR spectra were scanned on a Bruker WH-90 using TMS as internal standard and $CDCl_3$ as solvent.

3.1.3 Results and Discussion

Horiba et al.[3] had tried the separation of permethrin isomers on 2% XE-60 coated on 60-80 chromosorb W AW DMCS and found it could not give the baseline separation of permethrin. In the present study the separation of *cis* and *trans* isomers of MDV as well as permethrin was initially tried on QF-1 (5%), SE-30 (5%), XE-60 (3% and 5%). All these phases were coated on chromosorb W AW DMCS 60-80 mesh. The *cis* and *trans* isomers of MDV were partially separated from each other on SE-30 (5%) and QF-1 (5%), but a good separation was obtained on XE-60 (3%). The same column failed to separate the isomers of permethrin, hence XE-60 (5%) was finally chosen

for the separation of MDV and permethrin isomers.

A baseline separation was obtained for MDV isomers at 115°C and permethrin isomers at 210°C. The **FIGS. 3.2** and **3.3** show the chromatogram and ^{1}H-NMR spectrum for MDV and **FIGS. 3.4** and **3.5** show the chromatogram and ^{1}H-NMR spectrum of permethrin respectively. An integrator was used for calculating the percentage composition of *cis* and *trans* isomers and the values obtained were compared with the values obtained using ^{1}H-NMR. To determine the authenticity of this method a sample of MDV was enriched with the *cis* isomer and its GLC analysis and ^{1}H-NMR spectrum were taken. The *cis* isomer elutes before *trans* isomer by GLC and the *cis* isomer has higher chemical shift then the *trans* isomer as seen in ^{1}H-NMR. **FIG. 3.6** depicts the chromatogram and **FIG. 3.7** shows the ^{1}H-NMR spectrum of MDV enriched in *cis* isomer. The retention times, chemical shifts and values of *cis* and *trans* ratio are recorded in **TABLE 3.1.** The percentages of *cis* and *trans* isomers obtained by GLC and ^{1}H-NMR are comparable.

3.1.4 Conclusion

The separation of *cis* - *trans* isomers of permethrin, a valuable insecticide and of (2,2-dichlorovinyl) 2,2'-dimethyl carbomethoxycyclopropane (MDV), which is a valuable intermediate for the manufacture of permethrin and cypermethrin, was successfully obtained by GLC on XE-60 (5%) column. Previously the same phase was reported to be unsuitabe for this separation. The percentage composition obtained by GLC is comparable with the percentage composition obtained by ^{1}H-NMR.

