STUDIES IN CHROMATOGRAPHY

A THESIS
SUBMITTED TO THE
SHIVAJI UNIVERSITY, KOLHAPUR
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN CHEMISTRY

BY
Ms. AMRUTA S. TAMBE (M. Sc.)

DIVISION OF ORGANIC CHEMISTRY : TECHNOLOGY
NATIONAL CHEMICAL LABORATORY
PUNE 411008 (INDIA)

MARCH 1992

DEDICATED

TO

MY PARENTS

CERTIFICATE

Certified that the work incorporated in the thesis "Studies in Chromatography", submitted by Ms. Amruta S. Tambe, was carried out by the candidate under my supervision. Such material as has been obtained from other sources is duly acknowledged in the thesis.

(Dr. N.R. Ayyangar)

Supervisor

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude and reverence to Dr. N.R. Ayyangar, Ex. Head, Organic Chemistry - II Division, National Chemical Laboratory, Pune, for his inspiring guidance, helpful suggestions and profound interest throughout the progress of this work.

My sincere thanks are due to Ms. K. Zubaidha and Dr. Thomas Daniel for providing the samples of butenolides and chlorobenzophenones for the study. I am also thankful to Dr. P.P. Moghe for his cooperation and interest during the analysis of phenols.

I would like to express thanks to all my colleagues in the Organic Chemistry Division and in particular Mrs. Sujata Biswas for their help and cheerful cooperation on and off the field.

I take this opportunity to thank Dr. T.Ravindranathan, Head, Organic Chemistry:Technology division, for allowing me to carry out the research work in the laboratory.

Finally, I sincerely thank Dr. R.A. Mashelkar, Director, N.C.L., for permitting me to submit this work in the form of thesis.

Tambe.

(Ms. Amruta S. Tambe)

National Chemical Laboratory
Pune - 411008
March 1992

CONTENTS

	PREFACE	....	vi
CHAPTER 1 :	**CHARACTERIZATION OF SOLVENT PROPERTIES OF GAS CHROMATOGRAPHIC LIQUID PHASES : A GENERAL REVIEW**		
1.1	Retention Index System	....	2
1.2	Rohrschneider / McReynolds Phase Constants	....	5
1.3	Solvent Selectivity Triangle	....	11
1.4	Solubility Parameters	....	12
1.5	Spectroscopic Methods	....	12
1.6	Thermodynamic Approaches	....	13
1.7	Conclusion	....	15
	References	....	16
CHAPTER 2:	**CROWN ETHERS AS STATIONARY PHASES IN GAS CHROMATOGRAPHY**		
2.1	Applications of Crown Compounds in Analytical Chemistry	....	20
2.1.1	Separation Methods	....	20
2.1.2	Determination Methods	....	22
2.1.3	Applications of Crown Ethers in HPLC and GC	....	24
2.1.4	Crown Ethers in Synthetic Organic Chemistry	....	26
2.2	Comparison between Dibenzo-l8-Crown-6, Dibenzo-24-Crown-8 and Dicyclohexano-24-Crown-8 with respect to Polarity, Selectivity and Thermal Stability	....	26
2.2.1	Experimental	....	26
2.2.2	Results and Discussion	....	30
	References	....	49

...contd

CHAPTER 3: 4-(2'-PHENYLISOPROPYL)PHENOL ESTERS AS STATIONARY PHASES FOR GAS-LIQUID CHROMATOGRAPHY

3.1	Evaluation of 4-(2'-Phenylisopropyl)phenol Esters as Stationary Phases for Gas-Liquid Chromatography	....	51
3.1.1	Introduction	....	51
3.1.2	Synthesis	....	52
3.1.3	Experimental Methods	....	54
3.1.4	Results and Discussion	....	55
3.2	Application of Tri[4-(2'-phenylisopropyl)phenyl Phosphate (TPIPP) in Gas Chromatographic Analysis	....	62
3.2.1	Separation Between Benzoquinone, Phenol, Catechol and Hydroquinone	....	70
3.2.2	Separation Between Other Higher Boiling Isomers	....	71
3.2.3	Conclusion	....	71
	References	....	75

CHAPTER 4 : SYNTHESIS AND GAS CHROMATOGRAPHIC ANALYSIS OF *PARA* CHLOROBENZOPHE-NONE

4.1	Synthesis of *para* Chlorobenzophenone in Quantitative Yields by Friedel-Crafts Acylation Under Milder Reaction Conditions	....	77
4.1.1	Experimental	....	79
4.1.2	Procedure	....	79
4.2	Gas Chromatographic Method for the Analysis of Chlorobenzophenone Isomers	....	82
4.2.1	Experimental	....	83
4.2.2	Results and Discussion	....	84
4.2.3	Conclusion	....	85
	References	....	91

CHAPTER 5 : ENANTIOMERIC SEPARATIONS

5.1	Separation of Enantiomers by Gas Chromatography on Chiral Stationary Phases	....	92
5.2	Enantiomeric separations of β - γ Unsaturated Esters and Various Analogues of Butenolides on Known Chiral Phase	....	103
5.2.1	Experimental	....	104
5.2.2	Results and Discussion	....	104
5.2.3	Absolute Configuration	....	110
5.2.4	General Remarks	....	110
	References		112
	SUMMARY	....	117
	APPENDIX	....	119
	List of Publications	....	121

PREFACE

Chromatography, in its many forms is a well-known and widely used separative and analytical technique. The method was proposed in 1906 by Tswett, who employed it for the separation of colored substances and hence was named 'Chromatography'. It is essentially a physical method of separation in which the components to be separated are distributed between two phases, one of which is a stationary phase bed, and the other, a mobile phase. The chromatographic process occurs as a result of repeated sorption/desorption steps during the movement of the sample components along the stationary bed and the separation occurs due to the differences in distribution constants of the individual sample components. The various chromatographic techniques such as gas-liquid, liquid-liquid, gas-solid and liquid-solid, as the names indicate, differ in their stationary and mobile phases.

In gas chromatography the mobile phase is an inert gas and the stationary phase is either an adsorbent or a liquid distributed over the surface of an inert porous support. Separation takes place due to the selective interactions between the solute and the stationary liquid phase. The principal intermolecular forces involved between the solute and solvent are, namely, dispersion, induction, orientation and donor-acceptor interactions including hydrogen bonding. The sum of various intermolecular forces between a particular solute and liquid phase is a measure of polarity of the phase with respect to the solute. The magnitude of the individual interaction energies is a measure of phase selectivity. In chromatography, differences in selectivity assume importance, since these enable two solutes of equal polarity to be separated by a selective phase. Molecular size and shape should also be taken into account. Characterization of solvent properties of the liquid stationary phases in terms of polarity and selectivity is crucial because it makes possible to define the application areas for new phases and to identify phases having identical properties. This thesis deals with one of the most important and extensively used chromatographic techniques, i.e., gas-liquid chromatography. The technique has found widwspread use in qualitative and quantitative analysis of volatile compounds.

In the first Chapter, different methods of characterization of stationary phases in gas-liquid chromatography are reviewed. In the second and third Chapters crown ethers and phenyl isopro-

pylphenol esters are characterized as liquid stationary phases with respect to their polarity, selectivity and stability. These are absolutely new phases and can find various applications in analytical chemistry.

In the fourth Chapter we have developed a simple, fast, and an efficient gas-liquid chromatographic method for the analysis of chlorobenzophenone isomers. This is necessary in order to estimate the amounts of 2- and 3-chlorobenzophenone isomers in 4-chlorobenzophenone which is a starting material in the manufacture of 'Systral', an anti-Parkinson agent.

High resolution, precision and reproducibility are the merits of gas-liquid chromatography for enantiomeric resolution. Direct resolution of the enantiomers on chiral stationary phases is an attractive method for the separation of enantiomers. The fifth Chapter first reviews the various chiral stationary phases used in gas-liquid chromatography and subsequently reports the results of enantiomer separation of mintlactone and *iso* mintlactone on Chirasil-Val stationary phase. The assignment of absolute configuration and the order of elution of enantiomers is reported for the first time.

CHAPTER 1

CHARACTERIZATION OF SOLVENT PROPERTIES OF GAS CHROMATOGRAPHIC LIQUID PHASES : A GENERAL REVIEW

CHAPTER 1

CHARACTERIZATION OF SOLVENT PROPERTIES OF GAS CHROMATOGRAPHIC LIQUID PHASES : A GENERAL REVIEW

Introduction

In gas-liquid chromatography, separations take place because the solutes have different volatilities and/or interact to varied extents with the stationary liquid phase. The use of stationary phase is most important in gas chromatography due to following reasons:

(i) it makes possible the separation of solutes with very similar properties,

(ii) on the basis of their behaviour with a solute, conclusions may be drawn concerning the nature and the general characterization of the particular solute,

(iii) they contribute useful data to the knowledge of molecular interactions.

The principal interactions that affect the solubility of a solute in a liquid phase, and therefore retention are, dispersion, induction, orientation and donor-acceptor interactions including hydrogen bonding. Dispersion forces arise from the electric field generated by rapidly varying dipoles formed between nuclei and electrons at zero-point motion of the molecules, acting upon the polarizability of other molecules to produce induced dipoles in phase with the instantaneous dipoles forming them. Dispersion forces are universal and independent of temperature. Induction forces arise from the interaction of a permanent dipole with a polarizable molecule. Orientation forces originate from the interaction between two permanent dipoles. Induction and orientation forces decrease with increasing temperature. Donor-acceptor complexes involve special chemical bonding interactions that arise from the partial transfer of electrons from a filled orbital on the donor to a vacant orbital on the acceptor molecule, e.g., hydrogen-bonding interactions and coordination forces between π-electron-rich systems and metal ions.

Gas chromatographic stationary phases can be characterized in terms of their solvent strength (polarity), i.e., the capacity of a solvent for various intermolecular interactions and, solvent selectivity, the relative capacity of compared solvents for a particular intermolecular interaction.

1.1 Retention Index System

In order to overcome the problem of uniform reporting of the retention data, Kovats[1] proposed a system which uses *n*-alkanes as a series of standards. The retention of other substances has been expressed relative to these standards. The retention indices for normal paraffins are by definition equal to hundred times the number of carbon atoms in the molecule. An excellent review on the retention index system is presented by Ettre[2]. Retention index I of a substance x, on a particular stationary phase, at temperature T, can be calculated by equation :

$$I_T^{sp} = 100\left[n\left(\frac{\log R_x - \log R_z}{\log R_{z+n} - \log R_z}\right) + Z\right] \qquad (1)$$

Where,

R_x is the adjusted retention time of x,

R_z is the adjusted retention time of standard alkane with carbon number z and,

R_{z+n} is the adjusted retention time of standard alkane with carbon number $z + n$.

Kovats suggested seven rules correlating the molecular structure with the retention indices. These are:

1) In most homologous series the retention index of the higher members increases by 100 per CH_2 group introduced. For esters of some dibasic acids the increment is only 90-95.

2) On a nonpolar stationary phase the difference in retention indices (ΔI) of two isomers can be calculated from the difference of their boiling points (Δt_b) by;

$$\Delta I \approx 5\Delta t_b \qquad (2)$$

3) The retention index of an asymmetrically substituted compound can be calculated from the retention indices of the corresponding symmetrically substituted substances.

4) Similar substitution in similarly constructed compounds increases the retention indices by the same amount.

5) The retention indices of nonpolar substances (paraffins) remain almost constant for any kind of stationary phase.

6) The retention indices of any substance determined on various nonpolar stationary phases are identical or very close to each other.

7) If the retention index of a substance is determined on a polar and a nonpolar stationary phase, the difference in the retention indices (ΔI) is characteristic of the structure of the substance and can be predicted by adding up the individual increments pertaining to various adhering zones in the molecule. With the help of such a calculation, unknown substances can be identified by comparing the experimentally determined ΔI values computed for the possible structures.

By theory, the retention index of a substance depends only on the identity of the stationary phase and the column temperature, and should be independent of other chromatographic variables. The temperature dependence of the retention index value is a hyperbolic function described by an Antonine type relationship,

$$I(T) = A + \frac{B}{(T + C)} \tag{3}$$

Where,

$I(T)$ = retention index at temperature T,

T = column temperature (°K),

A, B and C are experimentally derived constants.

Sources of error in determining retention indices are primarily due to, (i) instrumental variations in temperature and carrier gas flow rates, (ii) inaccuracies in the measurement of retention times and the column dead time and, (iii) effects caused by support activity and impurities in the stationary phases[3,4]. In gas chromatography the directly measured property is the total retention

time t_R which is a sum of two factors: (i) dead time t_m, which is dependent on the system flow rate as well as on the void volume of the apparatus, and, (ii) the adjusted retention time t'_R which is independent of the equipment used and characterizes the separation process. Consequently,

$$t_R = t_m + t'_R \tag{4}$$

To find out the adjusted retention time, the dead time t_m must be known for a given column. Haken and coworkers[5] have reviewed different methods for estimation of dead time and calculation of Kovats indices. For t_m determination they have recommended the method proposed by Guardino *et al* [6] which uses the linear relation;

$$\ln t'_{Rz} = bI + C, (I = 100z) \tag{5}$$

An iteration is carried out on t_m with b and c estimated using a least-squares fit. Initial estimate of the dead time is used to determine the adjusted retention times. A linear regression then allows b and c to be calculated and thus retention indices can be determined. Subtracting these from the known values gives a sum of differences which is compared with the upper and lower limits. If the estimate of t_m is below the lower limit then the limits are reduced and the estimate of t_m is increased. When this estimate increases above the lower limit, it is decreased and the increment is lowered by a factor of 10. The procedure is repeated until the increment gets lowered than the required precision.

L.Ambrus[7] proposed a simple method for the accurate determination of column dead time which comprises of plotting a graph of t_{z+1} *versus* t_z, where t_z is the retention time of standard alkane with z number of carbon atoms and, t_{z+1} is the retention time of standard alkane with $z + 1$ number of carbon atoms. Slope of the logarithmic plot of the adjusted retention times of *n*-alkanes *versus* carbon number is constant over a wide range of carbon numbers, excluding the first few *n*-alkanes resulting in,

$$\frac{t'_{z+1}}{t'_z} = q \tag{6}$$

where z is the carbon number and q is the ratio of the adjusted retention times of successive members of the homologous series. Since,

$$t'_{z+1} = t_{z+1} - t_m \tag{7}$$

and

$$t'_z = t_z - t_m \tag{8}$$

upon dividing (7) by (8) we get;

$$\frac{t_{z+1} - t_m}{t_z - t_m} = q \tag{9}$$

or alternatively,

$$t_{z+1} = q\,t_z - t_m(q - 1) \tag{10}$$

Data points of the t_{z+1} *versus* t_z plot should therefore fit a straight line. Slope of the straight line is the relative retention q from which t_m can be calculated.

The retention index should be independent of stationary phase loading. In practice this is true within the normal range of phase loadings only if support interactions can be neglected and if the solute is retained entirely by partition. Sample size may also influence the retention. Polar phases show poor solvation properties for alkanes, which consequently exhibit variable retention times at moderate to large sample sizes. Alternative retention index scales based on standards other than alkanes are occasionally used. For example, fatty acid methyl esters in lipid analysis, androstane and cholestane for the calculation of steroid numbers and, naphthalene, phenanthrene, chrysene, and picene for polycyclic aromatic hydrocarbons [3,8,9,10]. Electron capture and flame photometric detectors do not respond significantly to *n*-alkanes. *n*-Alkyl bromides[11] or *n*-alkyl trichloroacetates[12,13] are used with the electron capture detector while *n*-alkyl sulfides are used with the flame photometric detector[14].

1.2 Rohrschneider/McReynolds Phase Constants

The most widely used method for stationary phase characterization was first proposed by Rohrschneider [15-18] and later modified by McReynolds[19]. The founding principle of the method proposed by Rohrschneider is that intermolecular forces are additive and their individual contributions to retention can be evaluated from the difference in retention index values of a series of test probes measured on the liquid phase to be characterized and on squalane, used as a nonpolar reference phase. Figure 1.1 shows a plot of the logarithm of the gas-liquid partition coefficient for a series of

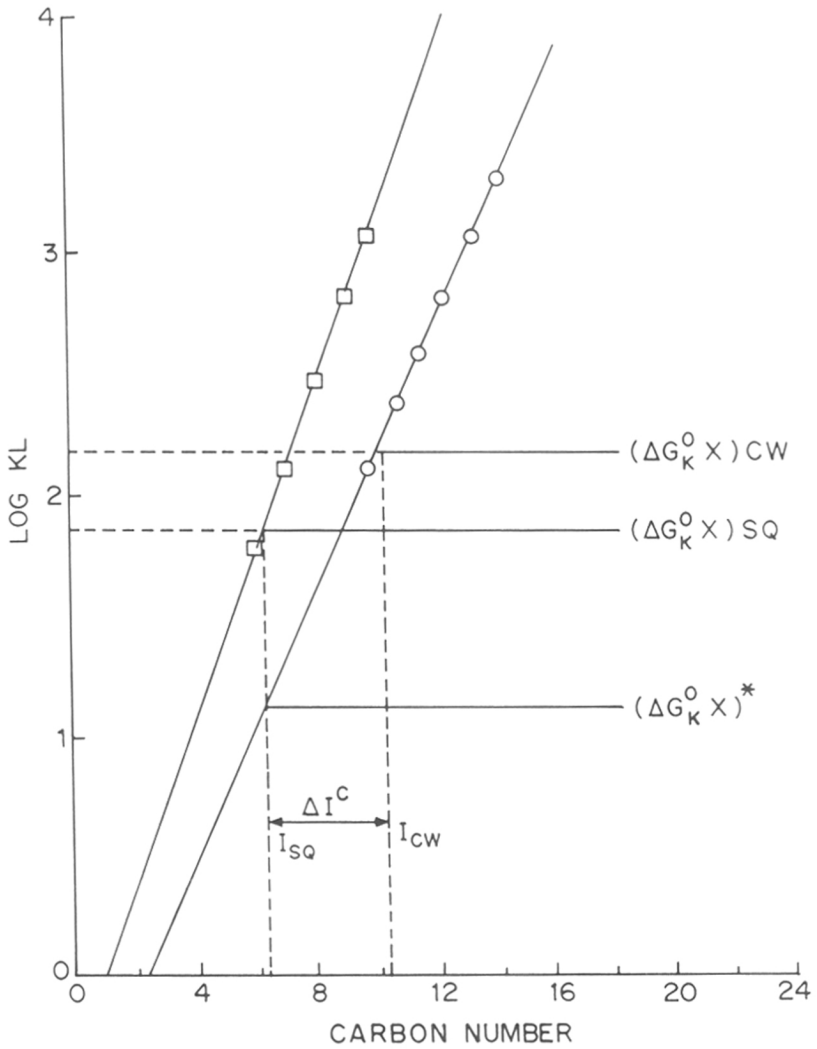


FIG. 1.1 : **DEFINITION OF ΔI^c (ΔI CORRECTED FOR INTERFACIAL ADSORPTION) ACCORDING TO ROHRSCHNEIDER (100 X CARBON NUMBER) (□) ARE FOR n-ALKANES ON SQUALANE AND (○) ARE FOR n-ALKANES ON CARBOWAX 20M.**

n-alkanes against their carbon number for squalane, the nonpolar reference phase, and carbowax 20M as an example of selective phase. In both the cases an approximately linear relationship exists, conforming to the general equation given as;

$$\log K_L^n = A + B(n) \tag{11}$$

where K_L^n is the gas-liquid partition coefficient for an *n*-alkane with n-carbon atoms. If dioxane is selected as a probe of proton-donor capacity, it will elute from carbowax 20M with an index value of I_{CW}^{DIOX} and from squalane with I_{SQ}^{DIOX}. Rohrschneider assumed that the retention index of a substance on a nonpolar phase, such as squalane is determined solely by the dispersive forces and that any difference in the retention index values for a polar phase and a nonpolar phase was due to polar interactions and can be expressed by equation,

$$I_{CW}^{DIOX} = I_{SQ}^{DIOX} + \Delta I \tag{12}$$

where ΔI is the retention index difference resulting from the polar interactions. A thermodynamic definition of ΔI was also provided by Rohrschneider is given as :

$$\Delta I(x) = \frac{100\,[\Delta G_K^o(x)]^{CW} - [\Delta G_K^o(x)]^*}{[\Delta G_k^o(CH_2)]^{CW}} \tag{13}$$

where $[\Delta G_K^o(x)]^{CW}$ is the partial molar Gibbs free energy of solution for dioxane on Carbowax 20M. $[\Delta G_K^o(x)]^*$ is equal to the partial molar Gibbs free energy of solution determined on Carbowax 20M for a hypothetical *n*-alkane coeluting with dioxane on squalane, and $[\Delta G_K^o(CH_2)]^{CW}$ is the partial molar Gibbs free energy of solution for a methylene group. Fundamental to the development of equation (13) is the equivalence between the free energy of the probe and the hypothetical *n*-alkane having the same retention properties (at a constant temperature). Rohrschneider selected benzene, ethanol, methyl ethyl ketone, nitromethane and pyridine as the set of test solutes to characterize the principal interactions responsible for retention in gas chromatography, i.e., dispersion, orientation, induction, and donor-acceptor complexation, and redefined ΔI as follows:

$$\Delta I = ax + by + cz + du + es \tag{14}$$

where a, b, ..., e are solute specific terms and x, y, ..., s are stationary phase characteristic terms. For benzene $a = 100$, $b = 0$, $c = 0$, $d = 0$ and $e = 0$. Therefore,

$$x = \frac{\Delta I}{100} \tag{15}$$

Similarly y, z, u and s are the $\Delta I/100$ terms for ethanol, methyl ethyl ketone, nitromethane and pyridine. The stationary phase selectivity constants are then tabulated in terms of their x, y, z, u and s values. McReynolds improved the applicability of the Rohrschneider method by making the following changes.