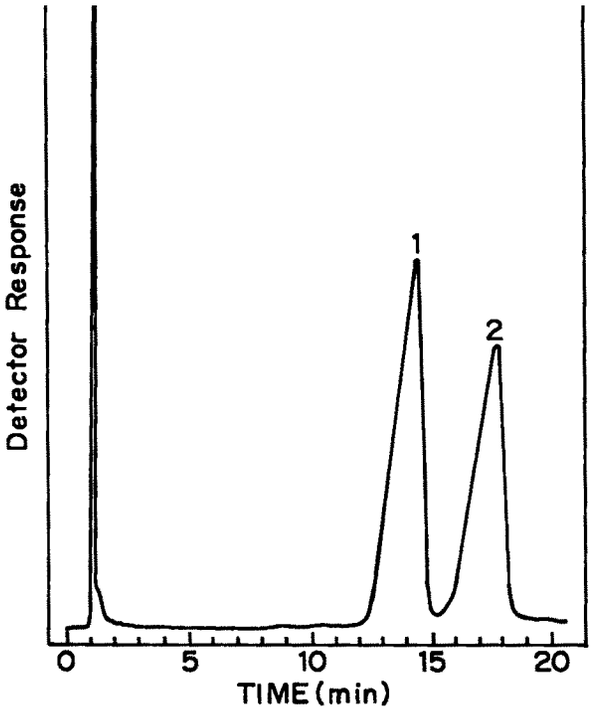


FIG. 3·2: SEPARATION OF MDV ISOMERS ON XE-60-5% COLUMN AT 115°C. PEAKS : 1- cis ; 2- trans

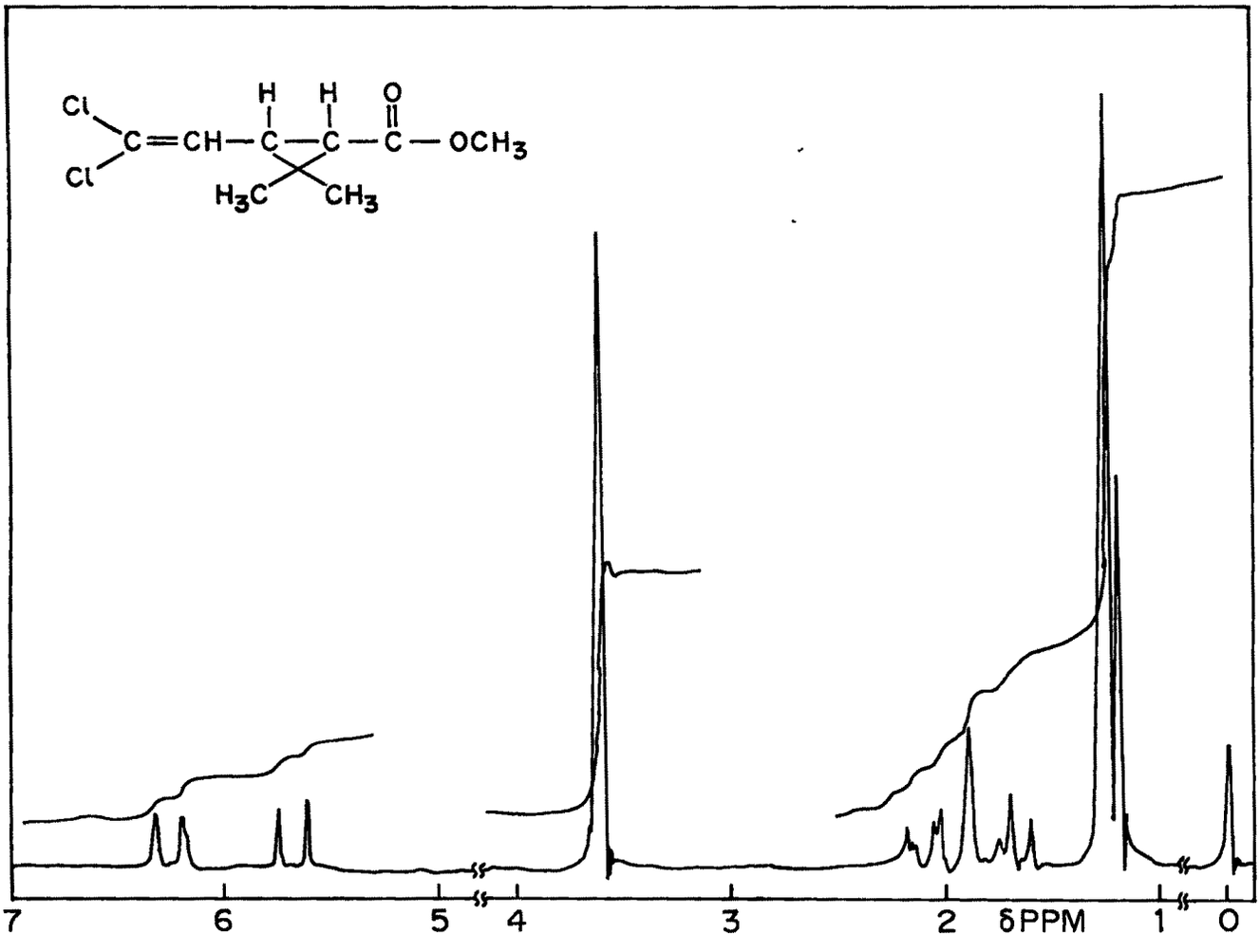


FIG. 3·3 ^{1}H NMR SPECTRUM OF MDV, CHEMICAL SHIFT (δ). cis = 6·25; trans = 5·67

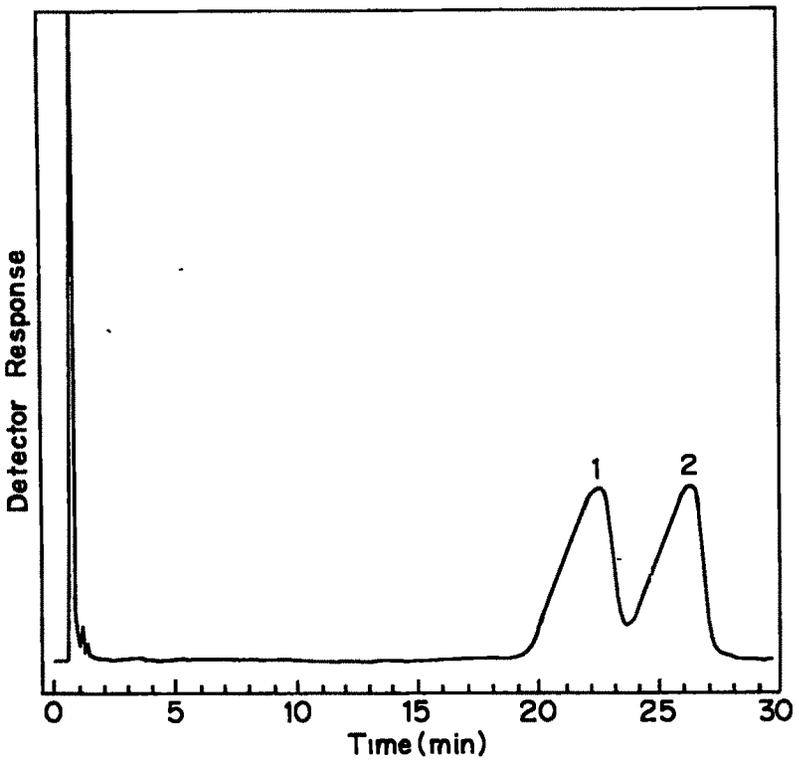


FIG.3·4: SEPARATION OF PERMETHRIN ISOMERS ON XE-60-5% COLUMN AT 210°C. PEAKS : 1- cis ; 2- trans

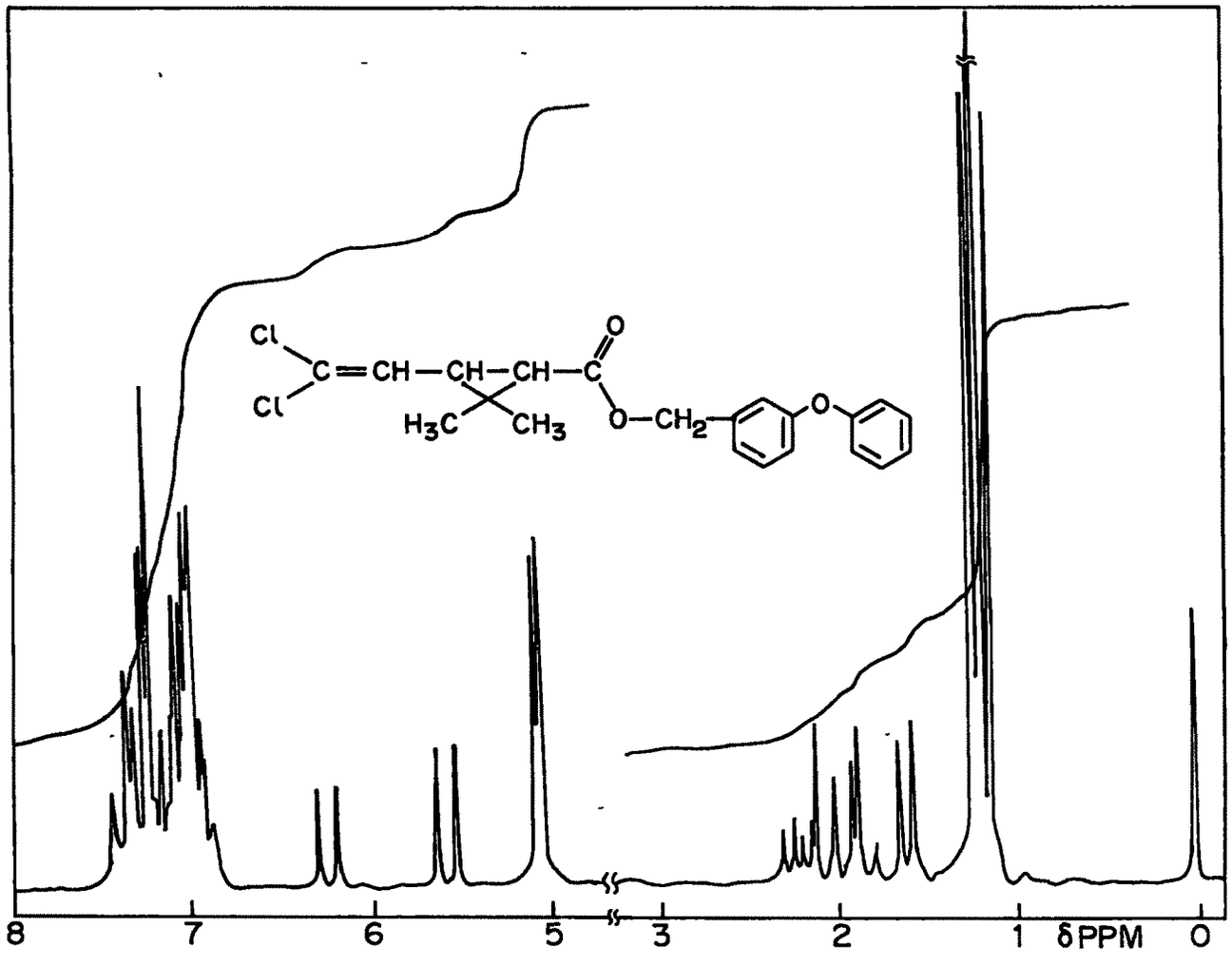


FIG.3·5: ^{1}H NMR SPECTRUM OF PERMETHRIN. CHEMICAL SHIFT (δ): cis = 6·25 ; trans = 5·68

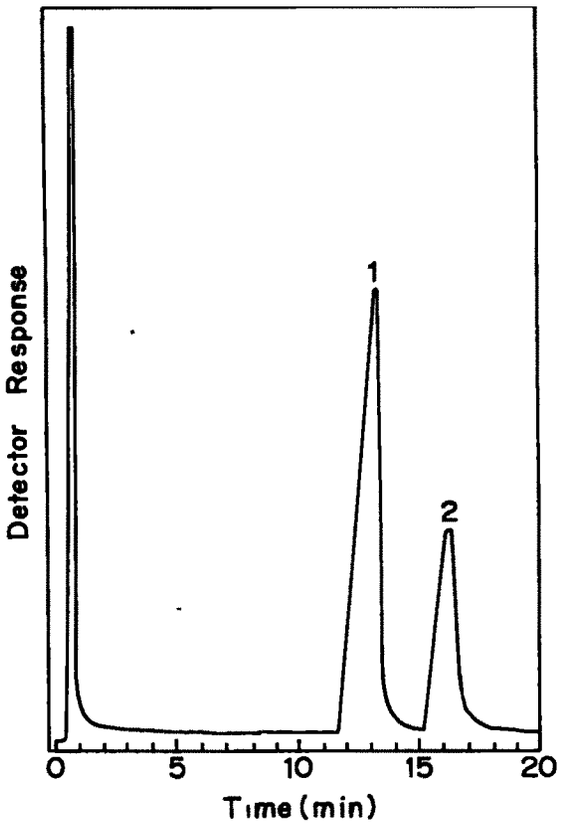


FIG.3·6: CHROMATOGRAM OF MDV ENRICHED IN cis ISOMER
PEAKS: 1- cis ; 2- trans

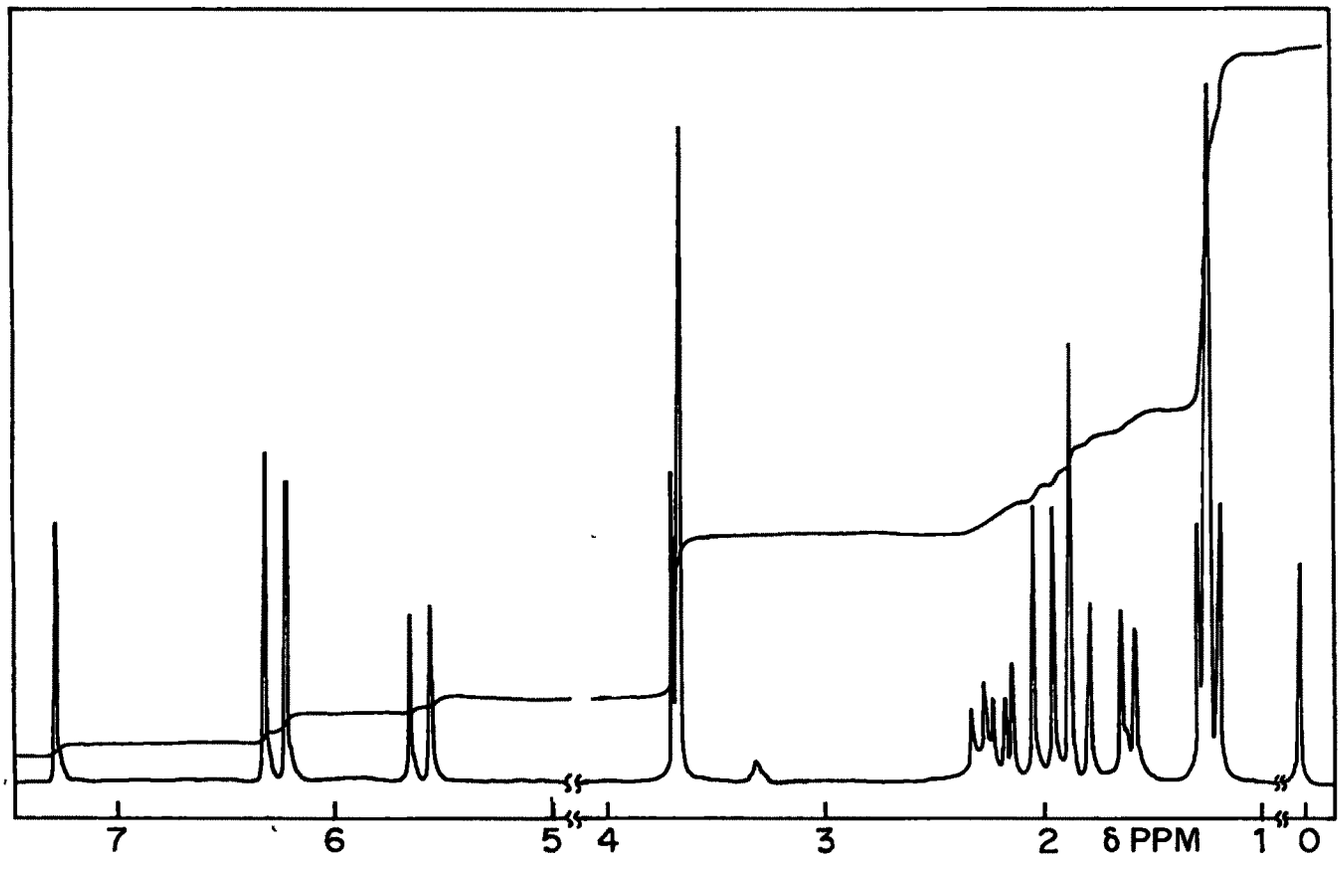


FIG.3·7: ^{1}H NMR SPECTRUM OF MDV ENRICHED IN cis ISOMER.
CHEMICAL SHIFT (δ). cis = 6·28 ; trans = 5·62

TABLE - 3.1

COMPARISION OF % COMPOSITON OF *cis-trans* ISOMERS OBTAINED BY GLC ON XE-60 5% COLUMN AND ^{1}H-NMR

Sample	Gas Chromatography				^{1}H-NMR			
	Temp. °C	Retention time, min.		% Composition*		Chemical Shift (δ)		% Composition
		cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
MDV[x]	115	13.94	17.27	54.90	44.87	6.25	5.67	54.5	44.5
MDV[y]	115	13.02	16.06	67.78	32.02	6.28	5.62	66.6	33.3
Permethrin	210	22.16	25.97	49.89	50.11	6.25	5.68	50.0	50.0

x = MDV as such
y = MDV enriched in *cis* isomer
* Mean of 8 determinations

3.2 Simultaneous Separation of Underivatised Hydroxylation Products of Phenol by GLC

3.2.1 Introduction

The process to manufacture dihydroxy benzenes and 1,4-benzoquinone by hydroxylation of phenol using hydrogen peroxide and zeolite catalyst was developed in our laboratory[4,5]. An inexpensive, simple and rapid method for separating these products with out any further chemical treatment became imperative. A thorough search through the literature revealed that there was no report so far about the simultaneous separation of underivatised 1,4-benzoquinone, phenol, catechol and hydroquinone. Dihydroxybenzenes have found wide applications in industrial processes. Hydroquinone is used as photographic developer and oxidation inhibitor. Catechol is used in preparation of dyes and medicines, photography, rubber, fur dyeing, speciality inks etc. Quinone (1,4-benzoquinone) is used as oxidising agent in manufacture of dyes.

Separation of catechol and hydroquinone was reported on 5% polyphenyl ether with six rings coated on chromaton N AW DMCS at 160°C. Two different columns were used by O'Grodnick et al.[7] for determination of benzene, phenol, catechol and hydroquinone in whole blood of rats and mice after derivatization of catechol and hydroquinone. Capillary columns, polar and non-polar were tried by Mayer and co-workers[8]. Wachowiak and Kenneth[9] separated phenol, 4-chlorophenol, anisole, catechol, resorcinol and 1-naphthol on Carbowax 20M and OV-17 after acetylation. Separation of dimethyl ethers of 1,2- and 1,3-dihydroxybenzene was done on 15% Apiezon L or poly (ethylene glycol) adipate column[10]. Cook et al.[11] reported separation of mono-substituted phenol isomers using liquid crystal stationary phases. Trimethylsilyl derivatives of cresols, phenol, catechol, resorcinol and hydroquinone were analyzed on GC-MS[12]. The process for the separation of dihydroxybenzene isomers using zeolites[13] has been patented.

The present work reports simultaneous separation of underivatized *o,p*, dihydroxybenzenes, phenol and 1,4-benzoquinone on a cyanosilicone column.

3.2.2 Experimental

Materials and Apparatus:

All the four compounds were 99% GC pure. A Hewlett-Packard (HP) model 5880 A series equipped with a level 4 integrator and computing system with flame ionization detector was used. The cyanosilicone phase was coated on chromosorb G AW DMCS at concentrations of 2-5% (w/w) and on chromosorb W AW DMCS at 5-10% (w/w). Solvent used for dispersion of stationary phase was acetone. The coated materials were packed into (1.8 m x 3 mm O.D.) steel columns.

3.2.3 Results and Discussion

Due to high polarity of phenolic compounds and decomposition or oxidation of phenols at high temperatures, derivatisation of the dihydric phenols is normally necessary for their separation in presence of phenol.