(i) Three of the probes suggested by Rohrschneider (ethanol, nitromethane, and 2-butanone) have low retention on many phases, frequently requiring gaseous hydrocarbons as bracketing standards for calculating retention indices. McReynolds replaced these with the less volatile solutes butanol, nitropropane, and 2-pentanone, thus making the determination of retention indices more convenient.

(ii) Increased the number of test solutes from 5 used by Rohrschneider to 10 so as to characterize the liquid-phase interaction better (see Table 1.1)[20].

(iii) Employed ΔI rather than $\Delta I/100$ values to measure the phase constants.

(iv) Used 120°C instead of 100°C for all experimental measurements.

Rohrschneider-McReynolds phase constants are commonly used by the vendors of chromatographic supplies to define the application areas for new phases and by users to identify phases having identical properties. In spite of the more or less universal adoption of the Rohrschneider-McReynolds phase constants, it has been suggested that these values may be unreliable due to a combination of theoretical and practical deficiencies in the protocol used for their calculation[21]. A brief pointwise discussion of the cited deficiencies is presented below.

Choice of Squalane as the Nonpolar Reference Phase :

The choice of squalane imposes some unnecessary restrictions on the method due to the temperature limit of squalane (120°C), poor oxidative stability and poor support deactivating characteristics. Hence McReynolds method can not be used to characterize those liquid phases with

TABLE 1.1

Interactions Characterized by McReynolds Probes

Symbol	Test Substance	Interactions Measured	Characteristic Substance Group
X'	benzene	primarily dispersion with some weak proton-acceptor properties	aromatics, olefins
Y'	butanol	orientation properties with both proton-donor and proton-acceptor	alcohols,nitriles, acids
Z'	2-pentanone	orientation properties with proton-acceptor but not proton-donor	ketones, ethers, aldehydes, esters, epoxides, dimethy-lamino derivatives
U'	nitropropane	dipole orientation properties; weak pro-ton acceptor	nitro and nitrile derivatives
S'	pyridine	weak dipole orientation with strong proton-acceptor capabilities; proton donor properties are absent	aromatic bases
H'	2-methyl-2-pen – tanol		branched-chain compounds, particu-larly alcohols
J'	iodobutane		halogenated com-pounds
K'	2-octyne		
L'	1,4-dioxane	proton-acceptor but not proton-donor capabilities; weak orientation properties	
M'	cis-hydrindane		

minimum operating temperatures above the maximum operating temperature of squalane (120°C). Also, the method provides no information about changes in phase polarity with increasing temperature above 120°C. Suggested solutions to this problem include the use of alternate thermally-stable reference phase[22-25] and a calculation method which does not require a reference phase[26]. Evans *et al.*[27,28] proposed characterizing the stationary-phase interactions in terms of selectivity indices, which are in effect, an extension of the McReynolds systems without the use of reference phase. It is assumed that the *n*-alkanes interact exclusively by dispersion forces in all phases, and that these forces increase in proportion to the molecular weight. The retention index can then be expressed in the form:

$$I \doteq I_m + I^* \tag{16}$$

Here I_m is defined as the retention index of hypothetical *n*-alkane having the same molecular weight (M) as the analyte and determined by the relationship, $I_m = [M - 2.016 / 0.14026]$ where I^* refers to the selectivity index determined by the combined effects of molecular shape and polar interactions.

Type and Number of Test Solutes Required for Stationary Phase Characterization :

No solute interacts by a single interaction. The original probes proposed by McReynolds are usually too volatile to provide accurate retention index values at temperatures much above 120°C. Alternative probes such as benzene and naphthalene derivatives[22,29,30] have been used at higher temperature. There is a controversy among chromatographers about the number of test solutes required for the stationary phase characterization. Common practice dictates that the first five McReynolds probes should be used.

Use of *n*-Alkanes as Retention Index Standards :

The choice of the *n*-alkanes as standard for measuring retention index differences is a poor one since on most polar phases it can not be assumed that their retention is not caused by mechanisms other than gas-liquid partitioning. Aue and Paramasigmani[31] concluded that the magnitude of the McReynolds phase constant was determined largely by the solubility of the *n*-alkanes in the stationary phase and only to a much smaller extent by the specific interactions of test solutes. Poole *et al.*[32] provided the confirmation of this result.

Selection of the Measurement Temperature :

If squalane is replaced by another phase with a higher operating temperature limit the test probes suggested by McReynolds are not sufficiently retained on most phases. Other probe sets suggested are, (i) benzene derivatives,[22] (ii) monofunctional naphthalene derivatives,[22,33] (iii) monofunctional biphenyl derivatives,[34] (iv) a mixture comprising of n-decane, naphthalene, dipyridyl and benzyl[30] and, (v) linatol, estragole and carvone[35].

Except for dispersive interactions the principal intermolecular forces between the solute and the liquid phase are temperature dependent and unlikely to change in an identical manner with temperature for all phases. The selectivity ranking of liquid phases at different temperatures may not be the same and at high temperatures could well be different from their ranking at 120°C. Ashes and Haken[36] reported a small increase in the value of phase constants or no significant change as a function of temperature.

Reliability of the Experimental Data of Rohrschneider and McReynolds :

Most stationary phase classification schemes and models to predict the retention as a function of sample properties are based on the original experimental data published by McReynolds or Rohrschneider. McReynolds article contains scant information concerning the experimental conditions used to determine the phase constants beyond the fact that all data were collected at 120°C. The probability of large errors in the data compilation is very high. Also the influence of interfacial adsorption on retention is not taken into account.

1.3 Solvent Selectivity Triangle

Snyder proposed the characterization of chromatographic solvents by the relative strength of hydrogen bonding interactions and orientation interactions[37,38]. He selected ethanol, dioxane, and nitromethane to measure the relative strength of the three intermolecular forces determined as retention index difference of the test solute on a polar phase and on squalane as a non-polar reference phase. The selectivity coefficients are calculated by equation (17) and plotted on the face of the selectivity triangle

$$X_i = \frac{\Delta I_i}{\Sigma \Delta I_i} \quad \text{(for i= e, d or n)} \tag{17}$$

where X_e is the selectivity parameter for proton acceptor interaction with retention index difference of ΔI_e. X_d is the selectivity parameter for proton-donor interactions with a retention index difference of ΔI_d, and X_n is the selectivity parameter for orientation interactions with retention index difference ΔI_n. The most selective stationary phases will have a large value of $\Sigma \Delta I_i$ and X_i values close to 0.3. Klee *et al.*[39] and Poole and Kersten[40] have used Snyder Triangle method for classification of commonly used stationary phases. Na and Rogers[41] noted that the position of a solvent within the selectivity triangle varied with the selection of the test solutes. Betts[42] used the absolute retention index value on each phase to calculate the selectivity indices and also changed the test solutes. Earlier, Brown[43] had plotted the retention volumes of various test solutes on different stationary phases as triangular coordinates to indicate selective stationary phase interactions.

Since retention index differences are used to calculate the selectivity indices, all the deficiencies pertaining to the Rohrschneider-McReynolds systems are applicable here.

1.4 Solubility Parameters

Karger *et al.*,[44,45] Tijssen *et al.*,[46] Laffort,[47] and Poole *et al.*[48] have employed the extended solubility parameter approach to liquid phase characterization based on the following equation;

$$\delta_T^2 = \delta_d^2 + 2\delta_{in}\delta_d + \delta_o^2 + 2\delta_a\delta_b \qquad (18)$$

where, δ_T is the total solubility parameter, δ_d is a measure of the ability of a substance to participate in dispersive interactions, δ_o is a measure of a substance to participate in orientation interactions, δ_{in} is a measure of the ability of a substance to induce a dipole moment in surrounding molecules, and, δ_a and δ_b are measures of the ability of a substance to function as a proton donor or acceptor respectively. The total solubility parameter δ_T is roughly equivalent to the polarity of a substance. The classical approach suffers from a major limitation that it applies only to interactions in non-polar systems.

1.5 Spectroscopic Methods

The Kamlet-Taft solvatochromic approach developed by Carr *et al.*,[49-51] and the development of selectivity indices from the correlation of gas chromatographic retention data with spectroscopic data by Hawkes and co-workers,[52] are two important contributions to chromatographic science. Hawkes *et al.* conducted dispersion, orientation, acidity and basicity measurements directly

from refractive indices and also from infrared, ultraviolet and nuclear magnetic resonance spectroscopy. The spectroscopic data were compared with the results obtained from gas chromatography to choose a series of test solutes suitable for evaluating the selectivity of stationary phases by a simple, reliable gas chromatographic technique.

According to solvatochromic approach, the observed property of a solvatochromic indicator XYZ in a solvent comprises of a contribution from the indicator in a chosen reference solvent XYZ_0. Additional contributions are those which depend on the solvent polarity or polarizability (Π^*) and, the hydrogen bond donor (α) and hydrogen bond acceptor (β) properties of the solvent. The correlation reads as:

$$XYZ = XYZ_o + s(\Pi^* + d\delta) + a\alpha + b\beta \qquad (19)$$

Here s, a, d and b are the measures of susceptibility of the indicator for a particular interaction and δ is the polarizability correction term. Solvatochromic approach has a sound theoretical basis and further extensions of the method to gas chromatography are anticipated.

1.6 Thermodynamic Approaches

In thermodynamic approaches the polarity of stationary phase is evaluated using thermodyanamic values such as ;

(i) the excess free energy of sorption of a methylene unit in an *n*-alkane or any other monofunctional homologues series,

(ii) the partial molar free energy of sorption of a methylene unit itself [$\Delta G(CH_2)$],

(iii) excess partial molar enthalpies and partial molar free energies of selected functional groups and,

(iv) the six parameter scale based on partial molar free energies of five test compounds and the methylene unit.

The partial molar free energy of solution is a total of the energies of intermolecular interactions, both specific and non-specific. The partial molar Gibbs free energy of solution, ΔG_K^o, for the test solutes can be calculated from gas-liquid partition coefficient K_L^x as :

$$[\Delta G_K^o(X)]^P = 2.303RT_c \log K_L^x \quad (20)$$

where $[\Delta G_K^o(X)]^P$ is the partial molar Gibbs free energy of solution for solute X on stationary phase P. The universal gas constant is denoted by R (1.987 cal mol^{-1} K^{-1}) and T_c is the column temperature (^oK). The difference $\delta[\Delta G_K^o(X)]_{SQ}^P$ between the partial molar Gibbs free energies of solution for solute X on stationary phase P, and squalane can be expressed as;

$$\delta[\Delta G_K^o(X)]_{SQ}^P = [\Delta G_K^o(X)]^P - [\Delta G_K^o(X)]^{SQ} \quad (21)$$

The partial molar Gibbs free energy of solution for a methylene group on phase P is defined as:

$$\Delta G_K^o(CH_2)^P = -2.3RT_cB_P \quad (22)$$

where B_P is the slope of *log* K_L *versus* carbon number for the *n*-alkane or 2-alkanones on phase P.

In fact the $\Delta G(CH_2)$ values estimate the ability of a stationary phase for dispersive interactions only. The ability of a stationary phase to undergo all types of interactions can not be measured correctly by only one parameter, i.e., $\Delta G(CH_2)$. Therefore, one should select a number of test compounds, simulating the best way possible all intermolecular interactions. Considering that five McReynolds test compounds adequately simulate the main types of intermolecular interactions, it was proposed to describe the polarity of sorbents using the partial molar Gibbs free energies of sorption for these compounds. The capacity of the stationary phase for dispersive interactions was measured by a sixth parameter namely, the partial molar Gibbs free energy of sorption of a methylene group.

According to Golovnya *et al.*,[53] the selectivity of two compared liquid phases for a particular solute can quantitatively be expressed as the difference in excess mixing energies. The larger this difference the greater the selectivity difference between the two phases for the tested interaction. The partial molar Gibbs free energy of solution can be computed from the specific retention volumes and retention index values reported by McReynolds as;

$$\Delta G_K^o + 2.3RT \log \rho = -2.3RT\left(\frac{I^x - 100n}{100}\right)B + \log\left(\frac{V_{gn}^o T}{273}\right) \quad (23)$$

where ρ is the density of the liquid phase at the column temperature T, I^x is the retention index of solute, and $B = (\log V_{gn+1}^o - \log V_{gn}^o)$. V_{gn}^0 is the specific retention volume of a normal hydrocarbon with n carbon atoms used to calculate I^x, and V_{gn+1}^o is the specific retention volume of a normal hydrocarbon with $n + 1$ carbon atoms.

The determination of selectivity as free energy difference for a solute on compared phases is the most logical approach to the general problem of a standardized scheme for evaluating selectivity differences.

1.7 Conclusion

Poole et al.[54] reported that the polarity scale derived from summing the ΔI values of the first five McReynolds constants does not correlate with the partial molar Gibbs free energy of solution for a methylene group using retention index values corrected for interfacial adsorption. A good correlation could be obtained between Snyder's 'P' polarity scale and the partial molar Gibbs free energy of solution per methylene group for liquid phases that retained *n*-alkanes, at least in part by partitioning.[40]

Recently some reviews[55,56] published the development on GC retention index scheme and evaluation of different solvent models[57]. Betts[58] reported the implications of solvent selectivity triangles in assessing stationary phases for GC. Fruton *et al.*[59] described a definitive example of anomalous selectivity differences between stationary phases resulting from the hydrocarbon index standards rather than the selectivity probes themselves. Li *et al.*[60] have reported an empirical scheme for the classification of gas chromatographic stationary phases based on solvatochromic linear solvation energy relationships. Golovnya and Polanuer[61] have compared methods for the determination of the polarity and selectivity of stationary phases in gas chromatography from a thermodyanamic viewpoint.

The main disadvantage of scales that use ΔI and I values is due to the large difference in the energetic equivalent of one index unit on different stationary phases.[53] The energetic equivalent of one index unit is calculated from :

$$\Delta G_{\text{i.u.}} = \frac{\Delta G(CH_2)}{100} = -RT \ln\left(\frac{t'_{n+1}}{t'_n}\right) \qquad (24)$$

where t'_{n+1} and t'_n are the adjusted retention times of two neighbouring homologues. The values of the energetic equivalent of a retention index unit at the same column temperature may vary from 0.24 to 5.2 J/mol.[54] This indicates that for two stationary phases a sorbate may have the same retention index but a different energy of sorption.

In spite of all the disadvantages, the McReynolds classification system is still the most popular approach. The thermodyanamic polarity scales also have some shortcomings and the classification of stationary phases in gas chromatography is still very much an active field. In Chapters 2 and 3 we have followed the McReynolds scheme for characterization of stationary phases.

REFERENCES

1. E. Kovats, A.E. Wehrli, *Helv. Chim. Acta,* **42** (1959) 2709.
2. L.S. Ettre, *Anal. Chem.,* **36** (1964) 31A.
3. L.S. Ettre, *Chromatographia,* **6** (1973) 489.
4. F. Vernon, J.B. Suratman, *Chromatographia,* **17** (1983) 597.
5. R.J. Smith, J.K. Haken, M.S. Wainwright, *J. Chromatogr.,* **334** (1985) 95.
6. X. Guardino, J. Albaiges, G. Firpo, R. Rodrigquez Vinals, M. Gassiot, *J. Chromatogr.,* **118** (1976) 13.
7. L. Ambrus, *J. Chromatogr.,* **294** (1984) 328.
8. J.K. Haken, *Adv. Chromatog.,* **14** (1976) 367.
9. M.L. Lee, D.L. Vassilaros, G.M. White, M. Novotny, *Anal. Chem.* **51** (1979) 768.
10. D.L. Vassilaros, R.C Kong, D.W. Later, M.L. Lee, *J. Chromatogr.,* **252** (1982) 1.
11. F. Pacholec, C.F. Poole, *Anal. Chem.*, **54** (1982) 1019.
12. K. Ballschmiter, M. Zell, *Z. Anal. Chem.*, **293** (1978) 193.

13. T. Schwartz, J. Petty, R. Kaiser, *Anal. Chem.*, **55** (1983) 1839.

14. L.N. Zotov, G.V. Golovkin, R.V. Golovnya, *J. High Resolu. Chromatogr. Chromatogr. Commun.*, **4** (1981) 6.

15. L. Rohrschneider, *Adv. Chromatogr.*, **4** (1967) 333.

16. L. Rohrschneider, *J. Chromatogr., Sci.*, **11** (1973) 160.

17. L. Rohrschneider, *J. Chromatogr.*, **22** (1966) 6.

18. L. Rohrschneider, *J. Chromatogr.*, **39** (1969) 383.

19. W.O. McReynolds, *J. Chromatogr.*, **8** (1970) 685.

20. C.F. Poole, S.A. Schuette, *Contemporary practice of chromatography*, Elsvier, Amsterdam, 1984, p. 46.

21. C.F. Poole, S.K. Poole, *Chem. Rev.*, **89** (1989) 377.

22. F. Vernon, C.O.E. Ogundipe, *J. Chromatogr.*, **132** (1977) 181.

23. J.K. Haken, F. Vernon, *J. Chromatogr.*, **186** (1979) 89.

24. F. Reido, D. Fritz, G. Tarjan, E. Kovats, *J. Chromatogr.*, **126** (1976) 63.

25. J.K. Haken, D.K.M. Ho, *J. Chromatogr.*, **142** (1977) 203.

26. J.K. Haken, D. Srisukh, *J. Chromatogr.*, **199** (1980) 199.

27. M.B. Evans, J.K. Haken, T. Toth, *J. Chromatogr.*, **351** (1986) 155.

28. M.B. Evans, J.K. Haken, *J. Chromatogr.*, **406** (1987) 105.

29. C.F. Poole, H.T. Butler, M.E. Coddens, S.C. Dhanesar, F. Pancholec, *J. Chromatogr.*, **289** (1984) 299.

30. R.D. Schwartz, R.G. Mathews, *J. Chromatogr.*, **126** (1976) 113.

31. W.A. Aue, V.J. Paramasigmani, *J. Chromatogr.*, **166** (1978) 253.

32. R.M. Pomaville, C.F. Poole, *Anal. Chem.*, **60** (1988) 1103.

33. M.E. Coddens, K.G. Fruton, C.F. Poole, *J. Chromatogr.*, **356** (1986) 59.

34. M.L. Lee, J.C. Kuei, N.W. Adams, B.J. Tarbet, M. Nishioka, B.A. Jones, J.S. Bradshaw, *J. Chromatogr.*, **302** (1984) 303.

35. T.J. Betts, G.J. Finucane, H.A. Tweedie, *J. Chromatogr.*, **213** (1981) 317.

36. J.R. Ashes, J.K. Haken, *J. Chromatogr.*, **84** (1973) 231.

37. L.R. Snyder, *J. Chromatogr.*, **92** (1974) 223.

38. L.R. Snyder, *J. Chromatogr. Sci.*, (1978) 223.

39. M.S. Klee, M.A. Kaiser, K.B. Laughlin, *J. Chromatogr.*, **279** (1983) 681.

40. B.R. Kersten, C.F. Poole, *J. Chromatogr.*, **452** (1988) 191.

41. P. Shah, H. Na, L.B. Rogers, *J. Chromatogr.*, **329** (1985) 5.

42. T.J. Betts, *J. Chromatogr.*, **354** (1986) 1.

43. I. Brown, *J. Chromatogr.*, **10** (1963) 284.

44. B.L. Karger, L.R. Snyder, C. Eon, *Anal. Chem.*, **125** (1976) 71.

45. B.L. Karger, L.R. Snyder, C. Eon, *Anal. Chem.*, **50** (1978) 2126.

46. T. Tijssen, H.A. Billiet, P.J. Schoenmakers, *J. Chromatogr.*, **122** (1976) 185.

47. P. Laffort, F. Patte, *J. Chromatogr.*, **126** (1976) 625.

48. P.H. Shetty, P.J. Youngberg, B.R. Kersten, C.F. Poole, *J. Chromatogr.*, **411** (1987) 61.

49. P.W. Carr, *J. Chromatogr.*, **194** (1980) 105.

50. J.E. Brady, P.W. Carr, *J. Phys. Chem.*, **86** (1982) 3052.

51. J.E. Brady, D. Bjorkman, C.D. Herter, P.W. Carr, *Anal. Chem.*, **56** (1984) 278.

52. W. Burns, S/J. Hawkes, *J. Chromatogr. Sci.*, **15** (1977) 185.

53. R.V. Golovnya, T.A. Misharina, *J. High Resolut. Chromatogr Chromatogr. Commun.*, 3 (1980) 51.

54. B.R. Kersten, C.F. Poole, K.G. Furton, *J. Chromatogr.*, **411** (1987) 43.

55. G. Tarjan, Sz. Nyiredy, M. Gyor *et al.*, *J. Chromatogr.*, **472** (1989) 1.

56. M.B. Evans, *J. Chromatogr.*, **472** (1989) 93.

57. S.K. Poole, B.R. Kersten, C.F. Poole, *J. Chromatogr.*, **471** (1989) 91.

58. T.J. Betts, *J. Chromatogr.*, **504** (1990) 90.

59. K.G. Furton and R. Morales, *J. High Resol. Chromatogr.*, **14** (1991) 62.

60. J. Li, A.J. Dallas and P.W. Carr, *J. Chromatogr.*, **517** (1990) 103.

61. Golovnya, R.V. and B.M. Polanuer, *J. Chromatogr.*, **517** (1990) 51.

CHAPTER 2

CROWN ETHERS AS STATIONARY PHASES IN GAS CHROMATOGRAPHY

CHAPTER 2

CROWN ETHERS AS STATIONARY PHASES IN GAS CHROMATOGRAPHY

2.1 Applications of Crown Compounds in Analytical Chemistry

Multidentate monocyclic ligands with any type of donor atoms are called coronands or 'crown compounds' while the term 'crown ether' is reserved for cyclic oligoethers exclusively containing oxygen as donor atom. Bridging of a classical monocyclic crown ether with an additional oligoether chain leads to a bicyclic type of ligand known as 'cryptands'. Noncyclic crown type compounds are known as 'podands'. The structures of these compounds are shown as [1], [2] and [3].