Initially we tried separation of the dihydric phenols, phenol and 1,4-benzoquinone on 3% Carbowax 20M coated on Porapak Q 80-100 mesh and 5% SE-30 coated on chromosorb W AW DMCS. Catechol, phenol and hydroquinone showed very good separation on 3% Carbowax 20M but benzoquinone showed a broad peak with nearly the same retention time as hydroquinone. The compounds phenol and 1,4-benzoquinone did not separate on 5% SE-30 column. Both catechol and hydroquinone showed post tailing indicating adsorption on the column. The cyanosilicone column at concentrations of 2 to 5% (w/w) and 5 - 10% (w/w) could separate all the four components such as 1,4-benzoquinone, phenol, catechol and hydroquinone without derivatisation. The best

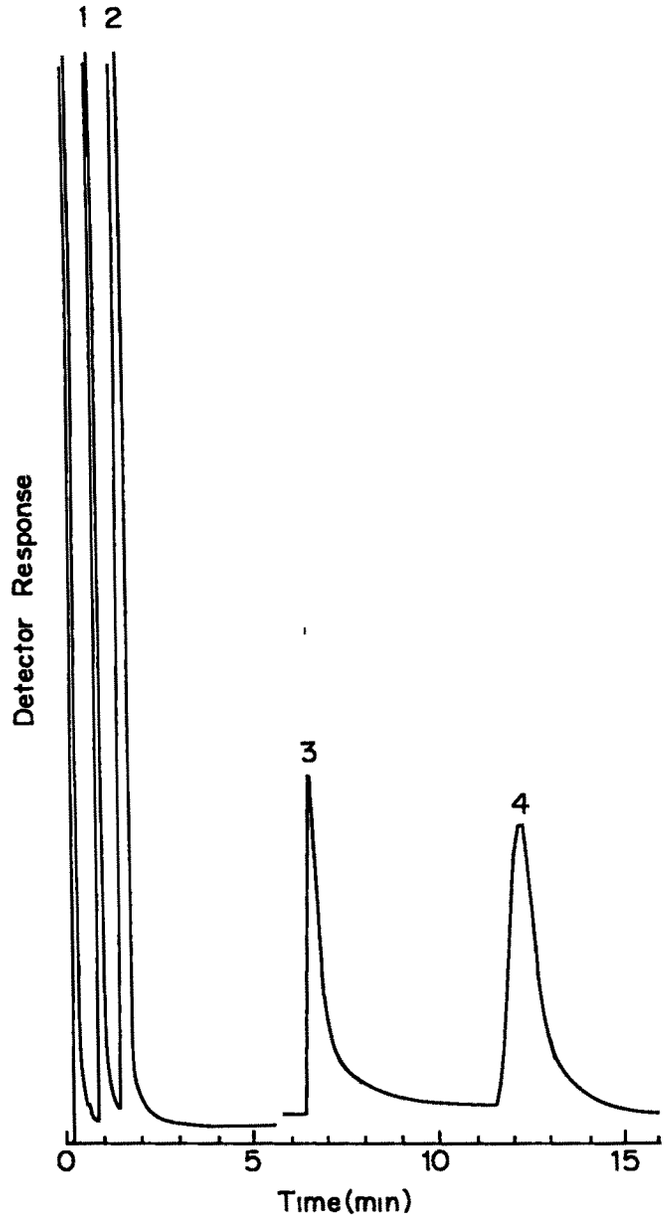


FIG.3·8. SEPARATION OF DIHYDROXYBENZENES, PHENOL AND 1,4 BENZOQUINONE ON CYANOSILICONE COLUMN (2-5% w/w) AT 180°C.

PEAKS 1) BENZOQUINONE; 2) PHENOL; 3) CATECHOL; 4) HYDROQUINONE

separation was achieved on (2-5% w/w) loading and can be seen in **FIG. 3.8** alongwith operating conditions. The order of elution was 1,4-benzoquinone, phenol, catechol and hydroquinone. A patent application for the separation process has been filed.[14]

3.3 A Separation Process for the Ammoximation Products of Cyclohexanone using GLC

3.3.1 Introduction

Cyclohexanone is used to manufacture cyclohexanone oxime which in turn is used for production of caprolactam. Cyclohexanone azine is another product of ammoximation of cyclohexanone by zeolite catalyst. It is used in the field of pharmaceuticals and pesticides. A new process to manufacture cyclohexanone oxime and azine by ammoximation of cyclohexanone using ammonia, hydrogen peroxide and zeolite catalyst was developed in our laboratory[15]. An analytical method became essential to separate the starting material from the products.

A Polish patent[16] describes a method to determine trace amounts of cyclohexanone in cyclohexanone oxime using steel column (0.5 m long x 5 mm i.d.) at 130°C packed with 15% silicone oil DC-200 on Celite 545 and flame ionization detector. Marshlakin et al[17] reported separation on GLC of cyclohexanone azine, octahydrocarbazole and tetrahydrocarbozole on a 10% silicone elastomer SE-30 on silylated diatomite (100-120) mesh packed in (2.1 m x 4 mm) column. Detector was TCD and column temperature was 200°C.

A gas chromatographic method was developed by us and a column was chosen which could separate all the three components simultaneously and within a reasonable time. A phenyl methyl silicone column with temperature programming separated the products, cyclohexanone azine and oxime from the starting material cyclohexanone.

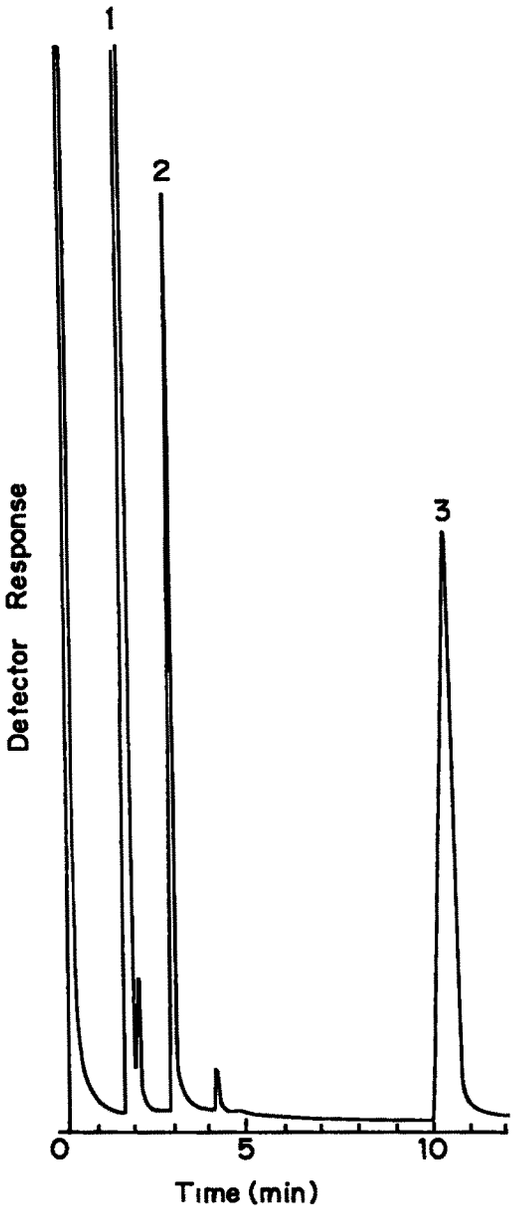


FIG.3·9 : SEPARATION OF CYCLOHEXANONE AMMOXIMATION PRODUCTS ON (3-5% w/w) METHYL-PHENYL SILICONE COLUMN. TEMP. PROG. FROM 100°C TO 170°C AT 20°min^{-1}
PEAKS : 1) CYCLOHEXANONE ; 2) OXIME ; 3) AZINE

3.3.2 Experimental

Materials and Apparatus

Cyclohexanone and cyclohexanone oxime were Aldrich grade 97-98% pure. Cyclohexanone azine was recrystallized before use.

A Hewlett Packard (HP) model 5880 A series instrument equipped with a level 4 integrator and computing system with flame ionization detector was used.

The phenyl methyl silicone phase was coated on chromosorb W AW DMCS at concentration of 3-10% (w/w). Solvent used for dissolving the phase was chloroform. The coated packing material was packed into (1.8 m long x 3 mm o.d.) stainless steel columns. The columns were conditioned before use.

3.3.3 Results and Discussion

A very good separation was obtained when a mixture containing the three components was loaded on a 5-10% (w/w) phenyl methyl silicone column at temperature programming from 120 to 160°C at the rate of 10°/min. Pattern of elution was cyclohexanone, cyclohexanone oxime and cyclohexanone azine. **FIG. 3.9** gives the chromatogram. This separation process has not been reported so far and a patent application for the process has been filed.[18]

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CHAPTER - 4

THIN LAYER CHROMATOGRAPHY ON SINTERED RODS AND FLAME IONIZATION DETECTION SYSTEM

CHAPTER - 4

THIN LAYER CHROMATOGRAPHY ON SINTERED RODS AND FLAME IONIZATION DETECTION SYSTEM

4.1 Introduction

Thin-layer chromatography (TLC) is the most versatile and flexible chromatographic method. Modern computer-controlled scanning instruments and automated sample applications and developers allow accuracy and precision in quantification that rival high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC). Conventional methods of quantitation of fractions resolved by TLC using techniques such as *in-situ* spectrophotometry or photodenistometry are of limited use to substances that contain weak or no chromophoric groups. Such fractions can be conveniently detected and quantified by vapour-phase detectors that are commonly used in GLC[1,2].

Three types of techniques for quantitative TLC with vapour phase detectors have become known so far. In one set of techniques TLC is carried out on narrow quartz plates known as chromatoplates. The chromatoplate after development in solvent is driven through a pyrolysis furnace where the substances separated on the chromatoplate are consecutively vaporized and the gaseous products are swept by the carrier gas to the flame ionization detector (FID).[3,4] The above technique was modified using chromatotubes.[5] This technique, also known as tubular TLC (TTLC), is a versatile method for separation and quantification of less volatile and weakly chromophoric substances that are difficult to analyze either by GLC, TLC or by *in-situ* spectrophotometry. Tubular TLC was modified further by replacing quartz with glass and coating the inside wall with cupric oxide. The organic substances fractionated in the chromatotube are combusted *in-situ* to carbon

dioxide and water and a thermal conductivity detector (TCD) monitors the carbon dioxide evolved from each fraction as separate peaks whose areas correspond to the carbon content of each resolved fraction.[6,7] In the second type of technique, separation of mixtures is carried out on an adsorbent-coated disk,[8,9] and the eluate is continuously conveyed by a moving wire to an FID.

The popularity and flexibility of the FID in GLC suggested that the organic carbon in a spot could be better assayed if introduced into a flame and determined through combustion. This is the third type of technique and was explored by Padley[10] in 1969. Thin quartz rods, coated with an adsorbent, such as silica or alumina embedded in porous sintered glass, were prepared by Okumura and Kadano[11] and are known as Chromarods. The Iatroscan TH-10 scanner[12] was developed to scan these rods and is described below.

4.1.1 Iatroscan TH-10

The working principle of a commercially available instrument, Iatroscan TH-10, designed to scan the adsorbent-coated Chromarods, is depicted in **FIG. 4.1.** A Chromarod, after development, is passed through the hydrogen flame, the carbon ions produced are collected and amplified and recorded as separate peaks. At a time ten Chromarods can be accommodated on the rod holder which in turn is placed on a moving frame which is housed in the instrument. The ion collector of the hydrogen flame is fixed above the plane of the frame, and the hydrogen flame jet is fixed below the plane. The frame, which can be moved at a preselected speed, once set in motion, moves automatically in a fixed pattern so that the top of the rod on the right passes through the fixed hydrogen flame and is burnt from top to bottom. The frame is then indexed so that the flame jet scans the space or "blank" position between rods as the frame returns itself to the starting level where the next Chromarod is ready to be scanned. The movement of the rods can be interrupted at any time which permits unique manipulation of samples with partial separation and quantitation,

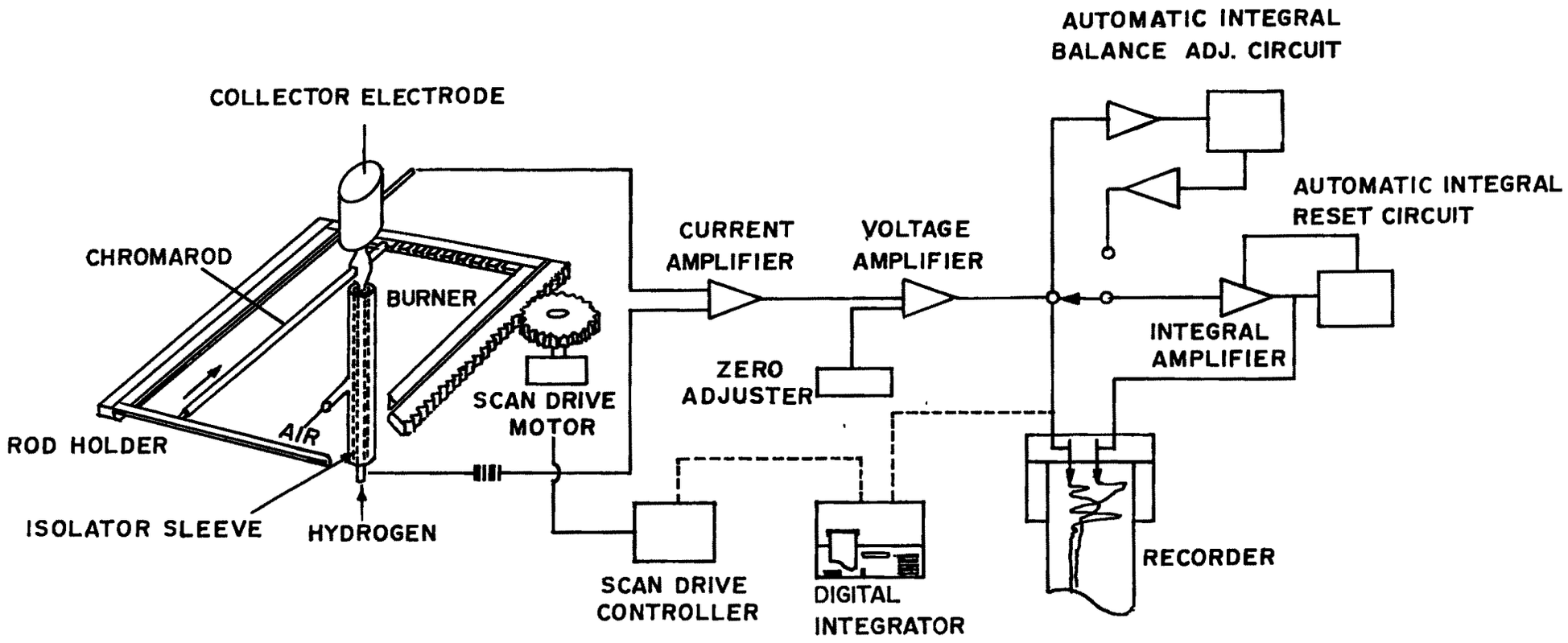

FIG. 4-1 : SCHEMATIC REPRESENTATION OF DETECTION METHOD

followed by redevelopment of one or more components into the freshly cleaned and activated separation medium on the Chromarod. This procedure has no parallel in conventional paper or thin-layer chromatography. As the rod is read from top to bottom the pattern of elution recorded is, fastest moving first and the slowest the last. Inversion of the rods will depict the actual elution order. In other words the least mobile material will be recorded first and the most mobile the last.

4.1.2 Chromarod

The Chromarod is a uniform quartz rod 0.9 mm in diameter and 152 mm in length. Of this, 148 mm are coated with a layer of special frit of soft glass and silica gel, particle size varying from 10 μm to 5 μm and baked at 800-1000°C. The rods are 75μm thick and due to the sharp interface between rod and frit tailing is eliminated. Such adsorbent-coated rods are commercially available with Newman-Howells Assoc. Ltd. UK. Chromarod S and SII are coated with silica gel having particle sizes 10μm and 5μm respectively. Recently Chromarod SIII has been introduced which is claimed to have better reproducibility. Chromarod A is coated with aluminium oxide having particle size 10μm. The alumina rod is normally used in circumstances where substances are prone to decomposition on silicas. It has also been found that the alumina material is very suitable for samples which fall into the stereoisomer group. Chromarods can be silver nitrate, boric acid and oxalic acid impregnated to enhance certain types of separation.

Before using the coated rods for analysis they must be meticulously cleaned free of any organic matter that may produce signals in the FID. This is very easily done by passing the rods through the flame jet of the FID or by 'blank-scanning' as it is popularly known. Another method for cleaning Chromarods suggested by Newman-Howells Assoc. Ltd.[13] is as follows:

1) Place the Chromarods in 60% H_2SO_4 acid solution and chromatograph the acid solution 100% up the rod.