The crown compounds consist of a series of lipophilic and hydrophilic structural elements. In hydrophilic media they behave as a fat droplet in water while in lipophilic media as a water droplet in oil. In the former case an endolipophilic cavity is formed whereas in the latter, an endohydrophilic one gets created. The hydrophilic electronegative cavity is ideally suited for alkali metal and alkaline earth metal cations according to their size. Because of the electroneutrality of the crown compounds the anion of the salt is bound to its cation. The preference for a certain salt is expressed by the value of the selectivity constant. When selectivity constants are high, separations and determinations of cations and anions are possible.[1]

2.1.1 Separation methods

Masking

1,4,7,10-Tetraazacyclododecane-N,N',N", N'''-tetraacetic acid [4] forms the strongest complex ever recorded for Ca[II], with pK = 15.85. The complexing agent is suitable for masking Ca[II] in the presence of Mg[II], Sr[II] and Ba[II].

Extractions

Extractions with monomeric cyclic polyethers are performed in presence of the soft picrate ion since the hardness of the anion of an extracted ion pair markedly affects the extraction coefficients. The extracted salts can be directly determined by photometry. Alkali metal picrates undergo extraction into benzene in the presence of 18-crown-6 in the order $K^+ > Rb^+ > Cs^+ > Na^+$. Soluble polymeric crown compounds show extraction coefficients upto 250 times higher than those of the corresponding monomers.[2]

Chromatography

In column chromatography monomeric cyclic polyethers adsorbed on silica gel or dissolved in the eluent may be used to separate cations and optically active compounds. Optically active primary amino ester salts are eluted by a chloroform solution of different derivatives of di(binaphthyl) 18-crown-6. They show complete chromatographic separation.[3,4]

Polymeric cyclic polyethers are more widely applied. They allow the separation of cations, anions and organic compounds. Numerous exchangers which are able to bind definite inorganic salts or organic compounds are obtained by condensation, substitution and copolymerization reactions with cyclic polyethers of different structure and ring size. They have various applications[5].

The exchangers can also be applied as adsorbents in thin-layer chromatography and electrophoresis. For this purpose poly (ethylene terephthalate) sheets[6] are coated with suspensions of powdered exchangers in poly (vinyl alcohol) solutions. Many organic solvents except chloroform, dichloromethane and dioxane can be used.

It is possible to separate certain neutral amino acids, e.g., isoleucine, valine, serine and α-aminobutyric acid by thin-layer electrophoresis on the exchanger layers.

Precipitate formation between crown ethers and phosphomolybdic acid gives a new class of ion exchangers which have a marked selectivity for the alkali metal ions. The use of crown ether-phosphomolybdic acid sorbents for metal ion separations has been reported[7]. Kolthoff[8] has published a comprehensive review paper on the application of macrocyclic compounds in chemical analysis.

2.1.2 **Determination methods**

Photometry:

All picrate extractions by cyclic polyethers offer the possibility of direct photometric determination of the extracted salts. The introduction of chromophoric groups into cyclic polyethers yields selective photometric reagents. These reagents are designed to give rise to specific colour changes on complexation of normally colourless metal ions such as the alkali and alkaline earth cations. A range of derivatives of this type have been developed for use as spectrophotometric analytical reagents for particular cations. Non-ionizable reagents are those which do not contain ionizable protons in their structure. The modified rings retain the metal ion discriminating ability which is characteristic of the corresponding simple crowns. Ionizable reagents incorporate a chromophoric group bearing an ionizable proton (or protons); in such systems the colour change is associated with ionization of the proton. The examples of non-ionizable [5] and ionizable [6] and [7] chromogenic crown ethers are shown on the next page.

Electrochemical methods:

Potentiometry

Sodium(I) and potassium(I) can be titrated with aqueous solutions of 2.2.1 cryptand and 2.2.2 cryptand respectively, using a cation selective electrode.

Conductometry

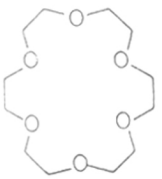
0.001 Mol/l CsCl can be determined by conductometric titration in methanol/chloroform (90/10 v/v) with 18-crown-6.

Polarography

The stability constants of dicyclohexano 18-crown-6 with sodium or potassium salts can be determined polarographically.

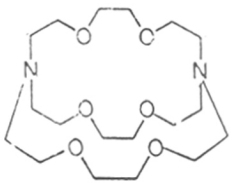
Ion sensitive electrodes

Depending on the number of coordination atoms and on the type of the chain ends, there are ion-sensitive carriers for Na(I)[9], Ca (II) and Ba (II)[10] [refer structures 8,9,10] respectively.



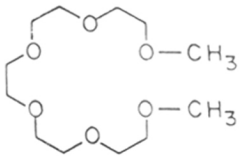
(1)

18-CROWN-6



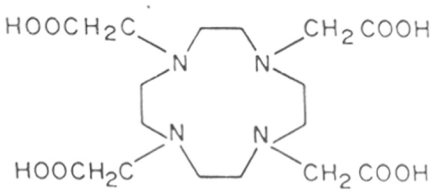
(2)

[2·2·2] CRYPTAND



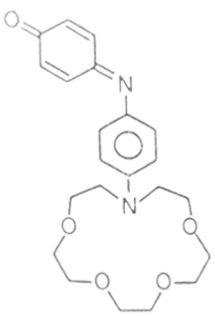
(3)

PENTAGLYME (PODAND)



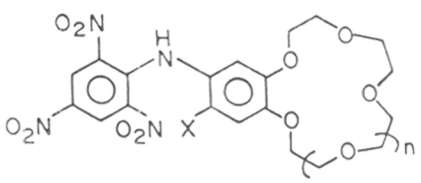
(4)

1,4,7,10-TETRAAZACYCLODODECANE-N,N′,N″,N‴-TETRAACETIC ACID

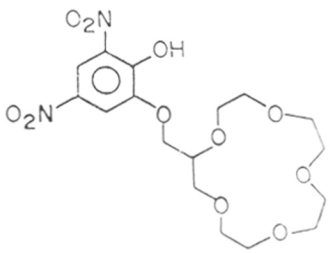


(5)

NON-IONIZABLE



(6)



(7)

IONIZABLE

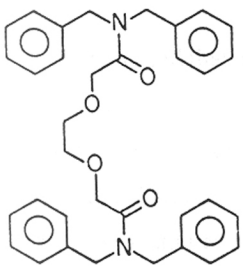
EXAMPLES OF CHROMOGENIC CROWN ETHERS

2.1.3 Applications of crown ethers in HPLC and GC

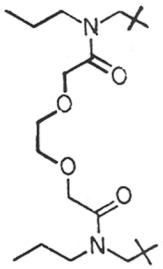
Crown ethers such as [11] form complexes not only with alkali metal ions and alkaline earth metal ions but also with salts of primary alkylamines. Cram and co-workers[11] have developed optically active crown ethers such as the optically active binaphthyl derivative [12] and have utilized it for racemate resolution. Alkylammonium ions $R - \overset{+}{N}H_3$ (where R is a chiral group) are drawn into the asymmetric cavity of the crown ether [12] and diastereomeric complexes with various degrees of stability are formed. Such suitably substituted ammonium salts can thus be separated by multiple distribution between an aqueous phase and the solution of the binaphthyl derivative [12]. Covalent bonding of optically active crown ethers on cross-linked polystyrene yields optically active adsorbents. These can be used to resolve several α-amino acids and their methyl esters as perchlorates into enantiomers by HPLC[12]. Blaschke[13] has reviewed the applications of chiral crown ethers.

Nakagawa *et al.*[14] have studied liquid chromatography with crown ether containing mobile phases. The effect of hydrophobicity and cavity size of the crown ether in crown ether-containing mobile phases on the retention of amino compounds in reversed phase HPLC has been studied. It has been pointed out that a protonated primary amino group fits well in the cavity of the 18-membered crown ether, whereas ortho diamino compounds fit in 24- and 27- membered crown ethers. Reversed phase HPLC of substituted anilines utilizing the molecular recognition ability of crown ethers has been compared with ion-pair chromatography[15].

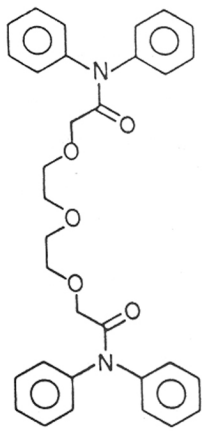
The use of crown ethers in gas chromatography has been rare. Vigalok and Bubachinkova[16] first reported the chromatographic characteristics of 18-crown-6. Ono[17] separated dichlorophenol isomers on 20% dibenzo-18-crown-6 coated on acid washed firebrick C_{22} (2.25m x 3mm I.D, SS Column). Li[18,19] attempted to separate hydrocarbons, alcohols, phenols, esters and amines on various crown ether stationary phases and pointed out that they are particularly suitable for the separation of mixtures containing constituents with high boiling points. The benzo crown ether compounds contain a large number of π-bonded aromatic rings, polar C-O bonds and nonpolar alkyls from which various types of intermolecular reactions can arise. Consequently they can separate polar compounds such as alcohols and phenols; medium or weakly polar compounds such as ketones and esters and nonpolar compounds such as hydrocarbons. Fine *et al.*[20] reported preparation and GC characterization of some silacrown ether stationary phases. Lee *et al.*[21] compared oligo (ethylene oxide) substituted polysiloxanes with polyethylene glycol as stationary phase for



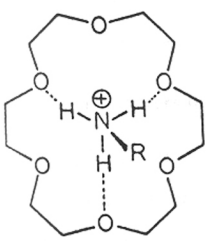
(8)



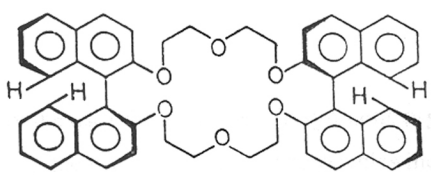
(9)



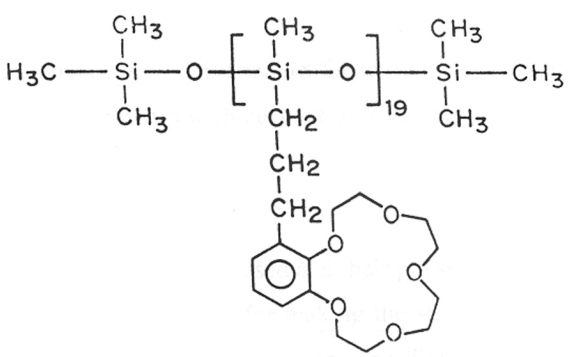
(10)



(11)



(12)



(13)

capillary GC and found that crown ether-poly-siloxanes have unique selectivity because of the size and shape of the crown ether cavity. Separations of aromatic hydrocarbons, chlorine-containing compounds etc. on carbochrome modified with dibenzo-18-crown-6 have been reported[22]. The chromatographic characteristics of some dipentadecyl crown ethers and n-undecyloxymethyl-18-crown-6 have been studied recently[23,24]. The inclusion properties of some crown ethers have been studied by GC[25] and the results indicate that the GC measurements can significantly contribute to the evaluation of properties of newly prepared crown ethers and to the characterization of their interaction with various organic substances. Preparation and GC characterization of some immobilized crown ether polysiloxane stationary phases has been published[26]. It has been reported that the crown ether-polysiloxanes are convenient for separating apolar and polar compounds, especially those having the ability to form hydrogen bonds with the oxygen atom in the crown ether ring. Zhang and co-workers[27] have reported poly(crown ether) stationary phases for open tubular capillary column chromatography. They have achieved separation of *ortho, meta* and *para* cresols on PAB15C5S [13] column at 136.6°C.

2.1.4 Crown ethers in synthetic organic chemistry

Apart from analytical applications, reports of the use of crown ethers in synthetic organic chemistry have been quite common. Reactions involving the use of polyether macrocycles include saponification, esterification, redox reactions, nucleophilic substitution, elimination, condensation, rearrangements, Wittig reactions, Cannizzaro reactions, Michel additions etc. The production of a 'free counter' ion in an organic solvent such as benzene or the solubilization of an inorganic reagent ($KMnO_4$) in organic solvent has formed the basis for many of these reports. Crown ethers are also used in phase transfer catalysis[28].

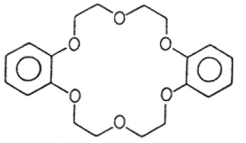
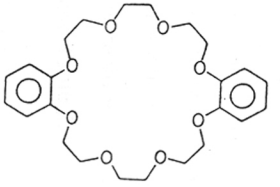
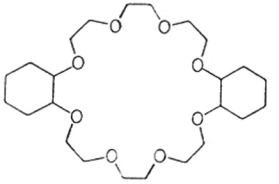
2.2 Comparison between Dibenzo-18-Crown-6, Dibenzo-24-Crown-8 and Dicyclohexano-24-Crown-8 with respect to Polarity, Selectivity and Thermal Stability

2.2.1 Experimental

The structures of the crown ethers used and their physical properties are given in Table 2.1. Aldrich grade crown ethers were used for making the stationary phases. Solvent used for dispersing the stationary phase on the solid support was dichloromethane. The crown ethers

TABLE 2.1

THE STRUCTURES AND THE PHYSICAL PROPERTIES OF THE CROWN ETHERS USED

	Name of the crown ether	Structure	Melting point	Cavity Size
1)	Dibenzo-18-crown-6 (DB18C6)		162-164°C	4 A° coplaner and symmetrical
2)	Dibenzo-24-crown-8 (DB24C8)		103-105°C	> 4 A° cylindrical and symmetrical
3)	Dicyclohexano 24-crown-8 (DCH24C8)		23-27°C	

(DB18C6, DB24C8 and DCH24C8) were coated on chromosorb WAW DMCS (80-100 mesh) at concentrations of 3%, 10% and 20%. The crown ethers were weighed depending on the desired concentrations, dissolved in dichloromethane and the resulting solution was poured at room temperature on weighed amount of chromosorb WAW DMCS (80-100 mesh). The mixture was shaken intermittently 4-5 times (2 hrs) and kept overnight. The solvent was removed with the help of rotavapor and the solids were oven dried. The coated supports were packed in stainless steel columns (1.8m x 3mm O.D.). The maximum operating temperatures for the stationary phases were first established using thermogravimetry and differential scanning calorimetry (DSC) and the phases were then tested chromatographically by the method given by Pulsifer *et al.*[29] Nitrogen used as the carrier gas at the flow rate of 30 cc/min was measured by Gasmet gas flow meter. The column void volumes were determined by the iterative method given by Guardino *et al.*[30] (pl. see Appendix). The efficiencies of the crown ether columns were determined at 170°C and compared with that of Carbowax 20M.

A Hewlett Packard Model 5880A Gas Chromatograph equipped with a level 4 integrator and computing system with flame ionization detector was used. The selectivities of crown ethers were characterized by injecting various aromatic positional isomers. The individual compounds which were used as the standard samples were 96-98% pure as found by GC. The boiling points of these compounds are given in Table 2.2 and Table 2.7.

Calculation of specific retention volume (Vg):

Specific retention volume *Vg* is the volume of gas (measured at 0°C) which has passed through a hypothetical ideal column, containing 1 gm of fixed phase, when the peak maxima emerges. This ideal column has a constant pressure (equal to the pressure at which the volume of gas is measured) throughout and zero dead volume. *Vg* can be estimated from the following equation:

$$V_g = T'_R \times f_c \times J \times \frac{P_o}{P_n} \times \frac{T_n}{T} \qquad (1)$$

where T'_R = corrected retention time (min)

f_c = carrier gas velocity (cc min^{-1})

J = James Martin factor $= \frac{3(P^2-1)}{2(P^3-1)}$

TABLE 2.2

POSITIONAL ISOMERS STUDIED

	Name of the compound	Boiling point	Melting point
1.	2,6-Dimethyl phenol	203°C	46-48°C
2.	2,5-Dimethyl phenol	212°C	75-77°C
3.	2,4-Dimethyl phenol	211°C	22-23°C
4.	2,3-Dimethyl phenol	217°C	73-75°C
5.	3,5-Dimethyl phenol	222°C	65-66°C
6.	3,4-Dimethyl phenol	227°C	65-68°C
7.	*o*-Cresol	191°C	32-34°C
8.	*m*-Cresol	203°C	8-10°C
9.	*p*-Cresol	202°C	32-34°C
10.	1-Chloro-2-nitrobenzene	246°C	33-34°C
11.	1-Chloro-3-nitrobenzene	236°C	42-44°C
12.	1-Chloro-4-nitrobenzene	242°C	83°C
13.	2-Chloroaniline	208°C	-
14.	3-Chloroaniline	230°C	-
15.	4-Chloroaniline	232°C	68-71°C
16.	2-Nitrophenol	-	-
17.	3-Nitrophenol	194°C/70mm	96-98°C
18.	4-Nitrophenol	279°C	113-115°C
19.	2-Nitroaniline	284°C	73-76°C
20.	3-Nitroaniline	-	112-114°C
21.	4-Nitroaniline	260°C/100 mm	148-149°C

$$P = \frac{\text{inlet pressure}(P_i)}{\text{outlet pressure}(P_o)}$$

P_n = normal pressure = 760mm

T_n = normal temperature = 273°K

T = temperature of the column in °K

The polarities of DB24C8 and DCH24C8 were measured at 120°C and that of DB18C6 at 180°C in terms of McReynolds constants. The absolute values of retention indices for McReynolds probes at 120°C on squalane, which is used as a standard reference were taken from the original reference[31]. Similarly, absolute values of the retention indices for test probes at 180°C on Apiezon MH, which is a standard reference phase, were also taken from the original reference[32].

2.2.2 Results and Discussion

Phase transition and thermal stability studies

The specific retention volume (V_g) for the test compound *p*-toluidine was calculated at 10°C intervals and a graph of *log* V_g *versus* 10^4 times the inverse of absolute temperature was plotted for *p*-toluidine on DB18C6 and DCH24C8 columns (Fig. 2.1). There is no phase transition in DCH24C8, as is indicated by the straight line. The temperature range from T_1 (140°C) to T_2 (153°C) is the transition range for DB18C6 as determined by GC. At temperatures below T_1, solute retention will depend essentially on adsorption processes, but as the temperature is raised above T_1, the stationary phase starts to melt and it attains the liquid state at T_2. At the latter temperature the retention of solutes is due to partition. This temperature matches with the phase transition temperature obtained by differential thermal analysis (DTA). Figure 2.2 shows the plot of *log* V_g *versus* 10^4 times the inverse of absolute temperature for acetonitrile on DB24C8. The phase transition temperatures obtained from the graph (90° and 105°C) are in good agreement with those obtained by DSC (85° and 107°C). DB24C8 becomes a true liquid at 105°C.