2) Immerse the rods in the same acid solution in test tubes and place in a sonic bath for 15 minutes.

3) Wash each rod thoroughly in distilled water and replace each rod in its position in the frame.

4) Finally, store rods in a constant humidity chamber (32% relative humidity).

Chromarod activity varies greatly with change in moisture content of the adsorbent (silica or alumina). This poses a great problem to obtain reproducible separation patterns. The activity can be regulated by the constant moisture method or the vacuum drying method.

1. Constant moisture method: The rods are placed in a covered glass jar containing distilled water. The filter paper, placed inside the jar, continuously absorbs water and maintains good hydration of the silica or alumina particles within the sintered coating.

2. Vacuum drying process: This process is performed after sample spotting and prior to solvent elution. However, this process is not applicable to samples of low boiling point that may evaporate during vacuum pumping.

4.1.3 Operation

Proper choice of operating variables, in both chromatography and scanning, are crucial for obtaining sensitivity of detection and reproducibility.[14] Hydrogen flow is kept at 160 ml/min and air supply at 2000 ml/min. The sample to be analysed is spotted on the rod about 2 cm from one end using 0.1 to 3 µl of volume containing 1-5 mg of sample. The end of the rod bearing the sample is immersed in the developing solvent in a closed chamber to a depth of about 1.5 cm. After development the rod is dried off the developer solvent and then passed through a hydrogen flame and scanned from top to bottom at a pre-selected speed. The combustion products are detected

and the signal generated is amplified and fed to a recorder. An integrator is built into the instrument, thus, a two-pen recorder is used giving curves and their integration as a vertical distance plot between horizontal moves from peak to peak. Thus the steps are:

(i) Sample dissolution

(ii) Sample spotting on rod

(iii) Solvent development

(iv) Drying

(v) Flame scanning

(vi) Curve plotting and integration

4.1.4 Applications

The Iatroscan TH-10 has proved particularly successful in areas of:

1. Crude drug analysis
2. Quality control in drug production
3. Analysis of lipids
4. Clinical analysis
5. Petrochemical, organic and polymeric materials
6. Analysis of vitamins
7. Cholesterol determination
8. Steroid determination, etc.

Practical application of Iatroscan TH-10 for analysis of opium alkaloids is discussed in the second section of this Chapter with special reference to peak pyrolysis method (PPM).

4.2 Separation of Opium Alkaloids by Thin-Layer Chromatography (TLC) Combined with Flame Ionization Detector (FID) using peak Pyrolysis Method (PPM)

4.2.1 Introduction

Few natural products have been studied so extensively as opium, and none has presented problems of such magnitude from chemical, biological and social viewpoints. The quantification of the alkaloids in opium has always presented problems. The methods of analysis reported so far include chromatographic as well as non-chromatographic techniques. Chromatographic methods include paper chromatography[15,16], thin-layer chromatography[17-33], gas-liquid chromatography[34-40], and high performance liquid chromatography[41-51]. Techniques for analysis other than chromatography are also available[52,53].

The first paper on thin-layer chromatographic separation of opium alkaloids was published by Borke and Kirsch[21] in 1953. Since then separation of the alkaloids on silica, alumina, carbomethoxy cellulose, impregnated silica and alumina, polyamide coated silica as various adsorbents, were tried with several polar, nonpolar and neutral solvent systems. Misra *et al.*[30] used glass fibre sheets to separate morphine, codeine, thebaine and some of its metabolites and congeners. Two dimensional TLC was used by Zakaria and co-workers[31]. Some alkaloids of opium were separated on silica gel sintered sticks by Okumura *et al.*[11] Solvent system used by them was chloroform-diethylamine (30 : 1) and flame ionization detector was used for detection. The drawback was that the sintered sticks had to be rechromatographed with lipophilic solvents such

as *n*-pentane to remove excess of diethylamine. Recently, Patzch *et al.*[54] have used high-performance TLC (HPTLC) to determine morphine, codeine and heroin on silica gel plates with post-column derivatization.

We are reporting here a rapid, relatively simple and reproducible method for the separation of the five major opium alkaloids, viz., morphine, codeine, thebaine, papaverine and narcotine (noscapine). TLC coupled with a FID combines the efficiency of TLC and the sensitivity of FID. It has the added facility allowing the use of the partial scanning or peak pyrolysis method (PPM)[55]. Analysis by TLC-FID does not require derivatization of samples and high operating temperatures as in gas chromatogaphy or the use of large volumes of solvent and maintenance of pH as in high performance liquid chromatography. As little as 2-3 μg of each compound is needed for detection without derivatization.

4.2.2 Experimental

Apparatus:

An Iatroscan TH-10 MK III analyzer (Iatron Labs., Tokyo, Japan) was equipped with a flame ionization detector and connected to a two-pen linear recorder (National).

Materials:

The alkaloids morphine, codeine, thebaine, papaverine and narcotine were obtained from Government Opium and Alkaloids Works Undertaking (Neemuch, M.P. India).

Procedure:

The detector was operated with a hydrogen flow rate 160 ml/min and air flow rate of 2000 ml/min. The recorder was used at 50-100 mV full scale deflection. The chart speed was kept at 12 cm/min and the scanning speed at 35s/scan. A new set of Chromarod S II silica rods (particle size 5 μm, Newman-Howells, UK) was used throughout.

The alkaloids were dissolved in methanol-dichloromethane (2 : 1 v/v). Spotting was done in aliquots of 0.25 μl to prevent spreading of the sample on the rod. Calibration was done by spotting different volumes (0.5 - 3 μl) of standard samples of the same concentration (2 mg/ml). Absolute calibration graphs were drawn for each standard sample as the peak area (abscissa) versus spot weight (ordinate). The calibration graphs were linear from 1 to 7 μg as can be seen in **FIG.4.2.** As long as analysis and calibration are performed under identical conditions, the graphs can be used to determine directly the weights (in μg) of the components provided that the peak areas of the components are known. When 1 μl of a sample containing a mixture of the alkaloids of concentration 24.08 mg/ml was spotted and the peak areas for the different components were detemined, the amounts (in μg) could be read directly from the calibration graph.

The alkaloids were separated using the partial scanning method between the stages of a two-step development system. Narcotine and papaverine were separated using benzene-acetonitrile-ethyl acetate (60 : 20 : 20, v/v) as solvent system in the first step and morphine, codeine and thebaine using ethyl acetate benzene-acetonitrile-ammonia solution (25 : 30 : 40 : 5, v/v) in the second step.

Dual development with benzene-ethanol (9.5 : 0.5 and 9 : 1, v/v) effected the simultaneous separation of the five opium alkaloids.