The DB18C6 and DCH24C8 columns were first conditioned at 190°C for 4 hrs. The specific retention volume (V_g) for octadecane was then determined at 160°C. Subsequently stepwise conditioning was done at 10°C intervals to establish whether there was any change in V_g. With 10% DB18C6 no change in the value of V_g was noted whereas a 2.3% decrease was observed for octadecane on 10% DCH24C8 after conditioning at 190°C for 2 hrs. The 10% DB24C8 column was

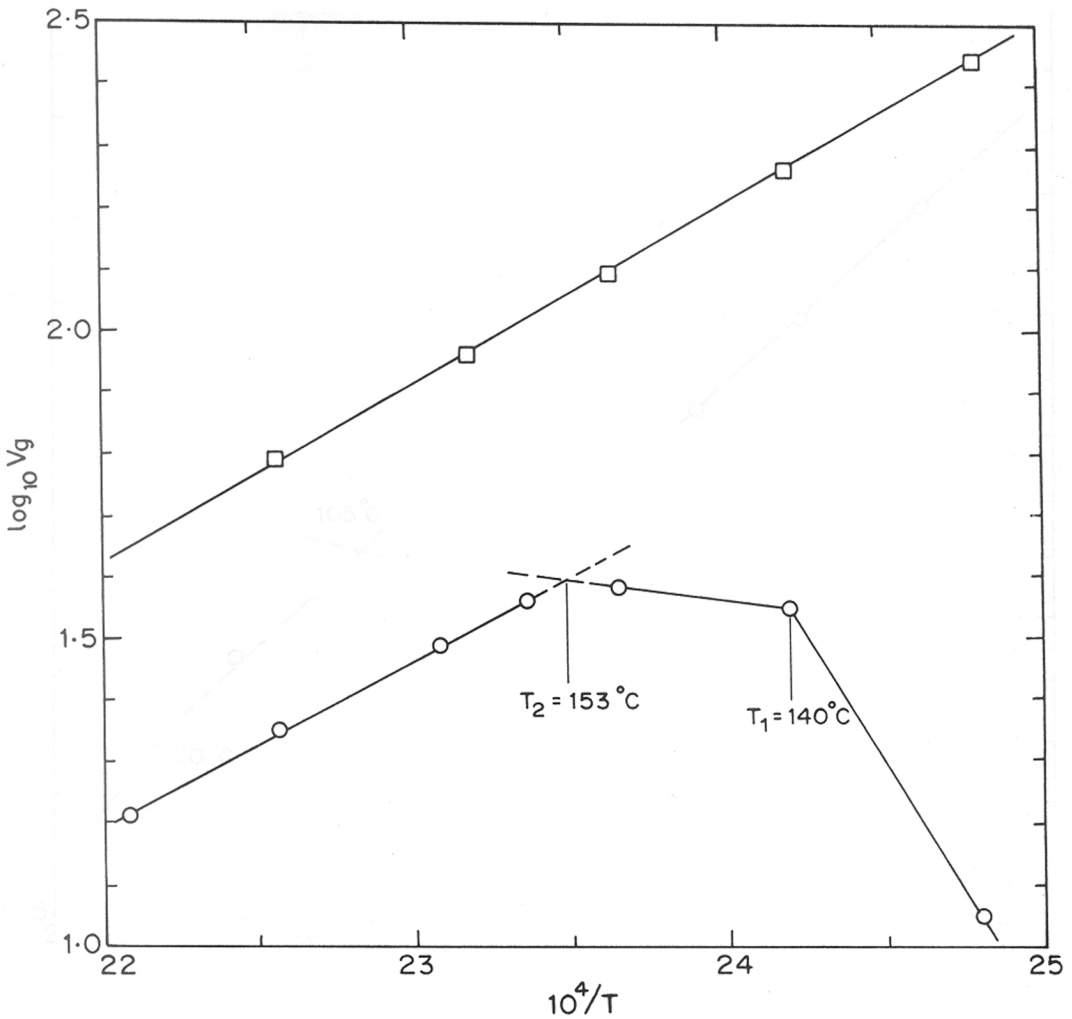


FIG. 2.1 : **PLOTS OF log (SPECIFIC RETENTION VOLUME) AGAINST INVERSE OF ABSOLUTE TEMPERATURE $X10^4$ FOR p-TOLUIDINE ON 10% DB18C6 (○) AND 10% DCH24C8 (□) COLUMNS**

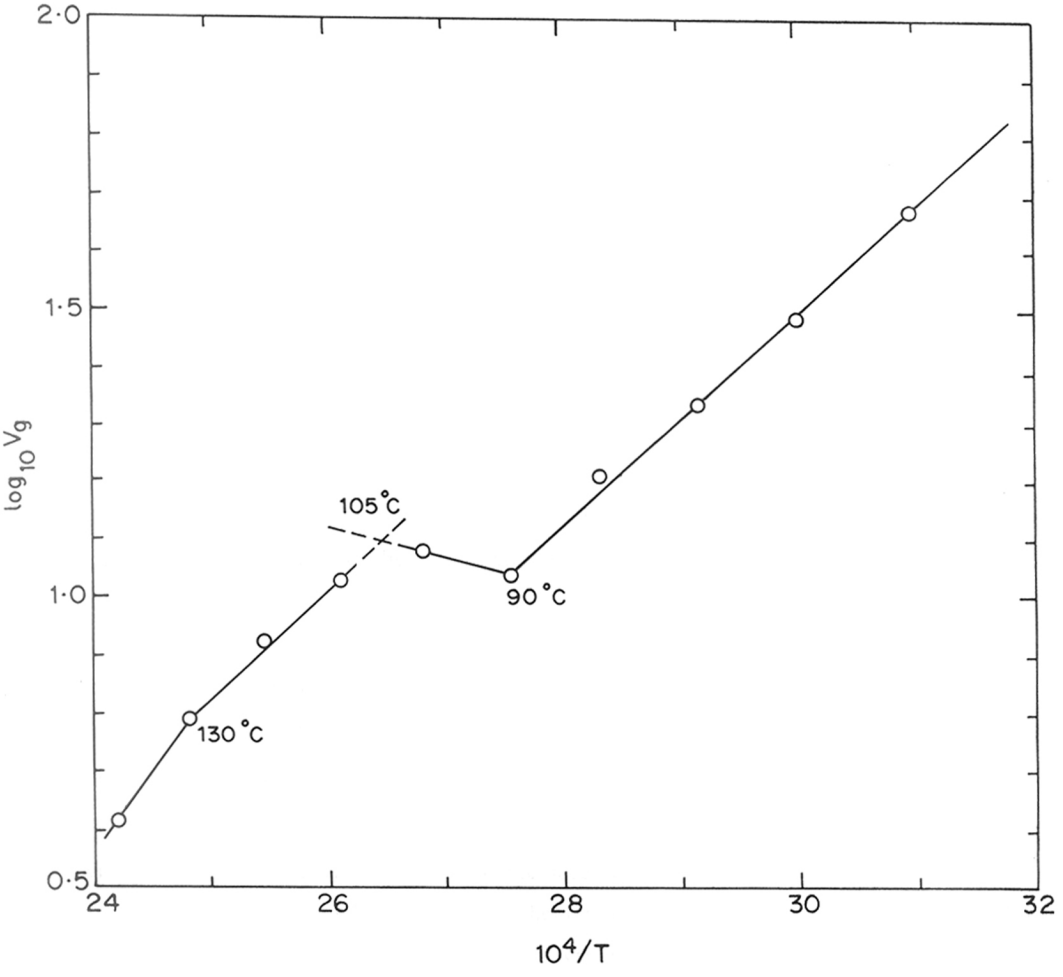


FIG. 2.2 : PLOT OF log (SPECIFIC RETENTION VOLUME) AGAINST INVERSE OF ABSOLUTE TEMPERATURE FOR ACETONITRILE ON 10% DB24C8 COLUMN

first conditioned upto 175°C and the specific retention volume for octadecane was determined at 140°C. No change in V_g was apparent after stepwise conditioning of the column at 150, 160, 170 and 175°C.

Table 2.3 gives a comparison of the phase transition temperatures and maximum operating temperatures for the different crown ethers. A comparison of efficiencies in terms of total number of plates (N) and height equivalent to a theoretical plate (HETP) between various crown ethers and carbowax 20M for 3 different solutes at 170°C is shown in Table 2.4. It can be seen that DCH24C8 is the most efficient column of all the crown ethers and carbowax 20M fares better than DCH24C8 only in the case of 3,4-dimethylphenol, i.e., alcohols.

Polarity and selectivity studies

McReynolds constants (ΔI), retention indices (I), capacity factors (k'), dead time values (t_m) and average polarities of the various crown ethers together with the ΔI values for carbowax 20M and tricresyl phosphate are given in Table 2.5. The comparison of ΔI values shows that average polarity of DCH24C8 is similar to that of tricresyl phosphate and the latter retains ketones and nitro compounds more strongly. Stationary phase DB24C8 has an average polarity similar to that of carbowax 20M and it retains proton accepting compounds much more strongly whereas Carbowax 20M retains alcohols more strongly.

The ring structure of the crown compounds provides selectivity for organic compounds with hydroxyl groups, which is based on the accessibility and availability of hydrogen bonding between the hydroxylic hydrogens and the ether oxygen atom[26]. Substances potentially capable of interacting with the cavities of the crown ethers were used as solutes. Since, Ono[17] had reported the separation of dichlorophenol isomers on 20% DB18C6, we compared the selectivities of the crown ethers with respect to dichlorophenols and similar compounds such as dimethylphenols, cresols, nitrophenols, nitrochlorobenzenes, chloroanilines etc. Stationary phase DB24C8 has the largest ΔI value for nitro compounds and accordingly it gives the best separation of nitrochlorobenzene isomers as is shown in Fig. 2.3. The analysis time is also much shorter (ca-7 min) than that for the same separation on carbochrome modified with DB18C6 (12 min)[22]. Previously we have reported the separation of nitrochlorobenzene isomers on 5% isopropylidenebisphenol diacetate column[33] (also see Chapter 3).

TABLE 2.3

COMPARISON OF PHASE TRANSITION TEMPERATURES AND MAXIMUM OPERATING TEMPERATURES

	Stationary phase	Temperature at which decomposition starts#	Maximum allowable temperature	Phase transition temperature		
				By DTA[a]	By DSC[b]	By GC[c]
1.	DB18C6	210°C	190°C	151°C	161.25°C	153°C
2.	DB24C8	185°C	175°C	60°C 80°C	85°C 107.5°C	90°C 105°C
3.	DCH24C8	199.5°C	180°C	--	--	--

\# This temperature was determined by thermogravimetry

a. DTA is differential thermal analysis

b. DSC is differntial scanning calorimetry

c. GC is gas chromatography

TABLE 2.4

COMPARISON OF EFFICIENCY IN TERMS OF TOTAL NO OF PLATES (N) AND HEIGHT EQUIVALENT OF THEORETICAL PLATES (HETP) BETWEEN VARIOUS CROWN ETHERS AND CARBOWAX 20M AT 170°C

	Octadecane		o-Nitrochlorobenzene		3,4-Dimethylphenol	
	N	(HETP) (cms)	N	(HETP) (cms)	N	(HETP) (cms)
10% DB18C6	331	0.54	465	0.38	243	0.74
10% DB24C8	798	0.23	1611	0.11	1247	0.14
10% DCH24C8	836	0.22	1697	0.106	1979	0.09
Carbowax 20 M	611	0.29	1527	0.118	2283	0.08

TABLE 2.5

CAPACITY FACTORS (k′), RETENTION INDICES (I), McREYNOLDS' CONSTANTS (ΔI) AND AVERAGE POLARITY FOR THE McREYNOLDS PROBES ON VARIOUS CROWN ETHERS ALONGWITH THE ΔI VALUES FOR CARBOWAX 20M AND TRICRESYL PHOSPHATE

Stationary phase		McReynolds Probes					Average Polarity
		Benzene	n-Butanol	2-Pentanone	Nitropropane	Pyridine	
DCH24C8 (t_m=0.268)	ΔI	153	333	194	331	291	260
	I	806	923	821	983	990	
	k′	1.31	2.47	1.43	3.40	3.55	
Tricresyl phosphate	ΔI	176	321	250	374	299	284
DB24C8 (t_m=0.254)	ΔI	286	454	366	544	607	452
	I	939	1044	993	1196	1206	
	k′	0.81	1.44	1.09	3.29	3.49	
Carbowax 20M	ΔI	322	536	368	572	510	462
DB18C6* (t_m=0.314)	ΔI	231	526	472	475	521	442
	I	1314	1558	1525	1580	1507	
	k′	0.56	2.57	2.09	2.95	1.87	

McReynolds constants for DB18C6 were found out at 180°C using high temperature probes suggested by Vernon and Ogundipe[32] i.e. n-butyl benzene, benzyl alcohol, acetophenone, nitrobenzene and aniline. ; t_m = dead time in minutes.

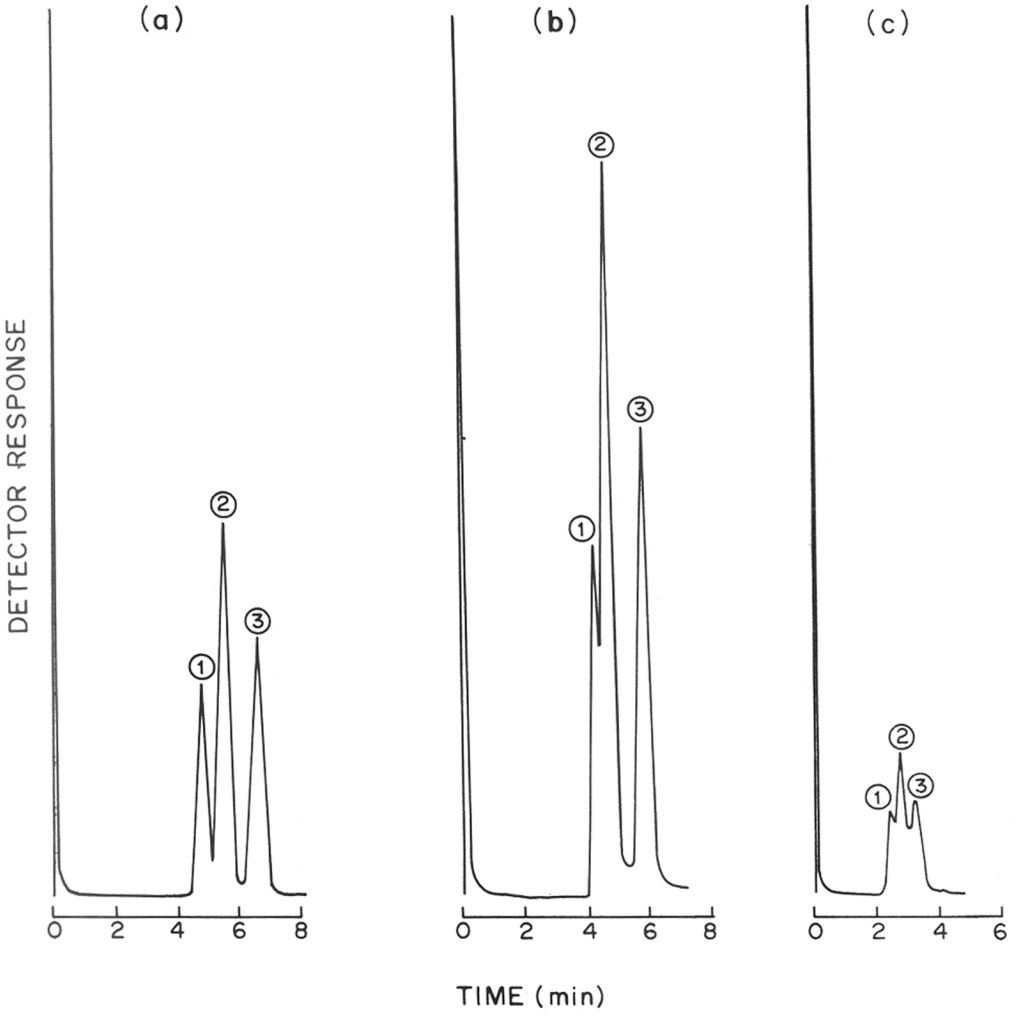


FIG. 2.3 : SEPARATION OF NITROCHLOROBENZENE ISOMERS ON VARIOUS CROWN ETHERS.

Oven temp. 170°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 210°C, Detector temp. 250°C.

Peaks : 1) 1-Chloro-3-nitrobenzene, 2) 1-Chloro-4-nitrobenzene, 3) 1-Chloro-2-nitrobenzene

(a) 10% DB24C8, (b) 10% DCH24C8, (c) 10% DB18C6

The separation of dimethylphenol isomers on the crown ether stationary phases is shown in Fig. 2.4. It was not possible to separate 2,4- and 2,5-dimethylphenol on any of the crown ethers and 2,3- and 3,5-dimethylphenol were only partially resolved. When the loading was increased to 20%, the peaks became broad and the separation did not improve much. Separations of *ortho*, *meta* and *para* cresols and -chloroanilines were also tried without any success for *meta* and *para* separation. When dichlorophenol isomers were injected on 10% DB18C6, post tailing was observed. The retention times of these isomers depend on the injected amount. The DCH24C8 and DB24C8 columns gave symmetrical peaks but without any separation.

Nitrophenol and nitroaniline isomers were injected on the 3% crown ether columns without any derivatization. *Ortho* isomers eluted immediately after the solvent peak whereas *meta* and *para* isomers were retained for a long time. The retention times of *para*-nitrophenol and *para*-nitroaniline were too long on DB24C8 and DCH24C8 at 170°C and 180°C respectively, indicating that these molecules must be fitting well in the cavities of crown ethers because of their rod like shapes. The *para* isomer shows a stronger hydrogen bonding interaction of the ether oxygen than the *ortho*-substituted compound, which forms intramolecular hydrogen bonds between the nitro oxygen atom and the phenolic or amino hydrogen atoms. Hence the relative retention of the *para*- and *ortho*- substituted pair on the crown ether phases is high.

The separation and analysis time for nitrophenols and nitroanilines on the 3% DB18C6 column is reasonable and one can use a DB18C6 column for the direct analysis of nitrophenols (coating can be even less, e.g., 1%). The analysis of phenols is important owing to its practical applications in biochemical, clinical, forensic and wood chemistry and also in food inspection and environmental pollution control. Table 2.6 gives the retention indices of various positional isomers on different crown ethers. The separations of nitrophenol and nitroaniline isomers on the crown ether phases are shown in Fig. 2.5 and Fig. 2.6 respectively.

Since DB24C8 column gives excellent separation of positional isomers of nitro compounds, we injected various isomers of nitrotoluene, dichloronitrobenzene, nitroanisole, anisidine, dichlorobenzene and phenylenediamine on 10% or 3% DB24C8 at 170°C. The retention data and relative retention values as well as the physical properties of these isomers are given in Table 2.7. The isomers follow elution sequence as is indicated by their boiling points. Figures 2.7 to 2.12 show the separations of positional isomers of nitrotoluene, dichloronitrobenzene, anisidine, nitroanisole

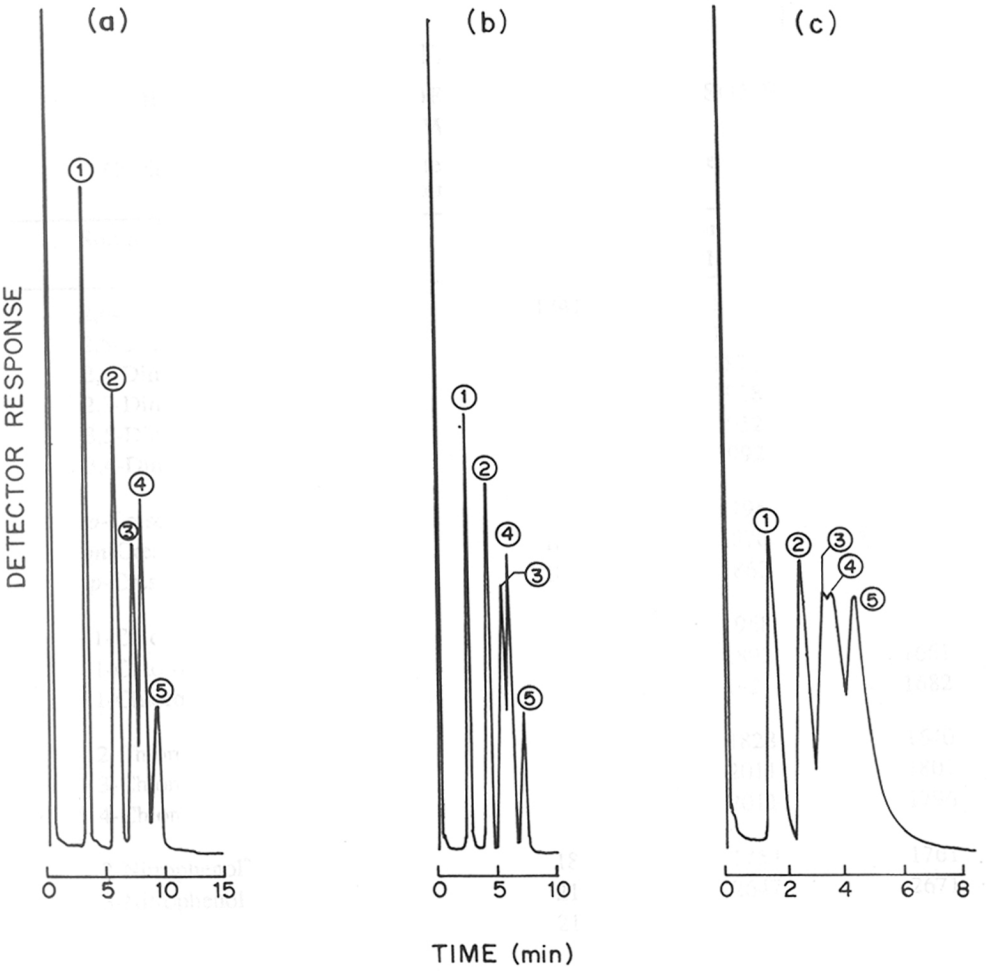


FIG. 2.4 : **SEPARATION OF DIMETHYLPHENOLS (DMP) ON VARIOUS CROWN ETHERS.**

Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 210°C, Detector temp. 250°C.

Peaks: 1) 2,6-DMP, 2) 2,4-+2,5-DMP, 3) 2,3-DMP, 4) 3,5-DMP, 5) 3,4-DMP.

(a) 10% DCH24C8 at 170°C (b) 10% DB24C8 at 170°C, (c) 10% DB18C6 at 160°C.

TABLE 2.6

RETENTION INDICES OF SOME POSITIONAL ISOMERS ON 10% CROWN ETHERS AT 170°C

Carrier gas nitrogen at a flow rate of 30 ml min^{-1}, Injection temperature 220°C; Detector temperature 250°C

Solute	Stationary Phase		
	DB18C6	DB24C8	DCH24C8
2,6-Dimethylphenol[a]	1741	1749	1593
2,5-Dimethylphenol	1858	1871	1724
2,4-Dimethylphenol	1858	1870	1721
2,3-Dimethylphenol	1919	1928	1767
3,5-Dimethylphenol	1935	1952	1789
3,4-Dimethylphenol	1976	1992	1820
o-Cresol	1785	1796	1593
m-Cresol	1858	1870	1655
p-Cresol	1849	1863	1651
1-Chloro-2-nitrobenzene	1983	1968	1728
1-Chloro-3-nitrobenzene	1918	1892	1661
1-Chloro-4-nitrobenzene	1949	1927	1682
2-Chloroaniline	1835	1828	1640
3-Chloroaniline	2010	2011	1801
4-Chloroaniline	2005	2011	1796
2-Nitrophenol[b]	1839	1754	1761
3-Nitrophenol	2130	2647	2671
4-Nitrophenol	2183	2798	2804
2-Nitroaniline	2057	2335	2312
3-Nitroaniline	2124	2533	2504
4-Nitroaniline	2230	2816	2774

a Dimethylphenols were injected at 160°C on DB18C6

b Nitrophenols were injected at 180°C on 3% DB18C6 and 3% DCH24C8 and at 170°C on 3% DB24C8.

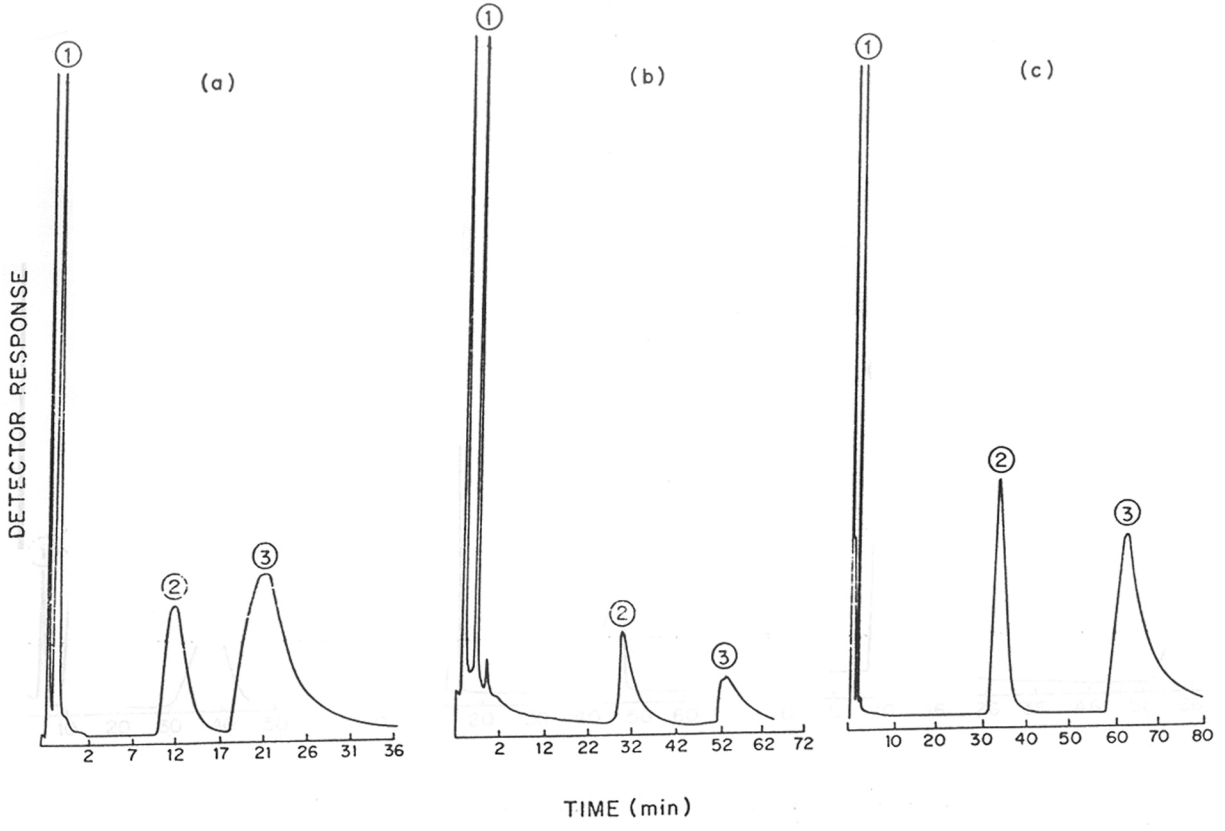


FIG.2·5:SEPARATION OF NITROPHENOL ISOMERS ON VARIOUS CROWN ETHERS. NITROGEN FLOW RATE 30 ml min^{-1}, INJECTION TEMP. 220°C, DETECTOR TEMP. 250°C
PEAKS: 1) 2-NITROPHENOL 2) 3-NITROPHENOL 3) 4-NITROPHENOL
(a) 3% DB18C6 AT 180°C (b) 3% DCH24C8 AT 180°C (c) 3% DB24C8 AT 170°C

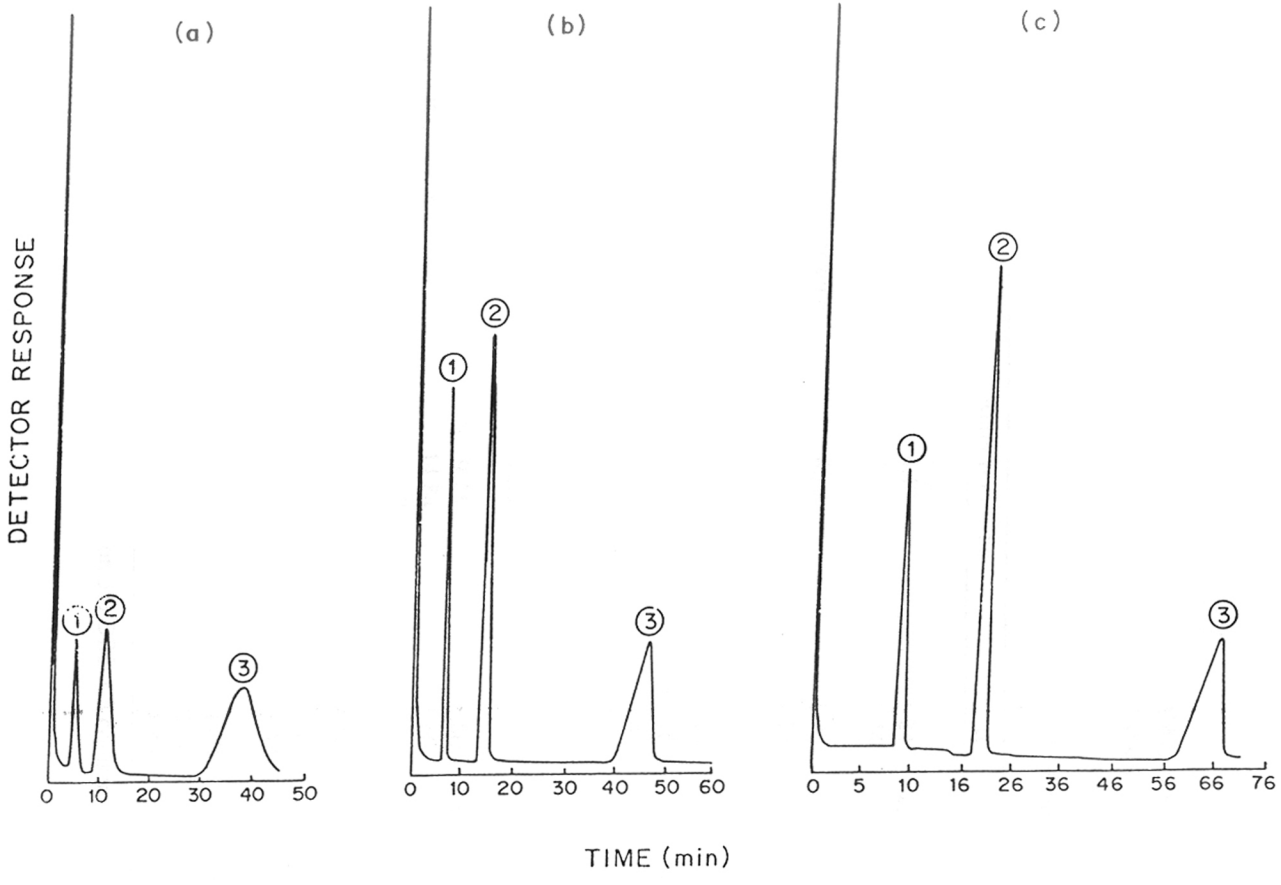


FIG.2·6:SEPARATION OF NITROANILINE ISOMERS ON VARIOUS CROWN ETHERS AT 170°C. NITROGEN FLOW RATE 30 ml min^{-1}, INJECTION TEMP. 210°C , DETECTOR TEMP. 250°C , PEAKS: 1) 2-NITROANILINE 2) 3-NITROANILINE 3) 4-NITROANILINE
(a) 3 % DB18C6 (b) 3 % DCH24C8 (c) 3 % DB24C8

TABLE 2.7

RETENTION TIMES (mins), RELATIVE RETENTION VALUES (α) AND PHYSICAL PROPERTIES OF THE POSITIONAL ISOMERS STUDIED ON 10% DB24C8 AT 170°C.

Carrier gas nitrogen at a flow rate of 30 ml min^{-1}, Injection temperature 210°C; Detector temperature 250°C

Solute	Retention time	α	BP °C	MP °C
2-Nitrotoluene	3.33	1.00	225°C	-
3-Nitrotoluene	4.13	1.24	230-231°C	15-16°C
4-Nitroltoluene	4.81	1.44	238	52-54
1-Chloro-3-nitrobenzene	4.98	1.00	236	42-44
1-Chloro-4-nitrobenzene	5.69	1.14	242	83
1-Chloro-2-nitrobenzene	6.76	1.36	246	33-36
3,4-Dichloronitrobenzene	10.71	2.15	255-56	41-44
2,3-Dichloronitrobenzene	13.46	2.70	257-58	61-62
2,5-Dichloronitrobenzene	16.95	3.40	258	29-32
o-Anisidine	4.75	1.00	225	5-6
m-Anisidine	9.73	2.05	251	-
p-Anisidine	7.25	1.53	240-43	57-60
3-Nitroanisole	9.25	1.00	121 at 8mm	36-38
2-Nitroanisole	14.84	1.60	273	9.5
4-Nitroanisole	16.77	1.81	-	-
1,3-Dichlorobenzene	3.85	1.00	172-73	-
1,4-Dichlorobenzene	4.40	1.14	173	54-56
1,2-Dichlorobenzene	5.70	1.48	179-180	-
2-Phenylenediamine*	3.60	1.00	256-58	103-105
3-Phenylenediamine	7.69	2.14	282-84	64-66
4-Phenylenediamine	5.23	1.45	267	143-45

* Phenylenediamine isomers were injected on 3% DB24C8 column.

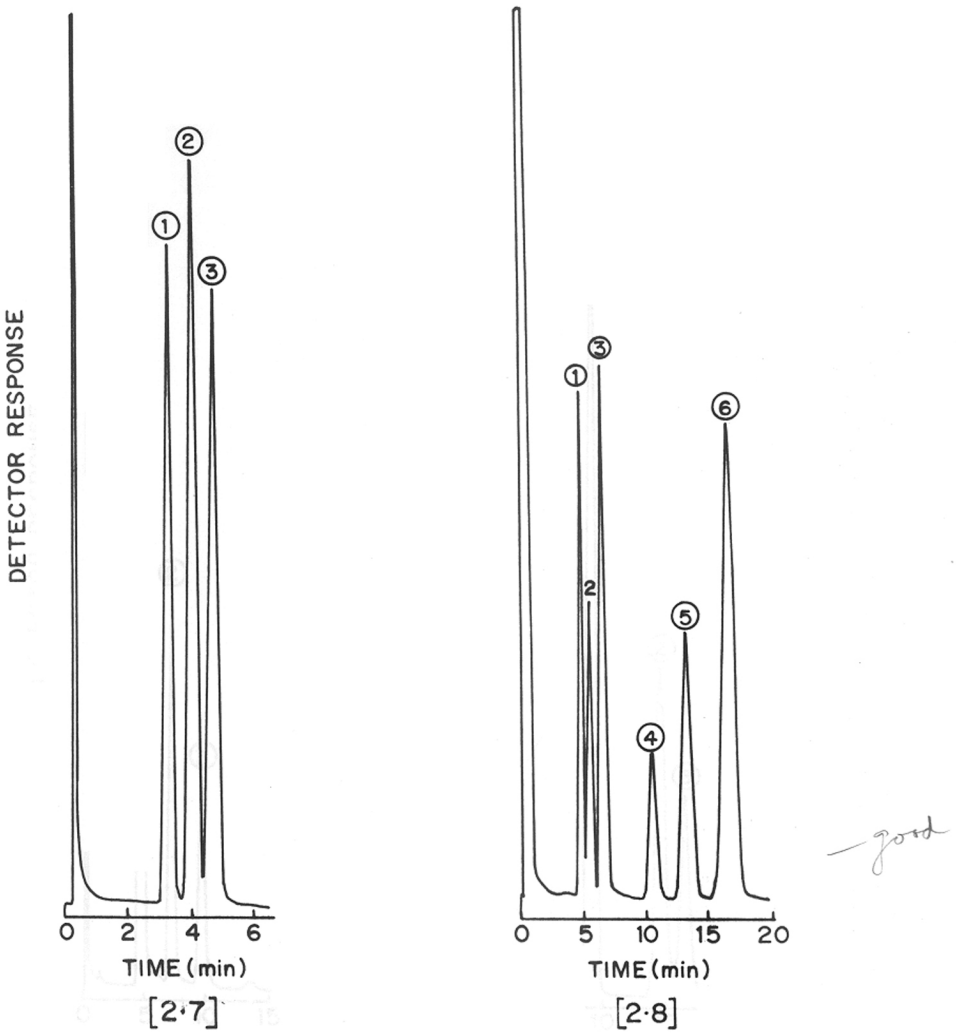


FIG. 2.7 : **SEPARATION OF NITROTOLUENE ISOMERS ON 10% DB24C8.**
Oven temp. 170°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 210°C, Detector temp. 250°C.
Peaks: 1) 2-nitrotoluene, 2) 3-nitrotoluene, 3) 4-nitrotoluene.

FIG. 2.8 : **SEPARATION OF NITROCHLORO AND DICHLORONITROBENZENE ISOMERS ON 10% DB24C8.**
Oven temp. 170°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 210°C, Detector temp. 250°C.
Peaks: 1) 1-chloro-3-nitrobenzene, 2) 1-chloro-4-nitrobenzene, 3) 1-chloro-2-nitrobenzene, 4) 3,4-dichloronitrobenzene, 5) 2,3-dichloronitrobenzene, 6) 2,5-dichloronitrobenzene.

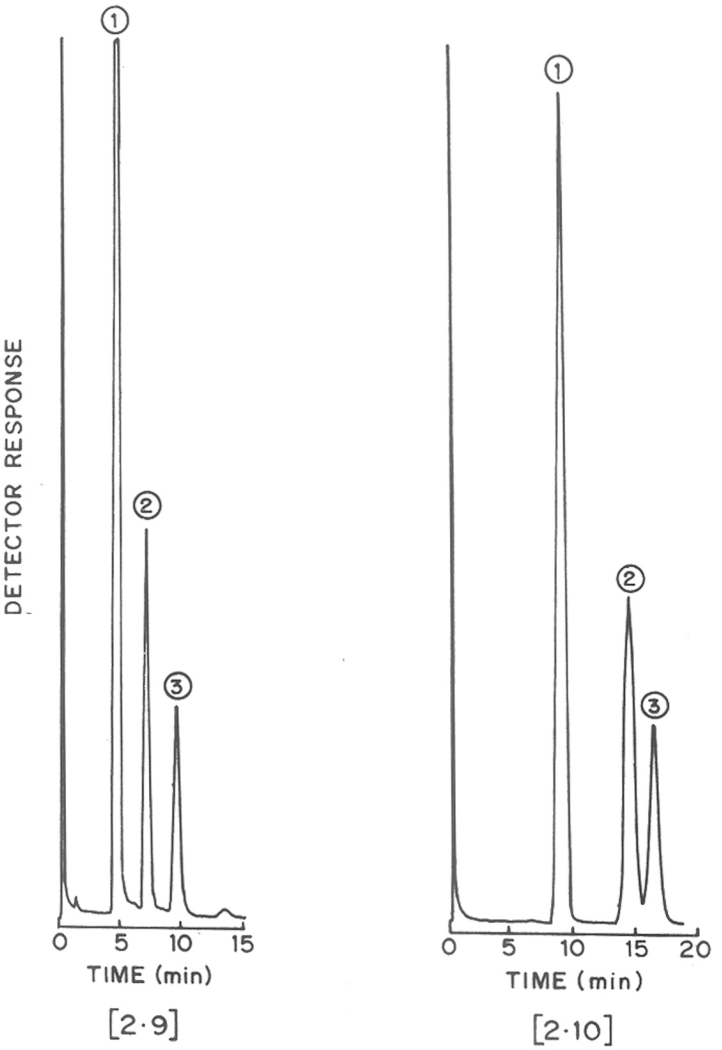


FIG. 2.9 : **SEPARATION OF ANISIDINE ISOMERS ON 10% DB24C8.**
Oven temp. 170°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 210°C, Detector temp. 250°C.
Peaks: 1) ortho-anisidine, 2) meta-anisidine, 3) para-anisidine.

FIG. 2.10 : **SEPARATION OF NITROANISOLE ISOMERS ON 10% DB24C8.**
Oven temp. 170°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 210°C, Detector temp. 250°C.
Peaks: 1) 3-nitroanisole, 2) 2-nitroanisole, 3) 4-nitroanisole.

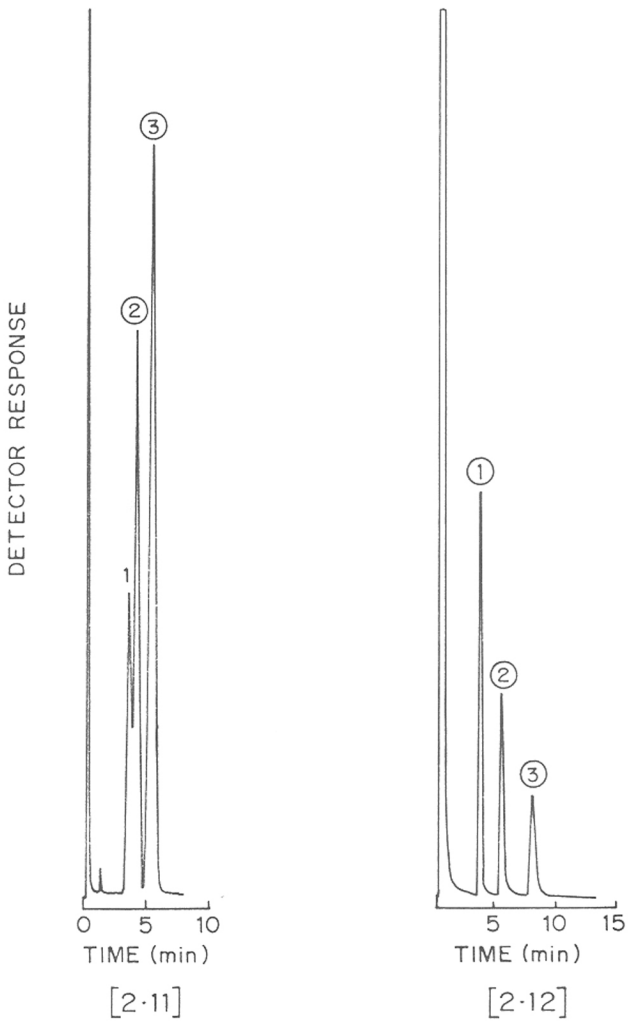


FIG. 2.11 : SEPARATION OF DICHLOROBENZENE ISOMERS ON 10% DB24C8.
Oven temp. 110°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 210°C, Detector temp. 250°C.
Peaks: 1) 1,3-dichlorobenzene, 2) 1,4-dichlorobenzene,
3) 1,2-dichlorobenzene.

FIG. 2.12 : SEPARATION OF PHENYLENEDIAMINE ISOMERS ON 3% DB24C8.
Oven temp. 170°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 210°C, Detector temp. 250°C.
Peaks: 1) 1,2-phenylenediamine, 2) 1,4-phenylenediamine,
3) 1,3-phenylenediamine.

dichlorobenzene and phenylenediamine respectively. All other isomers except phenylenediamine were separated on 10% DB24C8. Since phenylenediamine isomers take a long time to elute on 10% DB24C8, these were injected on 3% DB24C8.

A very good separation of benzoquinone, phenol, catechol and hydroquinone was obtained on 10% DB18C6 at 170°C in reasonable time (Fig. 2.13) and Table 2.8 gives the corresponding retention data alongwith relative retention values. These isomers take very long time to elute on DCH24C8 and DB24C8 and the behaviour can be explained by the strong hydrogen bonding interaction and the cavity size.

Since phenylenediamine isomers separated very well on 3% DB24C8, we injected 1,5- and 1,8 - diaminonaphthalene on the same column and it was found that 1,8-isomer takes about 70 minutes for elution while 1,5-isomer does not elute at all. This can be attributed to the fact that 24 membered crown ethers fit diamino compounds rather well than monoamino compounds[14]. Again the 1,5-isomer shows a stronger hydrogen bonding interaction of the ether oxygen than 1,8-isomer.

Table 2.9 presents retention times(t_R) and relative retention values (α) of amino compounds on 10% crown ether stationary phases and of phenylene diamine isomers on 10% DB18C6 at 170°C. It can be seen that diamino compounds are strongly retained as compared to monoamino compounds on DB18C6 and are eluted according to their boiling points. As stated above retention of diamino compounds on 24 membered crown ether phases is too high. Separation between *meta* and *para* isomers of toluidine and cumidine is possible only on 10% DCH24C8 as is indicated by the relative retention values. This unique selectivity of DCH24C8 may be attributed to the cavity size of the crown ether. The order of retention times of these isomers on the crown ether phases is DB24C8 > DCH24C8 > DB18C6.