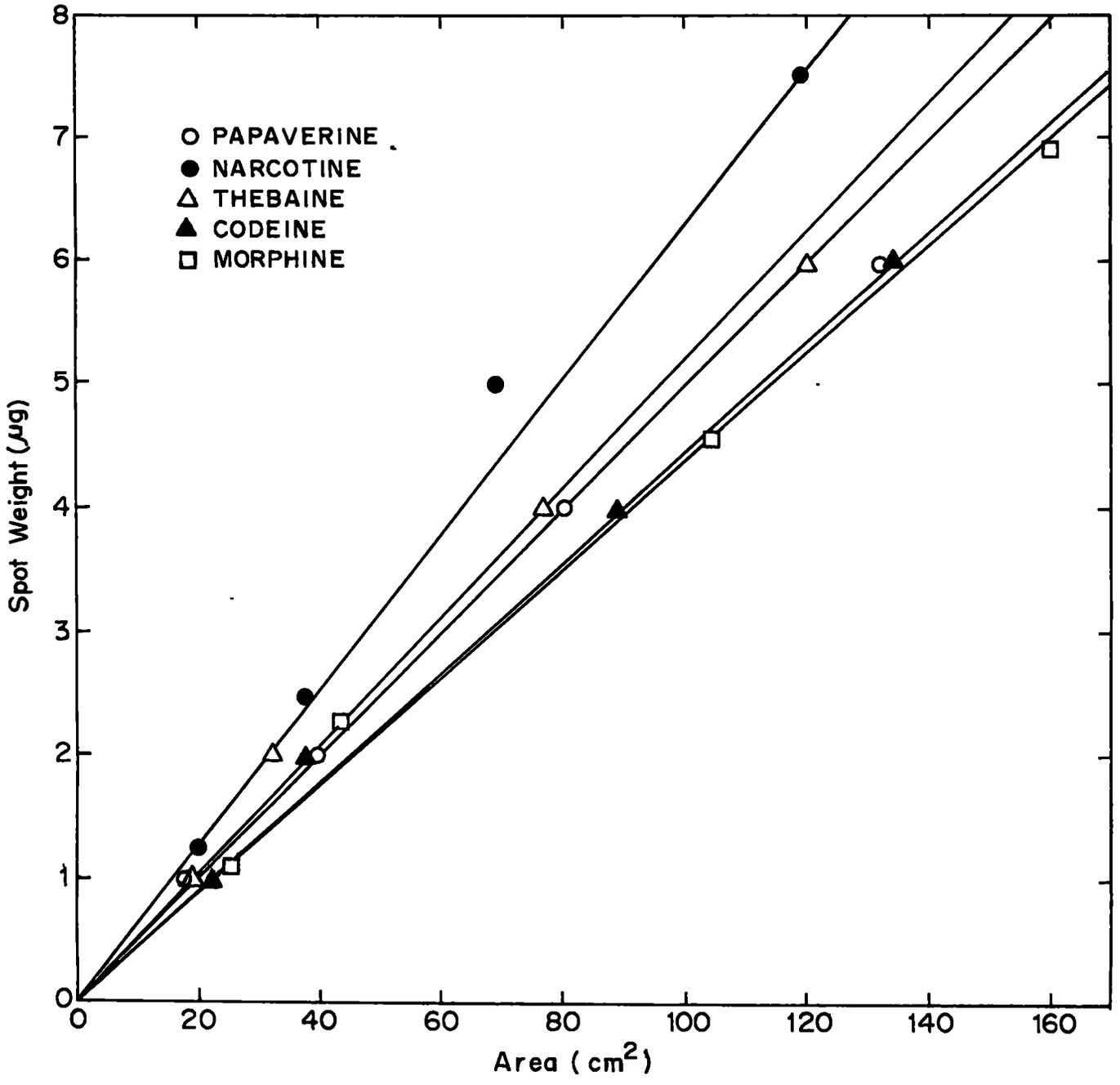

FIG. 4·2 : CALIBRATION CURVES FOR STANDARD OPIUM ALKALOIDS.

4.2.3 Results and Discussion

The R_F values of five major alkaloids on Chromarod S II silica rods are given in **TABLE 4.1.** Binary and multi-component solvent systems involving one- or two - step development systems were employed. The best multi-component system involving peak pyrolysis method between two-step development and a binary system which effects the separation of all five opium alkaloids simultaneously is reported. Results obtained by quantitative analysis are given in **TABLE 4.2.**

The fastest migrating alkaloid was narcotine, followed by papaverine, thebaine codeine and morphine. This pattern of elution was constant for both binary and multi-component systems. It should be noted that in the chromatograms the direction of development is from right to left and scanning is done from left to right. Hence, narcotine, which is the fastest moving compound, elutes as the first peak and morphine, which is very sluggish, elutes as the last peak. S and E denote the start and end of scans respectively and O is the point of sample application.

The quaternary system suggested by Steel[23] was investigated. This system consists of ethyl acetate-benzene-acetonitrile-ammonia solution in different ratios such as (a) (50 : 30 : 15 : 15 v/v) and (b) (25 : 30 : 40 : 5 v/v). System (a) gave only a moderate separation of morphine, codeine and thebaine and no separation of narcotine and papaverine. However, system (b) resolved the peaks of morphine, codeine and thebaine well, but there was no change in the separation of papaverine and narcotine. We therefore concluded that a less polar system such as benzene-acetonitrile-ethyl acetate (60 : 20 : 20 v/v), might give a clean separation of narcotine and papaverine. Hence, a two-step development sequence with partial scanning or peak pyrolysis method could be a successful approach to this problem.

TABLE 4.1

R_F VALUES OF OPIUM ALKALOIDS ON CHROMAROD SII SILICA RODS

Solvent System	Composition (% v/v)	Mode of development	R_F Values				
			Morphine	Codeine	Thebaine	Papaverine	Narcotine
Benzene-acetonitrile-ethyl acetate	60 : 20 : 20	Two-step development system with peak pyrolysis in between	0.13	0.15	0.18	0.41	0.56
Ethyl acetate-benzene-acetonitrile-ammonia solution	25 : 30 : 40 : 5	As above	0.24	0.33	0.49	-*	-*
Benzene-ethanol	9.5 : 0.5 then 9 : 1	Dual development	0.16	0.23	0.38	0.63	0.70

* Burnt during peak pyrolysis

TABLE 4.2

QUANTITATIVE ANALYSIS OF A MIXTURE OF OPIUM ALKALOIDS

Alkaloid	Peak area[a]	Amount applied (μg)	Amount found (μg)[b]	Recovery (%)	Standard deviation of peak area[a]
Narcotine	89.2	5.62	5.60	99.6	3.1
Papaverine	104.5	5.21	5.20	99.8	4.1
Thebaine	74.0	3.88	3.85	99.2	4.3
Codeine	147.3	6.56	6.55	99.8	1.5
Morphine	65.3	2.81	2.80	99.6	1.5

[a] Mean of six determinations

[b] From calibration graph.