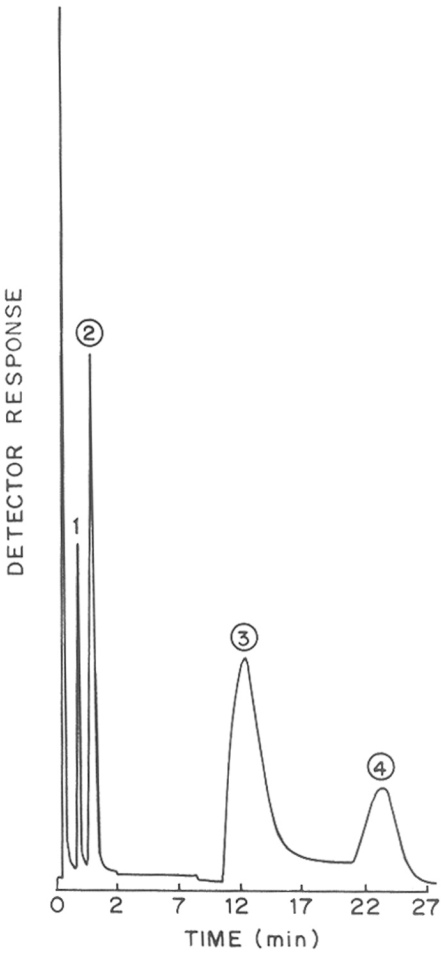


FIG. 2.13 : **SEPARATION OF BENZOQUINONE, PHENOL, CATECHOL AND HYDROQUINONE ON 10% DB18C6.**
Oven temp. 170°C, Carrier gas nitrogen flow rate - 40 ml/min, Injector temp. 210°C, Detector temp. 250°C.
Peaks: 1) benzoquinone, 2) phenol, 3) catechol, 4) hydroquinone.

TABLE 2.8

RETENTION TIMES AND RELATIVE RETENTION VALUES (α) OF SOME ISOMERS ON 10% DB18C6 AT 170°C.

Carrier gas nitrogen at a flow rate of 40 ml min^{-1}, Injection temperature 210°C; Detector temperature 250°C

Solute	Retention time	α	BP °C	MP °C
Benzoquinone	0.71	1.00	-	113-115
Phenol	1.18	1.66	182	40-42
Catechol	12.53	17.65	245	104-106
Hydroquinone	23.73	33.45	-	173-175

TABLE 2.9

RETENTION TIMES (t_R) AND RELATIVE RETENTION VALUES (α) OF AMINO COMPOUNDS ON 10% CROWN ETHER PHASES AT 170°C.

Carrier gas nitrogen at a flow rate of 30 ml min^{-1}, Injection temperature 220°C; Detector temperature 250°C

Solute	Stationary Phase					
	DB18C6		DB24C8		DCH24C8	
	t_R	α	t_R	α	t_R	α
p-Toluidine	1.15	1.00	2.72	1.00	2.06	1.00
m-Toluidine	1.22	1.06	2.97	1.09	2.35	1.14
o-Toluidine	1.79	1.56	4.69	1.72	2.98	1.45
o-Cumidine	1.52	1.00	3.77	1.00	2.53	1.00
m-Cumidine	-	-	-	-	2.90	1.15
p-Cumidine	1.78	1.17	4.50	1.19	3.10	1.23
2-Phenylenediamine	4.42	1.00	-	-	-	-
4-Phenylenediamine	6.45	1.46	-	-	-	-
3-Phenylenediamine	9.50	2.15	-	-	-	-

REFERENCES

1. E. Blasius and K.P. Janzen in F. Vogtle and E. Webber (Editors), *Host Guest Complex Chemistry Macrocycles, Synthesis, Structures, Applications*, Springer-Verlag, Berlin, Heidelberg, Tokyo, 1985, p. 189.
2. S. Kopolov, T.E. Hogen-Esch, J. Smid, *Macromolecules,* **6** (1973) 133.
3. R.C. Helgeson *et al., J. Am. Chem. Soc.,* **96** (1974) 6762.
4. R.L. Sousa, D.H. Hoffmann, L. Kaplan, D.J. Cram, *J. Am. Chem. Soc.,* **96**, (1974) 7100.
5. E. Blasius *et al., Talenta,* **27** (180) 127.
6. E. Blasius *et al., J. Chromatogr.,* **201** (1980) 147.
7. L.A. Fernando, M.L. Miles, L.H. Bowen, *Anal. Chem.,* **152** (1980) 1115.
8. I.M. Kolthoff, *Anal. Chem.,* **51** (1979), 1R.
9. D. Ammann, E. Pretsch, W. Simon, *Anal. Lett.,* **7** (1974) 23.
10. D. Ammann, E. Pretsch, W. Simon, *Helv. Chim. Acta.,* **56** (1973) 1780.
11. D.J. Cram, J.M. Cram, *Science* **183** (1974) 803.
12. G.D.Y. Sogah, D.J. Cram, *J. Am. Chem. Soc.,* **98** (1976) 3038.
13. G. Blaschke, *Angew. Chem. Int. Ed. Engl.,* **19** (1980) 13.
14. T. Nakagawa, H. Murata, A. Shibukawa, K. Murakami, H. Tanaka, *J. Chromatogr.,* **330** (1985) 43.
15. A. Shibuka, T. Nakagawa, A. Kaihara, K. Yagi, H. Tanaka, *Anal Chem.,* **59** (1987) 2496.
16. R.V. Vigalok, L.F. Bubachinkova, *Usp. Gaz. Khromatogr.,* (Kazan), **6** (1981) 190.
17. A. Ono, *Analyst* (London) **108** (1983) 1265.
18. R. Li, Wuhan Daxue Xuebao, *Zira Kexueban,* **4** (1985) 121.
19. R. Li, *Sepu,* **4** (1986) 304.
20. D.D. Fine, H.L. Gearhart II, H.A. Mottola, *Talenta,* **32** (1985) 751.

21. C.A. Rause, A.C. Finlinson, B.J. Tarbet, J.C. Pixton, N.M. Djordjevic, K.E. Markides, M.L. Lee, *Anal Chem.*, **60** (1988) 901.

22. E.V. Zagorevskaya, N.V. Kovaleva, *J. Chromatogr.*, **365** (1986) 7.

23. Y. Jin, R. Fu, Z. Huang, *J. Chromatogr.*, **469** (1989) 153.

24. C.Y. Wu, C.M. Wang, Z.R. Zeng, X.R. Lu, *Anal. Chem.*, **62** (1990) 968.

25. A. Kohoutova, E. Smokova-Keulemansova, L. Feltl, *J. Chromatogr.*, **471** (1989) 139.

26. C.Y. Wu, H.Y. Li, Y.Y. Chen, X.R. Lu, *J. Chromatogr.*, **504** (1990) 279.

27. A. Zhang *et al, J. Chromatogr.*, **521** (1990) 128.

28. L.F. Lindoy, *The chemistry of macrocyclic ligand complexes*, Cambridge, New York, New Rochelle, Melbourne, Sydney, 1989, p. 107.

29. M.A. Pulsipher, R.S. Johnson, K.E. Markides, J.S. Bradshaw, M.L. Lee, *J. Chromatogr. Sci.*, 24 (1986) 383.

30. X. Guardino, J. Albaiges, G. Firpo, R. Rodriguez-Vinals, M. Gassiot, *J. Chromatogr.*, **118** (1976) 13.

31. W.O.McReynolds, *J. Chromatogr. Sci.*, **8** (1970) 685.

32. F. Vernon, C.O.E. Ogundipe, *J. Chromatogr.*, **132** (1977) 181.

33. N.R. Ayyangar, A.S. Tambe, S.S. Biswas, *J. Chromatogr.*, **483** (1989) 33.

CHAPTER 3

4-(2'-PHENYLISOPROPYL) PHENOL ESTERS AS STATIONARY PHASES FOR GAS-LIQUID CHROMATOGRAPHY

CHAPTER 3

4-(2'-PHENYLISOPROPYL) PHENOL ESTERS AS STATIONARY PHASES FOR GAS-LIQUID CHROMATOGRAPHY

3.1 Evaluation of 4-(2'-phenylisopropyl)phenol Esters as Stationary Phases for Gas-Liquid Chromatography

3.1.1 Introduction

One of the by-products in the manufacture of phenol by the cumene hydroperoxide route is 4-(2'-phenylisopropyl) phenol (PIP). A possible use for esters of PIP is as stationary phases in gas-liquid chromatography (GLC), particularly if they show superior properties to other esters. Tricresyl and trixylenyl phosphates have been used for the separation of hydrocarbons, esters, ketones, alcohols[1], sulphur compounds[2], halogenated compounds[3], cresols[4], xylenols, fluorophenols and chlorophenols[5]. We have carried out the synthesis and gas-liquid chromatographic evaluation of a few PIP esters with a view to characterize their resolving abilities and evaluation of their McReynolds constants[6].

Di[4-(2'-phenylisopropyl) phenyl] maleate (DPIPM), n-butyl- [4-(2'-phenylisopropyl) phenyl] maleate (BPIPM), [4- (2'-phenylisopropyl) phenyl] acetate (PIPA), (4,4'-isopropylidenebisphenol) diacetate (IPBPDA) and tri [4-(2'-phenylisopropyl) phenyl] phosphate (TPIPP) were synthesized in our laboratory. Their purities were ascertained by NMR and IR spectroscopy, mass spectrometry and elemental microanalysis. The results of synthesis and chromatographic characteristics of [4- (2'-phenylisopropyl) phenyl] terephthalate (PIPT)[7] have already been published by us earlier. Though the synthesis of PIPA[8], IPBPDA[9,10] and TPIPP[11,12] was reported earlier by other workers, their spectral data were not available. Also, DPIPM and BPIPM are new esters and to our knowledge are not reported in the literature.

3.1.2 **Synthesis**

Preparation of Di [(phenylisopropyl) phenyl] maleate (DPIPM) :

This ester was prepared by the reaction of excess of PIP with maleic acid dichloride at low temperature. Maleic acid dichloride was prepared from maleic acid (5.8 gms, 0.05 mole) and phosphorous pentachloride (20.85 gms, 0.1 mole) by stirring at 120 - 25 °C for 4 hrs. To the mixture of 21.2 gms (0.1 mole) of PIP, 200 ml benzene, and 20.2 gms (0.2 moles) of triethylamine taken in a 500 ml round bottom flask, was slowly added the maleic acid dichloride solution (0.05 moles, 7.65 gms in 50 ml benzene) for 30 minutes at 30° - 35°C, with stirring maintaining the temperature (also with external cooling). After stirring for one hour, it was refluxed for two hours, cooled and washed with water, then concentrated and dried over sodium sulfate to yield 18.5 gms DPIPM (73.4% yield). Further purification was done by column chromatography using benzene : pet ether (50:50) as eluent. B.P. (observed) 220-30°C at 1 mm.

IR (neat) 1740, 1755 cm^{-1}. ^1H NMR (90 MHz, ($CDCl_3$): δ 1.66 (12H, S, C-CH_3), 6.55 ($2H, S, -\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}$), 7.2 (18H, m, Ar-H). MS : M^+ 504, base 119.

Anal. Calcd. for $C_{34}H_{32}O_4$: C, 80.95; H, 6.34

Found : C, 80.55; H, 6.38.

In order to ascertain that the synthesized ester is maleate only and not the fumarate, di (PIP) fumarate was prepared and the NMR of di (PIP) fumarate and di (PIP) maleate were compared. The NMR clearly shows that in the case of maleate, maleic acid protons show a singlet which is upfield from aromatic protons while in case of fumarate, fumaric acid protons merge with the aromatic protons to give multiplet ^1H NMR (90 MHz, $CDCl_3$) : δ 1.66 (12H, S, -CH_3); 7.25 (20H, m, Ar-H + $\overset{H}{\underset{|}{C}}=\underset{|}{\overset{}{C}}$).
H

Preparation of n-butyl [(phenylisopropyl) phenyl] maleate (BPIPM) :

Monobutyl ester of maleic acid was prepared by reaction of n-butanol (1 mole) and maleic anhydride (1.02 moles) at 80-90°C giving 62.6% yield in 2 hrs[13] and its' acid chloride was prepared by interaction with PCl_5 (20.85 gms, 0.1 mole). The solid acid chloride obtained was taken in 50 ml of dry benzene. In a 500 ml round bottom flask, 21.2 gms (0.1 mole) of PIP, 200 ml benzene, and 20.2 gms (0.2 mole) of triethylamine were taken and the acid chloride was slowly added to it

under stirring at 30-35°C. Further procedure was similar to that of DPIPM. The crude product (33.5 gms) was distilled under vacuum at 180-190°C at 2 mm to yield the pure product (26.35 gms, 72% yield).

IR (neat): 1740, 1760cm^{-1} ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.9 (3H, t, J = 7 Hz, $-CH_3$), 1.4 (4H, m, $-CH_2$), 1.66 (6H, s, C-CH_3), 4.2 (2H, t, J = 6Hz, $-COOCH_2$), 6.55(2H, s, $-\overset{H}{\underset{|}{C}}=\overset{H}{\underset{|}{C}}-$), 7.1 (9H, m, Ar-H). MS :M^+ 366, base 197.

Anal. Calcd. for $C_{23}H_{26}O_4$: C, 75.41; H, 7.10

Found : C, 75.44; H, 6.80

Preparation of [(phenylisopropyl) phenyl] acetate (PIPA) :

The title compound was prepared by acetylation of PIP (0.1 mole, 25.61 gms) with acetic anhydride (60 ml), acetic acid (5 ml) and a drop of concentrated sulphuric acid giving 23.05 gms of the product (90% yield). b.p. 120°C at 0.05 mm. Lit.[8] b.p. 170-173° at 3mm.

IR (neat) : 1750 cm^{-1} ^{1}H NMR (60 MHz, CCl_4) : δ 1.6 (6H, S, cumyl CH_3), 2.2 $\left(3H, S, -\overset{O}{\overset{\|}{C}}-CH_3\right)$, 7.0 - 7.5 (9H, m, Ar-H). MS : M^{+4} 258, base 197.

Anal. Calcd. for $C_{17}H_{18}O_2$: 80.31; H, 7.08

Found : C, 80.28; H, 7.17.

Preparation of (4,4'-isopropylidene bisphenol) diacetate (IPBPDA) :

The title compound was prepared from 4,4'-isopropylidene bisphenol (Bisphenol A, 22.8 gms, 0.1 mole), acetic anhydride (60 ml) and acetic acid (5 ml) in presence of catalytic amount of concentrated sulphuric acid in 86% yield by using the literature procedure[10]. m.p. 76°C. Lit.[9,10] m.p. 79.5 - 81.5°C and 80-82°C.

IR (nujol) : 1750 cm^{-1}. ^{1}H NMR (60 MHz, CCl_4) : δ 1.6 (6 H, S, cumyl CH_3), 2.2 (6H, S, $-\overset{O}{\overset{\|}{C}}-CH_3$), 6.9 - 7.3 (8H, d of quartet, Ar-H). MS : M^+ 312.

Anal. Calcd. for $C_{19}H_{20}O_4$: C, 73.07 ; H, 6.41

Found : C, 73.37; H, 6.84.

Preparation of tri [(phenylisopropyl) phenyl] phosphate (TPIPP) :

Synthesis of the phosphate ester is similar to that of DPIPM, the only difference being the use of phosphorous oxychloride in place of maleic acid dichloride. To the mixture of 21.2 gms (0.1 mole) PIP, 200 ml benzene and 20.2 gms (0.2 moles) of triethylamine taken in a 500 ml round bottom flask, 5.11 gms (0.033 moles) phosphorous oxychloride diluted with 20 ml benzene was slowly added under stirring, and maintaining the temperature with external cooling. Work up procedure was similar to that of DPIPM. The crude product was crystallized from acetone to yield 17.5 gms of the pure product (yield 77.2%). Melting point 145°C. Lit[12] m.p. 144°C.

IR (nujol):1180 cm^{-1} ^1H NMR (90 MHz, $CDCl_3$):δ1·6 (18H, S, C-CH_3), 7.15 (27H, M, Ar-H). MS : $(M + 1)^+$ 681, base 119.

Anal. Calcd. for C_{45} H_{45} O_4 P : C, 79.41; H, 6.61; P, 4.55

Found : C, 79.47, H, 7.07; P, 4.23.

3.1.3 Experimental Methods

IR spectra was recorded on a Pye Unicam SP 3-300 spectrophotometer. NMR spectra were obtained on Jeol-60 and Bruker WH90 spectrometers using TMS as an internal standard. Mass spectral analyses were carried out using Finnigan Mat 1020 mass spectrometer. Thermogravimetric analysis was recorded on STA 409 apparatus from Netzsch Geratbau, Selb, F.R.G. The PIP esters were used as GLC substrates at a concentration of 5% (w/w) on Chromosorb WAW DMCS (60/80 mesh). Chloroform was used as the solvent for maleates and the phosphate, and acetone for the acetate, and diacetate to disperse the esters on chromosorb support. The weighed amounts (0.5263 gms) of PIP esters were dissolved in chloroform or acetone and the resulting solution of each ester was poured at room temperature on 10 gms of chromosorb WAW DMCS (60-80 mesh). The mixture was shaken intermittently and kept overnight. The solvent was removed by rotavapor and solid dried in the oven. The coated supports were packed in aluminium columns (1.8 x 6 mm I.D.). The DPIPM, BPIPM and IPBPDA columns were conditioned at 140°C, and the PIPA and TPIPP columns at 120°C and 200°C respectively.

A Hewlett-Packard 700 gas chromatograph with a flame ionization detector and an HP 3800A integrator was used. The details of the GC conditions are given with the figures (3.1 to 3.7).

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[14] and also by the iterative method given by Guardino *et al.*[15] (for details refer to Chapter 1). The BASIC language program developed to find out dead time values of various stationary phases by the iterative method is listed in Appendix. To investigate the selectivity of these PIP esters, different aromatic positional isomers were injected at 140°C. The standard samples analyzed were of 97 to 99% purity.

3.1.4 Results and Discussion

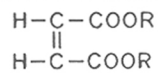
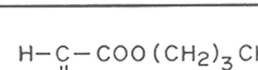
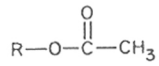
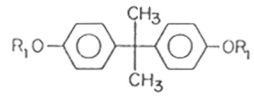
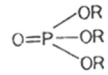
The structures of the synthesized five esters and their physical properties are given in Table 3.1. Graphs of the logarithm of corrected retention times *versus* carbon number for *n*-alkanes plotted for all five phases were found to be linear (Fig. 3.1). Although TPIPP is a solid at 120°C, a linear graph was obtained with TPIPP. Since the retention index concept utilizes this plot for n-alkanes and the retention index of a substance is simply the number of carbon atoms (conveniently multiplied by 100) of a hypothetical n-alkane that would have identical retention characteristics, we decided to determine McReynolds constants for TPIPP at 120°C and to compare these with the values for the other ester phases. When the esters IPBPDA and TPIPP were used below their melting points, the separation takes place due to adsorption and not by partition. In most instances, the shapes of the peaks and separations on these ester phases were satisfactory and no peak tailing was observed.

The McReynolds constants for the stationary phases determined at 120°C are given in Table 3.2. The absolute values of the retention indices on squalane, tricresyl phosphate and other standard stationary phases were taken from the original reference[6]. Table 3.2 also lists the dead times obtained by graphical and iterative methods and these values show good agreement.