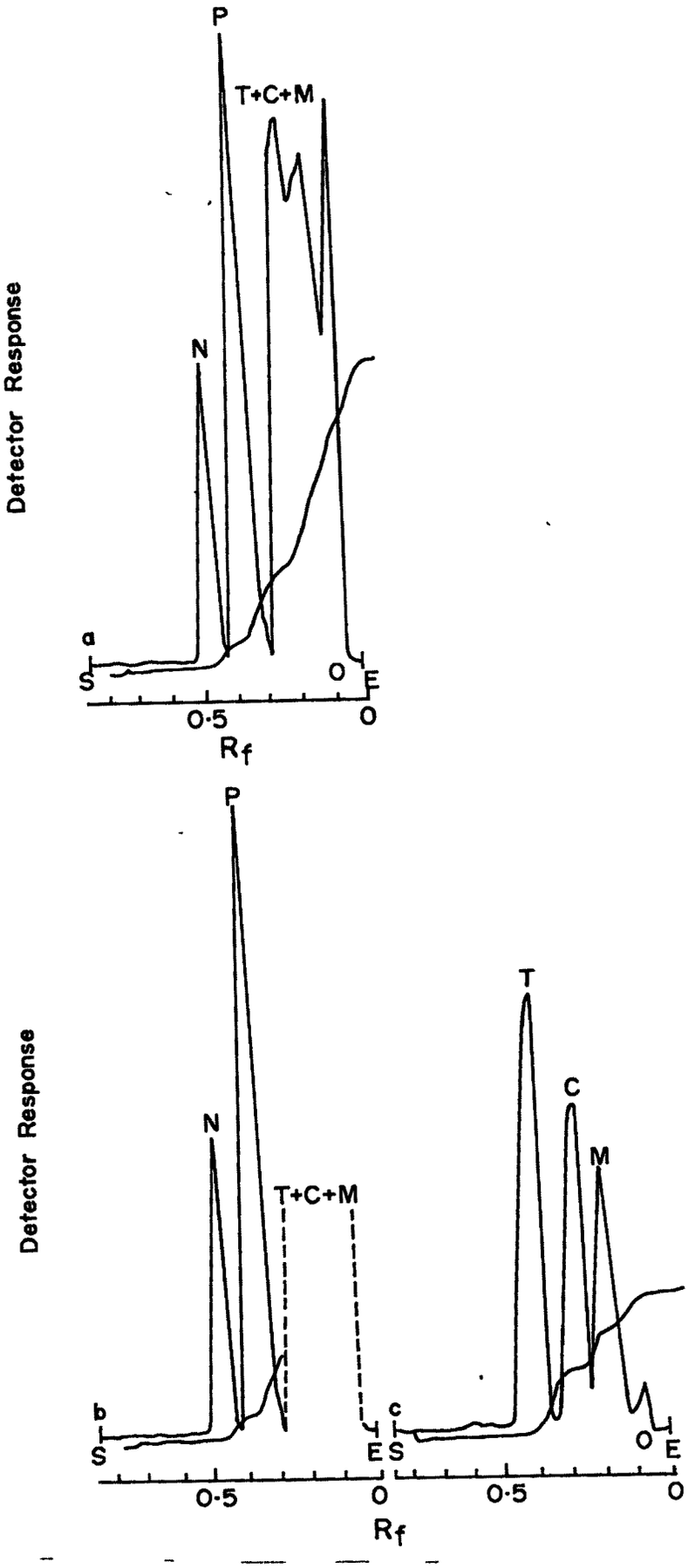

FIG. 4.3: (a) COMPLETE SCAN OF CHROMAROD ≤ II IN BENZENE-ACETONITRILE-ETHYL ACETATE (60 : 20 :20 v/v) (b) PARTIAL SCAN (c) REDEVELOPMENT IN ETHYL ACETATE-BENZENE-ACETONITRILE-AMMONIUM HYDROXIDE (25 : 30 : 40 : 5 v/v). P = PAPAVERINE, N = NARCOTINE, T = THEBAINE, C = CODEINE, M = MORPHINE

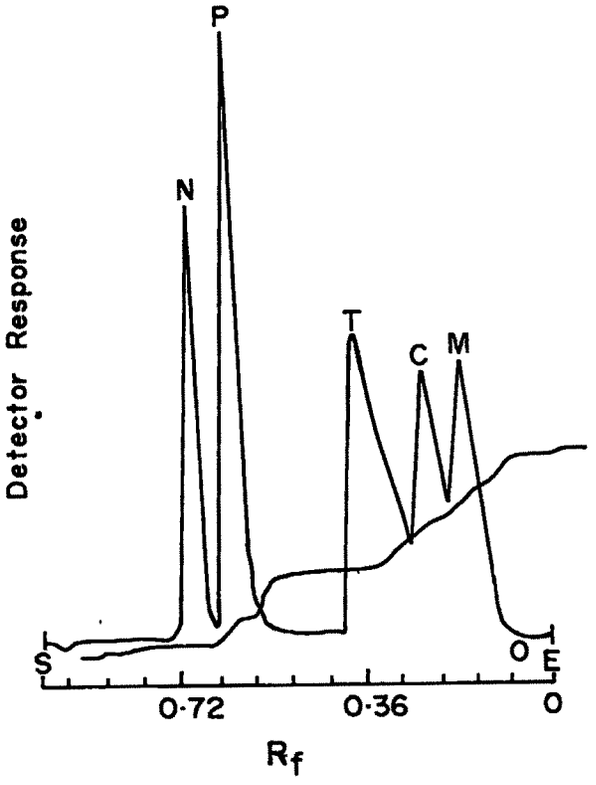

FIG. 4.4: SIMULTANEOUS SEPARATION OF THE FIVE MAJOR ALKALOIDS OF OPIUM ON CHROMAROD SII DEVELOPED TWICE IN BENZENE-ETHANOL SYSTEM COMPRISING OF TWO DIFFERENT COMPOSITION (X) (9.5 : 0.5 v/v) AND (Y) (9 : 1 v/v). P = PAPAVERIN, N = NARCOTINE, T = THEBAINE, C = CODEINE, M = MORPHINE

The first development was done with benzene-acetonitrile-ethyl acetate (60 : 20 : 20 v/v). This resulted in the fastest migrating compounds i.e. narcotine and papaverine, to migrate into the upper part of the rod, morphine, codeine and thebaine remaining near the point of application and appearing as a forked peak, as can be seen in **FIG. 4.3a.** The scan stop screw was then set at a point between the resolved and unresolved compounds and the remaining rods were partially scanned, as shown in **FIG. 4.3b.** In this way, narcotine and papaverine were determined and this part of the rod was reactivated so that a second development could be performed with a more polar solvent sytem, taking the unresolved compounds (morphine, codeine and thebaine) as the new origin. The second development of the rods was done with ethyl acetate-benzene-acetonitrile-ammonia solution (25 : 30 : 40 : 5 v/v), which gave a good separation of morphine, codeine and thebaine, as is seen in **FIG. 4.3c.** The rods in both these systems were developed upto 10 cm for a period of 35 min. each and scanned at 35s per scan.

The binary solvent system suggested by Mary *et al.*[24] proved to be quite satisfactory but with a little modification. They used benzene-ethanol in (8 : 2 v/v) combination which separated all the five alkaloids on TLC. This composition, however, did not succeed in separating the five alkaloids on Chromarods. A modified composition of benzene-ethanol (9.5 : 0.5 and 9 : 1 v/v) with dual development separated the five alkaloids simultaneously. The spotted rods were first developed upto 10 cm in (9.5 : 0.5 v/v) composition, removed and dried, and again developed upto 10 cm in (9 : 1 v/v) composition. The chromatogram showing separation of the five opium alkaloids simultaneously is seen in **FIG. 4.4.**

4.2.4 Conclusion

All the five major opium alkaloids could be separated without derivatization on Chromarod S II silica rods using peak pyrolysis method (PPM) between the stages of a two-step

development system. The first development with benzene-acetonitrile-ethyl acetate (60 : 20 : 20 v/v) helps to move the less polar compounds away from the point of application. The remaining unresolved compounds are then separated with a more polar solvent system, ethyl acetate-benzene-acetonitrile-ammonia solution (25 : 30 : 40 : 5 v/v). The best binary system was benzene-ethanol (9.5 : 0.5 and 9 : 1 v/v), which could separate all five alkaloids simultaneously.

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SUMMARY

The thesis deals with chromatographic techniques such as gas liquid chromatography (GLC) and thin layer chromatography coupled to a flame ionization detection systems (TLC-FID). It is divided into four chapters.

The first chapter reviews the different stationary phases available to the chromatographer. The different stationary phases can be clubbed together into four groups. The four groups are discussed in detail.

The second chapter explores the use of N-alkyl aminonitrobenzophenone derivatives as stationary phases in GLC. The following aminonitrobenzophenone derivatives were used as substrates and evaluated:

1. 4-Ethylamino-3-nitrobenzophenone (4-ETHA-3-NB)
2. 4-Isopropylamino-3-nitrobenzophenone (4-IPA-3-NB)
3. 4-n-Butylamino-3-nitrobenzophenone (4-BUTA-3-NB)
4. 4-Dimethylamino-3-nitrobenzophenone (4-DMA-3-NB)
5. 4-Cyclohexylamino-3-nitrobenzophenone (4-CYHA-3-NB)
6. 2-Cyclohexylamino-5-nitrobenzophenone (2-CYHA-5-NB)

The polarities (Mc Reynolds constants) of these derivatives were determined and compared with standard stationary phases. These phases have polarity ranging from very polar (4-ETHA-3-NB) to medium polar (2-CYHA-5-NB). The phase (4-CYHA-3-NB) has a high melting point, so its polarity was determined at 180°C. All the phases except (4-DMA-3-NB) can be used in the separation of various positional isomers.

Chapter 3 also deals with gas chromatography. Permethrin, which is a very well known synthetic pyrethroid and its intermediate (2,2-dichlorovinyl)2,2'-dimethyl-carbomethoxycyclopropane (MDV) have been separated on a packed XE-60 column. The results obtained by GLC were compared with ^{1}H-NMR.

The second and third sections of this chapter informs about the separation processes for the Hydroxylation products of phenol and Ammoximation products of cyclohexanone on conventional GLC columns.

Instrumental thin layer chromatography is discussed in Chapter 4. The working principle, operation and applications of the TLC-FID instrument is detailed here. The second section describes the separation of opium alkaloids with special reference to peak pyrolysis method.