The ΔI values in Table 3.2 show that the polarity of IPBPDA is comparable to that of cyclohexanedimethanol succinate (CHDMS). Out of the five test probe values for CHDMS, four (A,B,D and E) are very close to those of IPBPDA and the latter retains ketones more strongly than the former. PIPA and Ucon-50-HB-280X differ in their polarity towards ketones (C) and alcohols (B); ketones are retained more strongly on the former whereas alcohols are retained more strongly

TABLE 3.1

STRUCTURES AND PHYSICAL PROPERTIES OF PIP ESTERS

Ester	Structure	BP °C		MP (°C)	
		Observed	Reported	Observed	Reported
Di-[4-(2'-Phenylsopropyl) phenyl maleate (DPIPM)[a]		220 (1mm Hg)	-	Liquid	-
n-Butyl-[4-(2'-phenylisopropyl) phenyl] maleate (BPIPM)[a]		180 (2 mm Hg)	-	Liquid	-
[4-(2'-Phenylisopropyl) phenyl] acetate (PIPA)		120 (0.05mm Hg)	170-173 (3mm Hg)[8]	Liquid	-
(4,4'-Isopropylidenebisphenol) diacetate (IPBPDA)		-	-	76	79.5-81.5[9,10]
Tri[4-(2'-Phenylisopropyl)phenyl] phosphate (TPIPP)		-	390-400 (0.1 mm Hg)	144	145[12]

a These esters are not reported in the literature ; R = (Phenylisopropyl) phenyl ; $R_1 = CH_3-\overset{O}{\overset{\|}{C}}-$

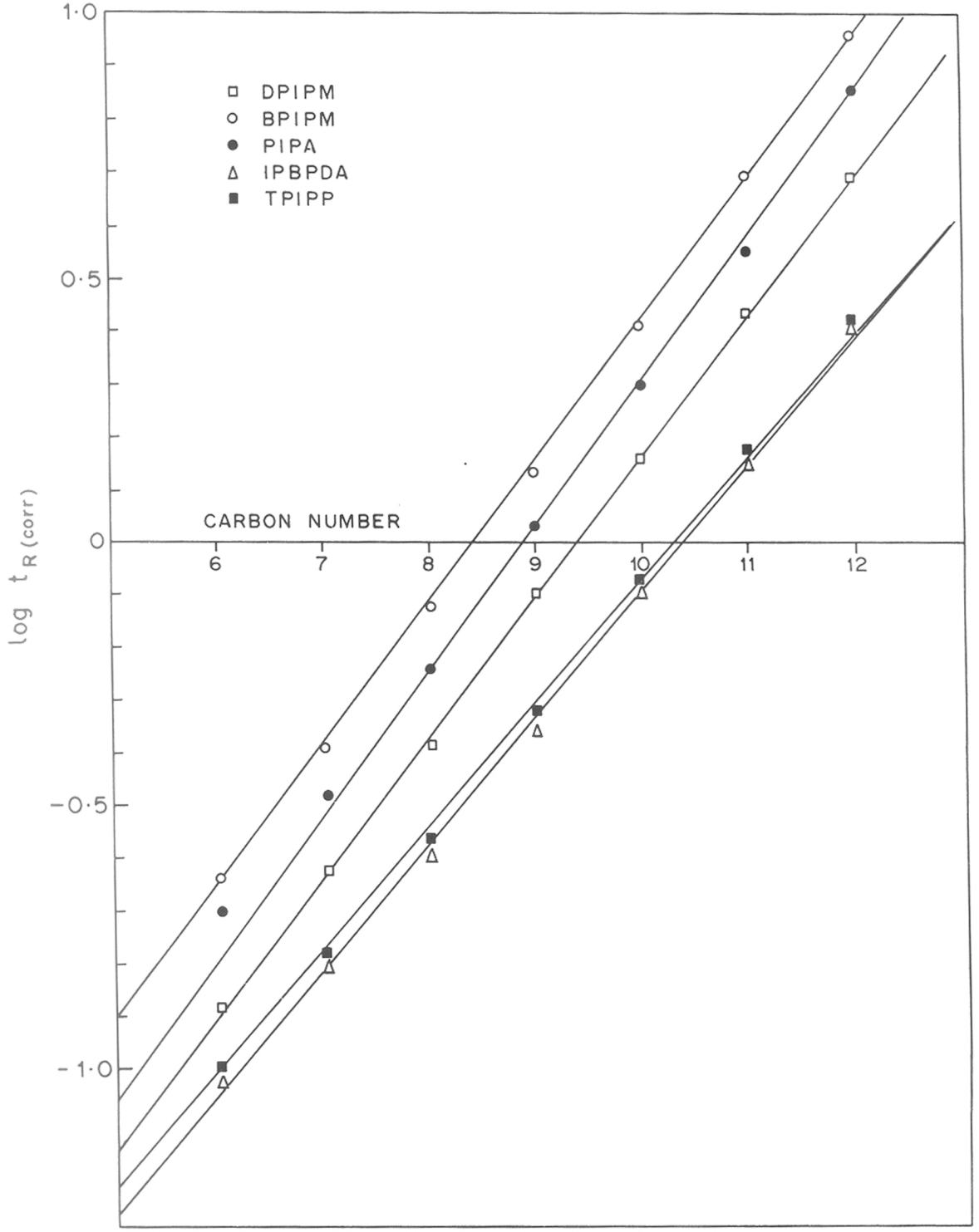


FIG. 3.1 : PLOT OF log $t_{R(corr)}$ Versus NUMBER OF CARBON ATOMS

TABLE - 3.2

McREYNOLDS' CONSTANTS AND DEAD TIME DETERMINATIONS FOR VARIOUS STATIONARY PHASES AT 120°C

t_m = Dead time obtained by the graphical method[14]; t'_m = dead time obtained by the iterative method[15]; I = retention index calculated considering t_m as dead time value = [I_R test phase - I_R squalane for test probe solutes;] I' = retention index calculated considering t'_m as dead time value = [I'_R test phase - I_R squalane for test probe solutes]

Stationary phase (5%)	McReynolds' probes															
	Benzene (A)		1-Butanol (B)		2-Pentanone (C)		Nitropropane (D)		Pyridine (E)							
	ΔI	ΔI'	ΔI	ΔI'	ΔI	ΔI'	ΔI	ΔI'	ΔI	ΔI'	t_m (min)	$\sum_1^5 \Delta I$	t'_m (min)	$\sum_1^5 \Delta I'$	b+	r#
IPBPDA	264	265	426	426	379	379	492	492	481	481	0.63	2042	0.629	2043	0.2400	1.823
CHDMS	269		446		328		493		481			2017			0.2351	1.718
PIPA	196	188	298	293	269	265	352	351	314	314	0.60	1429	0.649	1411	0.2660	1.929
Ucon-50-HB-280X	177		362		227		351		302			1419			0.2540	1.794
DPIPM	166	166	219	219	226	226	334	334	294	294	0.66	1239	0.659	1239	0.2660	1.846
OS-124	176		227		224		306		283			1216			0.2660	1.845
BPIPM	64	56	169	164	143	134	266	265	202	201	0.72	844	0.749	0824	0.2660	1.881
OV-17	119		158		162		243		202			842			0.2551	1.799
TPIPP	203	201	311	310	255	253	379	379	328	328	0.61	1476	0.619	1471	0.2330	1.780
Tricresyl phosphate	176		321		250		374		299			1420			0.2630	1.832

\+ The constant "b" is the slope of the curve obtained from the plot of log t_{RC} versus carbon number

\# The constant "r" is calculated from the square root of the ratio of corrected retention time for dodecane to corrected retention time for decane.

on the latter. Compared with OS-124, DPIPM retains nitro compounds more strongly and the A,B,C and E values are comparable. BPIPM and OV-17 differ only slightly for all other test probes except benzene (A). With TPIPP, three values (B, C and D) are very close to those for tricresyl phosphate and it retains aromatic and proton accepting compounds more strongly than tricresyl phosphate. As can be seen from the sum of ΔI values, BPIPM is the least polar and IPBPDA is the most polar of the new ester phases. The esters PIPA and DPIPM are of intermediate polarity. Table 3.2 gives b and r values for the new ester phases as well as for the standard 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[6]. Improved separations are expected for any series of homologous compounds using the phase with the higher r value even though both the phases have identical McReynolds constants. The esters IPBPDA, PIPA and BPIPM have higher b and r values than the respective standard phases while TPIPP has lower b and r values than Tricresyl phosphate. These values are equal for DPIPM and OS-124 phases.

Table 3.3 gives the McReynolds constants for TPIPP at 120°C and 180°C. The retention indices on Apiezon MH at 180°C were taken from the original reference[16]. It can be seen that the overall polarity of TPIPP at 180°C is greater than at 120°C, i.e., below its melting point. Alcohols, ketones, nitro compounds and proton acceptor compounds are retained much more strongly at 180°C, as can be seen from the McReynolds constants. Nitro compounds are most strongly retained at 120°C whereas alcohols are most strongly retained at 180°C, as is evident from the relative differences in the McReynolds constants. This means that at 180°C, forces between TPIPP and solutes with proton donor and proton acceptor groups are more significant than the dipole orientation forces between TPIPP and solutes with weak proton acceptor groups.

Of all the esters, TPIPP showed exceptional thermal stability and could be used for the separation of high boiling compounds. Thermogravimetric analysis (Fig. 3.2) shows that the decomposition starts after 200°C and is complete at 480°C. Hence the column can be safely used up to 200°C. This is a significant improvement over similar phosphate esters such as tricresyl phosphate (maximum 125°C) and tri (2,4-xylenyl) phosphate (maximum 150°C).

TABLE 3.3

McREYNOLDS' CONSTANTS FOR TPIPP (5%)

Solute	ΔI^a	Solute	ΔI^b
Benzene (A)	203	1-Butylbenzene (A)	150
1-Butanol (B)	311	Benzyl alcohol (B)	438
2-Pentanone (C)	255	Acetophenone (C)	391
1-Nitropropane (D)	379	Nitrobenzene (D)	423
Pyridine (E)	328	Aniline (E)	400
	$\sum_{1}^{5} \Delta I$ = 1476		$\sum_{1}^{5} \Delta I$ = 1802

[a] $\Delta I = I_{TPIPP} - I_{squalane}$ (120°C).

[b] $\Delta I = I_{TPIPP} - I_{Apiezon\ MH}$ (180°C).

The absolute values of the retention indices observed for Apiezon MH at 180°C are 1-butylbenzene : 1083, benzyl alcohol : 1032, acetophenone : 1053, nitrobenzene : 1105, pyridine : 986.

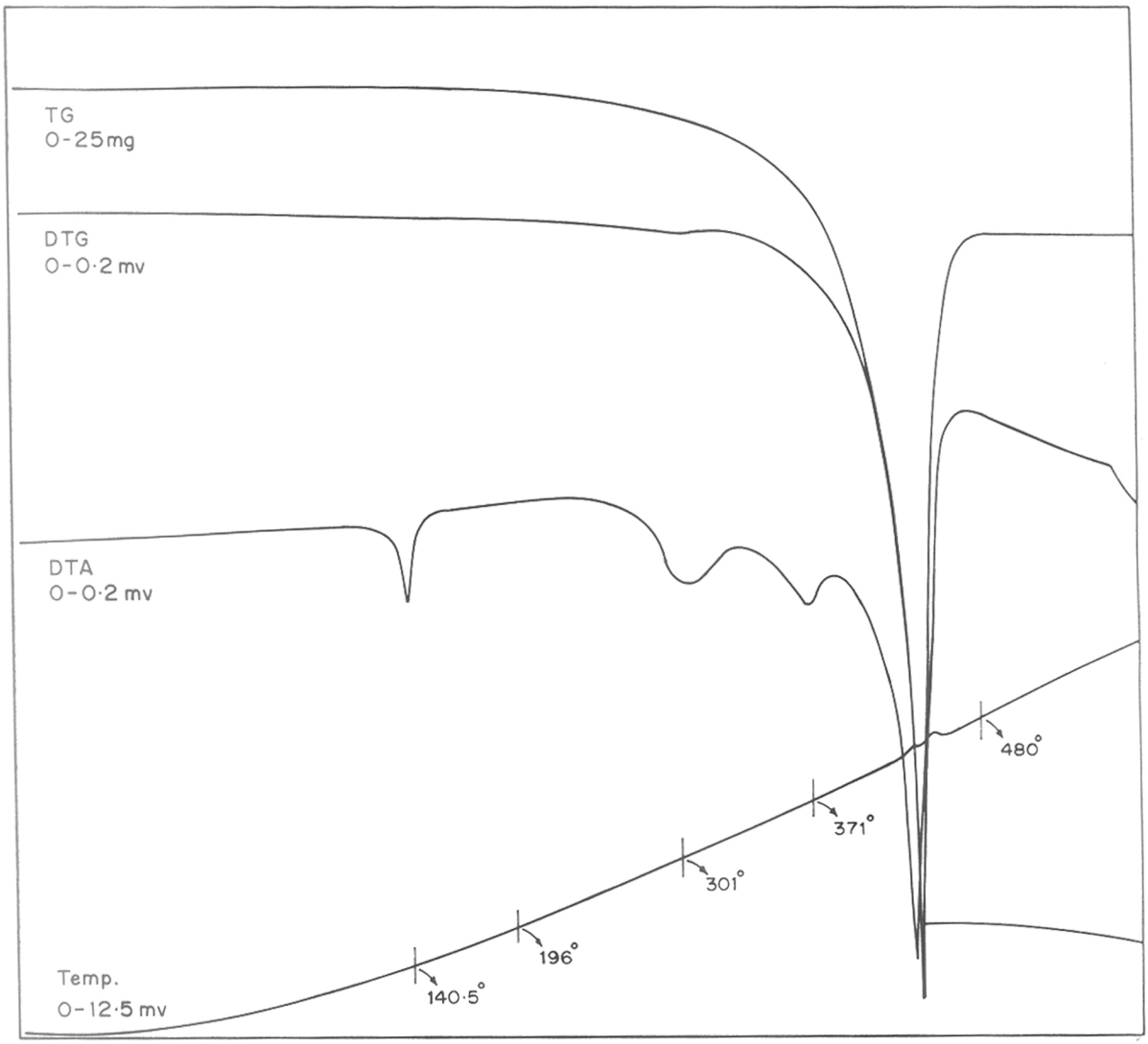


FIG. 3.2 : **THERMAL ANALYSIS OF TRI[4-(2'-PHENYLISOPROPYL)PHENYL] PHOSPHATE**

Table 3.4 lists the retention times for some aliphatic alcohols and aromatic hydrocarbons studied on packed columns of these new esters except TPIPP (since TPIPP is a solid below 144°C). The melting point of IPBPDA ester is 76°C, hence alcohols were not injected on this phase; only xylene isomers were injected at 80°C. Figure 3.3 shows the separation of some aliphatic alcohols on PIPA.

Table 3.5 indicates a good separation between *ortho* (slowest) and *p*-nitrochlorobenzenes on DPIPM and TPIPP, whereas the *meta* (fastest) and *para* isomers were poorly resolved. On the other hand, with BPIPM and PIPA, *ortho* and *para* nitrochlorobenzenes were poorly resolved and the *meta* and *para* isomers showed a near baseline separation. These results suggest that the presence of two or more diaromatic phenylisopropyl groups in an ester helps to resolve the *ortho* and *para* isomers, whereas the combined presence of a cumyl (PIP) group and the long chain alkyl group has effected the separation of *meta* and *para* isomers. The IPBPDA column gave a complete separation of *ortho*-, *meta*- and *para*-nitrochlorobenzene (Figure 3.4) and a near baseline separation of *ortho*-, *meta*-, and *para*-dichlorobenzene (Figure 3.5). Unfortunately, the separation of *meta*- and *para*-chloroaniline, *meta*- and *para*-cresol and 2,4- and 2,5-xylenols could not be achieved on any of the columns. Baseline separation of *ortho*-chloro-, *para*-chloro- and 2,4-dichlorophenol was obtained on TPIPP (Figure 3.6). Table 3.6 gives the retention times and relative retention values for these and α and β isomers of naphthol and naphthylamines on 5% TPIPP at 190°C.

3.2 Application of Tri[4-(2'-Phenylisopropyl)phenyl] Phosphate (TPIPP) in Gas Chromatographic Analysis

Since TPIPP is more polar than tricresyl phosphate and is suggested as an improvement over other phosphate esters due to its thermal stability, we tried out separations of higher boiling isomers on TPIPP. It was coated on various solid supports at different concentrations. The specifications of various TPIPP columns are presented in Table 3.7. Dimethyl phenol, naphthol, chlorobenzophenone and dihydroxybenzene isomers were injected on these columns at different temperatures.

TABLE 3.4

RETENTION TIMES (min) FOR ALIPHATIC ALCOHOLS AND AROMATIC HYDROCARBONS ON PIP ESTER PHASES

Carrier gas Nitrogen at a flow rate of 30 ml min^{-1}, Injection temperature 140°C; Detector temperature 200°C

Solute	Temperature°C	Stationary Phase 5%			
		DPIPM	BPIPM	PIPA	IPBPDA
Aliphatic alcohols	50°C				
Methanol		1.60	1.28	1.69	-
Ethanol		1.85	1.82	2.51	-
iso-Propanol		1.99	2.10	3.07	-
n-Propanol		3.39	3.41	5.20	-
ter-Butanol		2.14	2.39	3.40	-
iso-Butanol		4.50	5.71	8.11	-
n-Butanol		6.63	8.70	12.83	-
Aromatic hydrocarbons	50°C				
Benzene		5.37	6.05	5.02	-
Toluene		12.45	13.09	11.65	-
Ethyl benzene		26.30	28.80	24.13	-
Xylene isomers	80°C				
ortho Xylene		15.87	16.08	18.57	9.64
meta Xylene		12.65	12.75	14.97	7.91
para Xylene		12.12	12.14	14.48	7.13
α *m/p* xylene		1.04	1.05	1.03	1.11
α *o/m* xylene		1.25	1.26	1.24	1.22

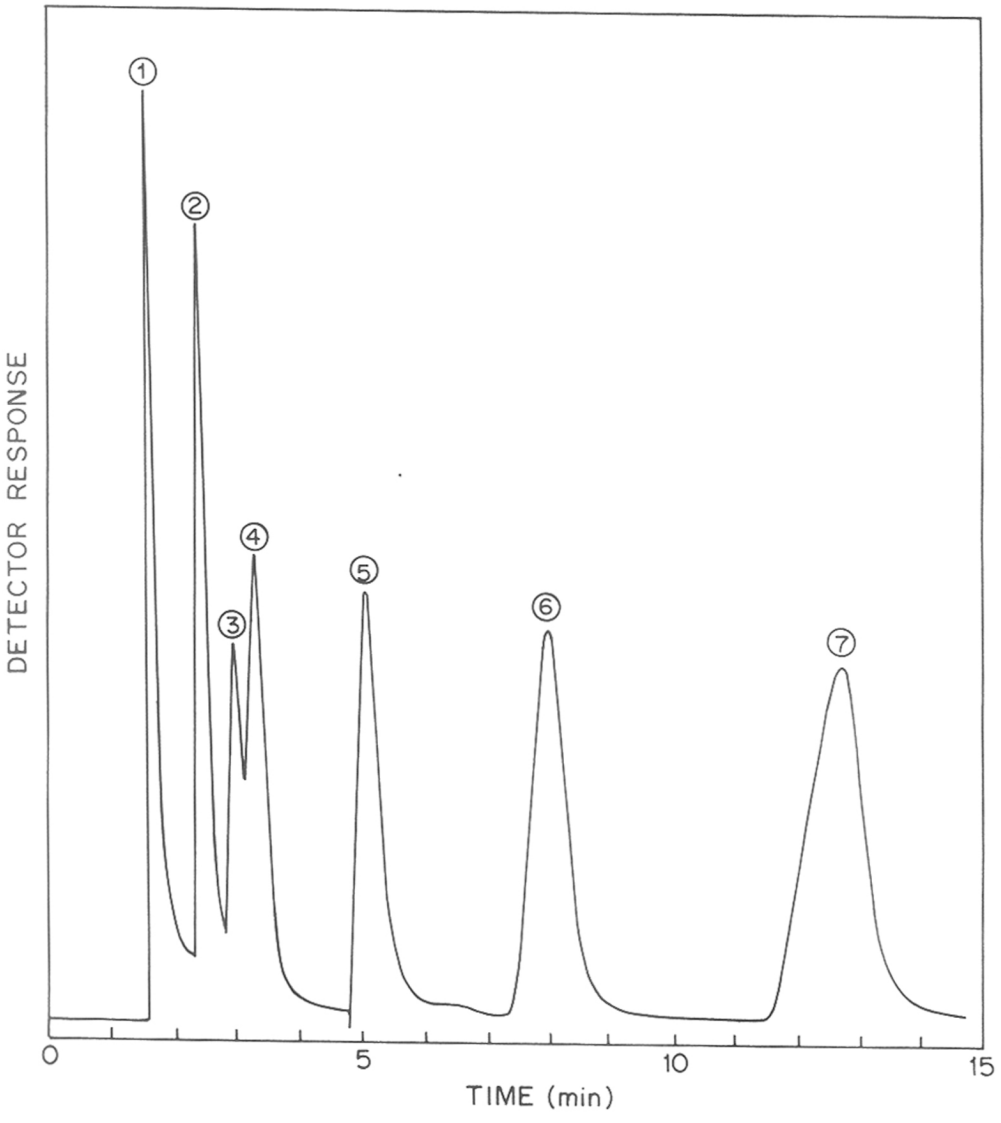


FIG. 3.3 : **SEPARATION OF ALIPHATIC ALCOHOLS ON PIPA - 5%.**
Oven temp. 50°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 140°C, Detector temp. 200°C.
Peaks: 1) methanol, 2) ethanol, 3) iso-propanol, 4) tert-butanol, 5) n- propanol, 6) iso-butanol, 7) n-butanol.

TABLE 3.5

RETENTION TIMES (min) AND RELATIVE RETENTION VALUES (α) FOR VARIOUS POSITIONAL ISOMERS OF BENZENE ON NEW ESTER PHASES

Carrier gas Nitrogen at a flow rate of 40 ml min^{-1}, Injection temperature 180°C; Detector temperature 300°C

	Stationary phase 5%				
Solute	DPIPM	BPIPM	PIPA	IPBPDA	TPIPP
Temperature °C	140	140	120	140	140
o-Nitrochlorobenzene	19.09	20.36	34.20	21.00	19.59
m-Nitrochlorobenzene	15.20	16.04	25.60	16.00	14.53
p-Nitrochlorobenzene	16.56	18.00	29.60	18.80	15.79
α *p/m*	1.09	1.12	1.16	1.18	1.09
α *o/p*	1.15	1.13	1.16	1.12	1.24
o-Chloroaniline	7.12	9.55	12.99	11.12	7.54
m-Chloroaniline	12.86	18.03	27.00	22.46	15.18
p-Chloroaniline	13.80	18.60	28.00	23.52	14.90
α *m/o*	1.81	1.89	2.08	2.02	2.01
α *p/m*	1.07	1.03	1.04	1.05	1.02
o-Cresol	4.71	7.21	30.35	9.99	8.00
m-Cresol	5.70	9.08	33.00	9.78	10.35
p-Cresol	5.60	8.82	34.45	9.79	9.88
α *p/o*	1.19	1.22	m/o 1.09	1.02	1.20
α *m/p*	1.02	1.03	p/m 1.04	1.00	1.05
o-Dichlorobenzene*	9.20	11.81	6.63	13.26	7.20
m-Dichlorobenzene	6.84	9.19	5.08	9.55	6.14
p-Dichlorobenzene	7.92	9.97	5.60	11.04	6.16
α *p/m*	1.16	1.08	1.10	1.16	1.00
α *o/p*	1.16	1.18	1.18	1.20	1.17
2,4-Dimethylphenol[a]	7.83	14.87	-	14.08	14.16
2,5-Dimethylphenol	7.86	14.58	-	13.38	14.16
2,6-Dimethylphenol	6.04	10.22	-	9.63	8.88
3,4-Dimethylphenol	10.89	22.88	-	19.81	22.40
α 2,4/2,5	1.00	1.02	-	1.05	1.00
α 2,5/2,6	1.30	1.43	-	1.39	1.59
α 3,4/2,4	1.39	1.54	-	1.41	1.58

* Dichlorobenzenes at 110°C on all the ester phases

[a] Dimethylphenols were not injected on PIPA as these isomers take very long to elute

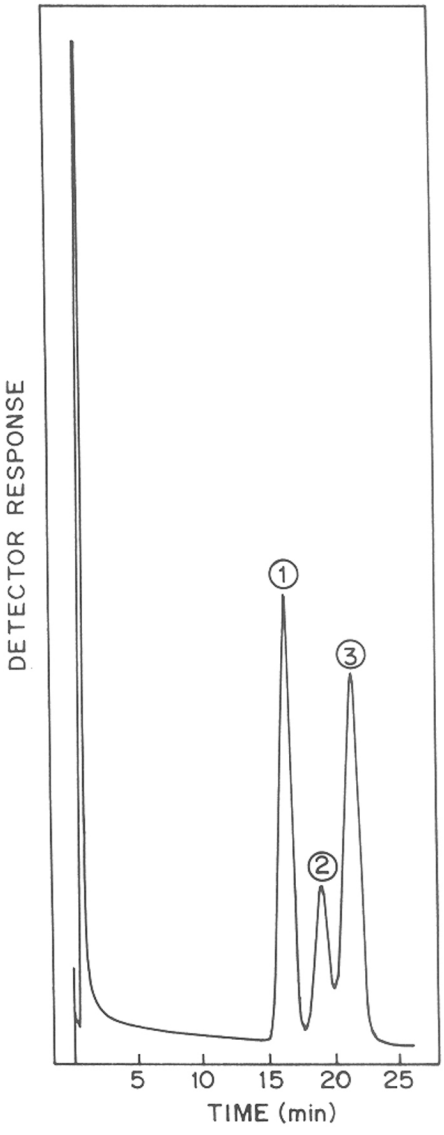


FIG. 3.4 : **SEPARATION OF NITROCHLOROBENZENE ISOMERS ON IPBPDA 5% Oven temp. 140°C, Carrier gas nitrogen flow rate - 40 ml/min, Injector temp. 180°C, Detector temp. 300°C.**
Peaks : 1) 1-Chloro-3-nitrobenzene, 2) 1-Chloro-4-nitrobenzene, 3) 1-Chloro-2-nitrobenzene

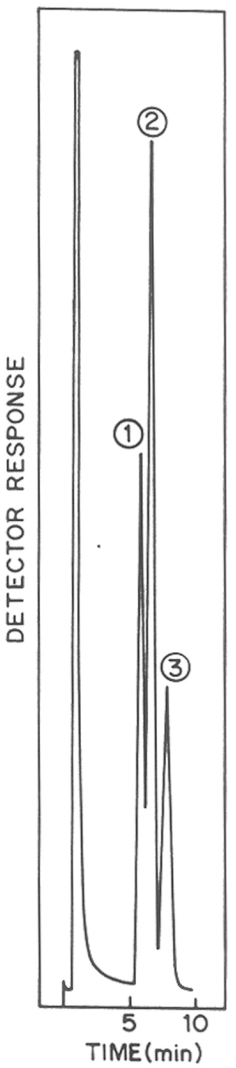


FIG. 3.5 : **SEPARATION OF DICHLOROBENZENE ISOMERS ON IPBPDA 5%**
Oven temp. 110°C, Carrier gas nitrogen flow rate - 40 ml/min, Injector temp. 180°C, Detector temp. 300°C.
Peaks: 1) 1,3-dichlorobenzene, 2) 1,4-dichlorobenzene,
3) 1,2-dichlorobenzene.

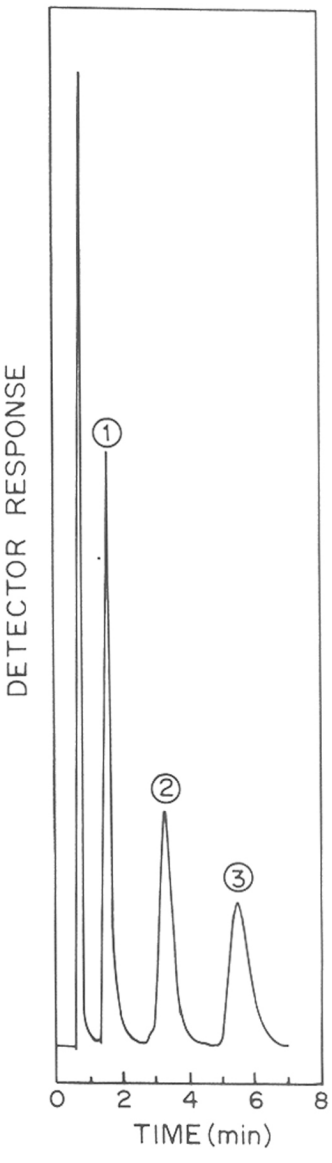


FIG. 3.6 : **SEPARATION OF 2-CHLORO, 4-CHLORO AND 2,4-DICHLORO-PHENOL ISOMERS ON TPIPP-5%**
Oven temp. 190°C, Carrier gas nitrogen flow rate - 40 ml/min, Injector temp. 230°C, Detector temp. 300°C.
Peaks: 1) 2-chlorophenol, 2) 2,4-dichlorophenol, 3) 4-chlorophenol.

TABLE 3.6

RETENTION TIMES (min) AND RELATIVE RETENTION VALUES (α) OF HIGHER BOILING ISOMERS ON TPIPP (5%) AT 190°C

Carrier gas Nitrogen at a flow rate of 40 ml min^{-1}, Injection temperature 230°C; Detector temperature 300°C

Solute	Retention time	α
1-Naphthylamine	14.40	1.00
2-Naphthylamine	15.80	1.10
1-Naphthol	21.60	1.00
2-Naphthol	24.20	1.12
2-Chlorophenol	1.50	1.00
4-Chlorophenol	5.52	3.68
2,4-Dichlorophenol	3.31	2.21

TABLE 3.7

SPECIFICATIONS OF VARIOUS TPIPP COLUMNS

Solid Support	Loading%	Length ft.	I.D.	Column No.
Chromosorb WAW DMCS 80/100 mesh	3	6	1/8"	I
	12	6	1/8"	II
Chromosorb G AW 80/100 mesh	10	6	1/8"	III
Porapak Q 80/100 mesh	5	2	1/8"	IV
	10	2	1/8"	V

3.2.1 Separation between Benzoquinone, Phenol, Catechol and Hydroquinone

Dihydroxybenzenes have found wide applications in industrial processes. Hydroquinone is used in photographic developers and in the production of polymerization inhibitors and rubber and food antioxidants. Important uses of resorcinol include : production of adhesives for rubber, starch,tyres, wood, dyestuff synthesis, UV absorbers and pharmaceuticals. Catechol is used in fur dyeing, leather processing, photographic developers, and in the fabrication of polymerization inhibitors, perfumes, pharmaceuticals and pesticides. Benzoquinone is used as an oxidizing agent in the manufacture of dyes.

Since, in our laboratory, a process to manufacture hydroxybenzenes and 1,4-benzoquinone by hydroxylation of phenol using hydrogen peroxide and zeolite catalyst is being developed, it became essential to find out an analytical method for the separation of hydroxylation products. Separation of catechol and hydroquinone is reported on 5% polyphenyl ether coated on chromaton N (AW DMCS) at 160°C[17]. O'Grodnick *et al.*[18] have used two different columns for the determination of benzene, phenol, catechol and hydroquinone in whole blood of rats and mice. Catechol and hydroquinone are separated as their trifluoroacetyl derivatives. Separation of free polyhydric phenols by capillary gas chromatography has been reported where only catechol and hydroquinone were separated alongwith other polyhydric phenols[19]. Wachowiak and Kenneth[20] 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[21]. Cook *et al.*[22] reported separation of monosubstituted phenol isomers using liquid crystal stationary phases. Complete separation was achieved when *n*-alkyl ether derivatives were formed. Trimethylsilyl derivatives of *ortho*, *meta* and *para* cresol, phenol, catechol, resorcinol and hydroquinone were analyzed by capillary gas chromatography with mass spectrometer detector using *ortho* chloro phenol as an internal standard[23]. High performance liquid chromatography has also been used for the separation of phenols. Raghavan[24] reported separation of hydroxyphenols from phenol and benzoquinone on a C18 column with 4% dioxane-water as the mobile phase in 25 minutes. Good resolution of hydroxybenzenes including phenol, *ortho* cresol, *meta*- and *para*- cresol, resorcinol, catechol and hydroquinone was obtained by Yeatts *et al.*[25] using C18 column and 40% (v/v) acetonitrile in water buffered with

sodium acetate-acetic acid at pH 4.6. The process for the separation of catechol, hydroquinone and resorcinol has been patented[26]. Cheng and Yuehua[27] separated phenol, *ortho*-, *meta*- and *para*-hydroxy phenol isomers by using perfluoramide bonded stationary phase.

Because of the high polarity of phenolic compounds and the decomposition or oxidation of phenols at high temperature, derivatization of dihydroxybenzenes is necessary for the separation in the presence of phenol. In the present work, simultaneous separation of 1,4-benzoquinone, phenol, catechol and hydroquinone has been achieved on TPIPP. An efficient and rapid gas chromatographic method has been proposed where derivatization of the phenols is not necessary.

Table 3.8 presents the retention times and relative retention values (α) of benzoquinone, phenol and dihydroxybenzene isomers on the prepared TPIPP columns. Column II gives the best separation and a patent application for the separation process has been filed[28].

3.2.2 Separation between Other Higher Boiling Isomers:

The retention times and α values of some other higher boiling isomers on Column I and II are presented in Table 3.9. *Ortho* and *para* isomers of chlorobenzophenone can be separated very well, but resolution between *ortho* and *meta*; and *meta* and *para* isomers is partial. A very good separation between α and β naphthol can be obtained on Column I (Figure 3.7). All the other dimethyl phenol isomers can be separated on both the columns, except 2,4- and 2,5-dimethyl phenol, as well as 2,3 - and 3,5-dimethylphenol.

3.2.3 Conclusion

TPIPP has shown very promising results in the separation of various positional isomers of phenolic compounds and is particularly very useful for the separation of hydrolysis products of phenol. The PIP esters such as PIPA, IPBPDA and TPIPP have shown promising results as GC stationary phases. The TPIPP ester is more polar than tricresyl phosphate and thermally the most stable. It is suggested as an improvement over other phosphate esters that can be used only upto 125°C. We could achieve separation of higher boiling isomers on this ester phase.

TABLE 3.8

RETENTION TIMES t_R (min) AND RELATIVE RETENTION VALUES (α) OF PHENOLS ON VARIOUS TPIPP COLUMNS

Injection temperature 240°C, Detector temperature 250°C

Column No.*	I		II		III		IV		V	
Temperature °C	180		200		200		200		200	
Flow rate (ml/min)	25		45		30		30		30	
Solute	t_R	α	t_R	α	t_R	α	t_R	α	t_R	α
Benzoquinone	0.59	1.00	0.82	1.00	1.50	1.00	2.97	1.00	2.26	1.00
Phenol	1.03	1.75	1.40	1.71	2.84	1.80	3.17	1.07	3.07	1.36
Catechol	5.26	8.92	6.74	8.22	23.50	15.67	10.89	3.67	12.90	5.71
Hydroquinone	10.07	17.07	11.62	14.17	29.66	19.77	18.79	6.33	19.79	8.76
Resorcinol	12.19	20.49	13.95	17.01	-	-	-	-	20.81	9.21

* See Table 3.7

TABLE 3.9

RETENTION TIMES t_R (min) AND RELATIVE RETENTION VALUES (α) OF SOME POSITIONAL ISOMERS

Carrier gas Nitrogen at a flow rate of 30 ml min^{-1}, Injection temperature 240°C, Detector temperature 250°C

Column No.	TPIPP - I			TPIPP - II		
Solute	Temp °C	t_R	α	Temp °C	t_R	α
2-Chlorobenzophenone	180	16.00	1.00	200	20.20	1.00
3-Chlorobenzophenone		17.60	1.06		21.40	1.06
4-Chlorobenzophenone		18.90	1.14		23.02	1.14
1-Naphthol	180	15.22	1.00	200	20.16	1.00
2-Naphthol		17.54	1.15		22.73	1.13
2,6-Dimethylphenol	150	2.60	1.00	150	5.86	1.00
2,4-Dimethylphenol		4.00	1.54		9.18	1.57
2,5-Dimethylphenol		4.01	1.54		9.18	1.57
2,3-Dimethylphenol		4.93	1.90		11.74	2.00
3,5-Dimethylphenol		5.28	2.03		11.89	2.03
3,4-Dimethylphenol		6.07	2.33		14.09	2.40

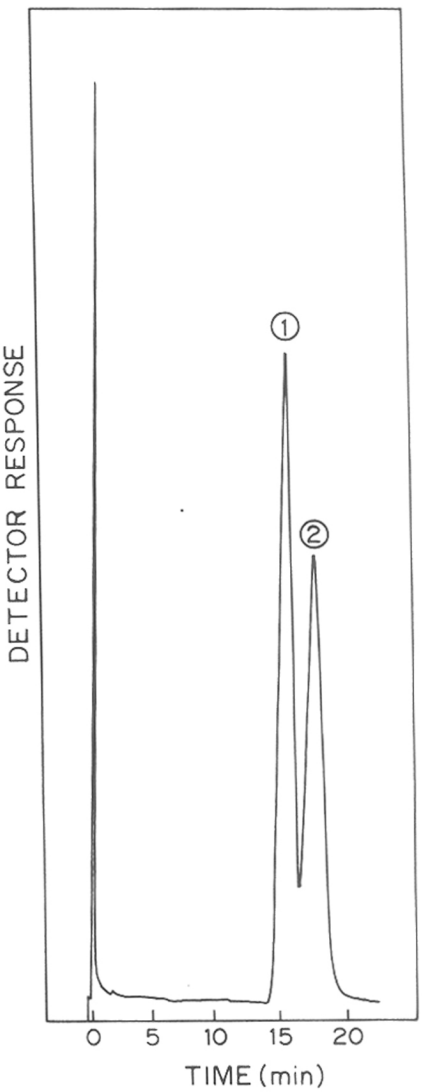


FIG. 3.7 : **SEPARATION OF 1- and 2-NAPHTHOL ON COLUMN No. I**
Oven temp. 180°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 220°C, Detector temp. 250°C.
Peaks: 1) 1-naphthol, 2) 2-naphthol.

REFERENCES

1. J.S. Lewis, H.W. Patton, W.I. Kaye, *Anal. Chem.*, **28** (1956) 1370.
2. S.A. Ryce, W.A. Bryce, *Anal. Chem.*, **29** (1957) 925.
3. D.M. Ruthven, C.N. Kenney, *Analyst* (London), **91** (1966) 603.
4. V.T. Brooks, *Chem. Ind.* (London), **42** (1959) 1317.
5. S.J.R. Lindsay, R.O.C. Norman, G.K. Radda, *J. Gas Chromatogr.*, 2 (1964) 146.
6. W.O. McReynolds, *J. Chromatogr. Sci.*, **8** (1970) 685.
7. N.R. Ayyangar, M.D. Deo, K.V. Srinivasan, *Indian J. Technology*, **24** (1986) 759.
8. D.N. Andreevskii, M.I. Aleksandrova, *J. Appl. Chem.* USSR, **34** (1961) 2181.
9. B.B. Corson, W.J. Heintzelmen, L.H. Schwartzman, H.E. Tiefenthal, R.J. Lokken, J.E. Nickel, G.R. Atwood, F.J. Pavlik, *J. Org. Chem.*, **23** (1958) 544.
10. M. Levine, S.C. Temin, *J. Polym. Sci.*, **28** (1958) 179.
11. E.V. Kuznetsov, L. Minimullina, *J. Appl. Chem.*, USSR, **32** (1950) 490.
12. V.S. Tsivunin, R.G. Iranova, G.K. Kamai, *J. Gen. Chem.*, USSR, **38** (1968) 1021.
13. B. Yu. Gordinskii, V.M. Shimanskii, R.S. Vishnyakova, *J. Appl. Chem.*, USSR, **40** (1967) 1820.
14. L. Ambrus, *J. Chromatogr.*, **294** (1984) 328.
15. X. Guardino, J. Albaiges, G. Firpo, R. Rodriguez-Vinals, M. Gassiot, *J. Chromatogr.*, **118** (1976) 13.
16. F. Vernon, C.O.E. Ogundipe, *J. Chromatogr.*, **132** (1977) 181.
17. P. Buryan, J. Macak, *J. Chromatogr.*, **150** (1979) 246.
18. J.S. O'Grodnick, G.D. Dupre, B.J. Gulzia, S.H. Blake, R.A. Kuna, *J. Chromatogr. Sci.*, **21** (1983) 289.
19. H. Mayer, J. Nolte, B. Paschol, *Fresenius Z. Anal. Chem.*, **315** (1983) 708.

20. R. Wachowiak, A.C. Kenneth, *Anal. Chem.*, **51** (1979) 27.

21. V. Vahessaar, Eesti NSV Tead. Akad. Toim., *Keem Geol.*, **17** (1968) 124 (Russ) CA 69 : 56847Y.

22. L.E. Cook, R.C. Spangelo, *Anal. Chem.*, **46** (1974) 122.

23. E.J. Nanni, M.E. Lovette *et al. J. Chromatogr.*, **505** (1990) 365.

24. N.V. Raghavan, *J. Chromatogr.*, **168** (1979) 523.

25. L.B. Jr. Yeatts, G.B. Hurst, J.E. Gaton, *Anal. Chim Acta.*, **183** (1988) 348.

26. A.H. Zinman, US 4,82,049, 2 May 1989, Appl. 173, 853 28 May 1983 pp 7. CA 111 : 173743h.

27. W.J. Cheng, M. Yuchua, *Sepu* 4 # 5 (1986) 291, Ch. CA 106 : 78001c.

28. P.P. Moghe, A.S. Tambe *et al.* 625/DEL/90, Appl. June 22, 1990.

CHAPTER 4

SYNTHESIS AND GAS CHROMATOGRAPHIC ANALYSIS OF *PARA* CHLOROBENZOPHENONE

CHAPTER 4

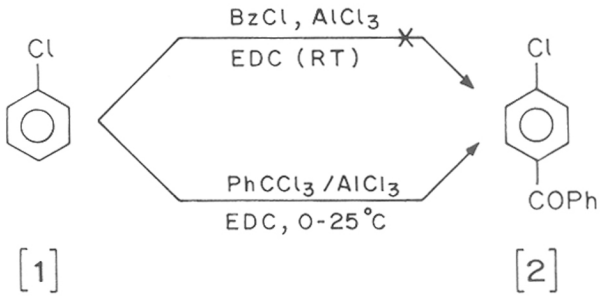
SYNTHESIS AND GAS CHROMATOGRAPHIC ANALYSIS OF *PARA* CHLOROBENZOPHENONE

4.1 Synthesis of *Para* Chlorobenzophenone in Quantitative Yields by Friedel Crafts Acylation Under Milder Reaction Conditions.

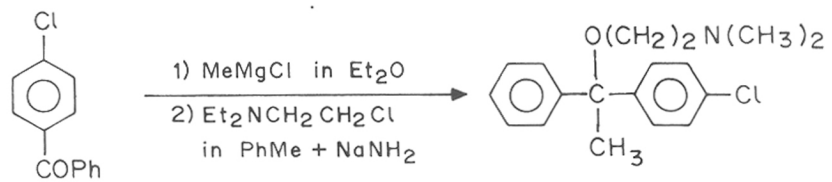
Chlorphenoxamine or β-dimethylaminoethyl (*p*-chloro-α-methylbenzhydryl) ether hydrochloride which is known as systral [3] is one of the important drugs used as anti-parkinson agents. It can be manufactured from 4-chlorobenzophenone. Friedel-Crafts reaction of benzene with 4-chloro benzoyl chloride or the benzoylation of chlorobenzene using benzoyl chloride are the two different routes for the synthesis of 4-chlorobenzophenone. Morley[1] reported benzoylation of halobenzenes catalyzed by Iron (III) sulphate. He observed that the yield is low when the concentration of the catalyst is increased. Also, the amount of acid-chloride consumed increases, whereas the benzophenone yield decreases due to side reactions. He also reported that the yields obtained with active metal oxides were generally higher than those obtained by the traditional Friedel-Crafts synthesis[2]. For efficient benzoylation excess of the substrate is required. It should be noted that 2- as well as 4- isomers were formed. Although there are few patents,[3,4] none mentions the yield or specific reaction conditions.

We report an efficient synthesis of 4-chlorobenzophenone starting from chlorobenzene without formation of any other isomer. Therefore, the product can directly be used for the synthesis of systral avoiding the need for isomer separation.

Recently, the existence of a remarkably stable phenyldichlorocarbenium tetrachloroaluminate complex derived from benzotrichloride or (trichloromethyl) benzene under Friedel-Crafts acylation conditions has been reported from our laboratory[5]. It has been stated that (trichloromethyl) benzene is an effective benzoylating agent for the preparation of substituted benzophenones under milder reaction conditions[6]. We have carried out the benzoylation of chlorobenzene using (trichloromethyl) benzene as benzoylating agent. The solvent used is dichloroethane and anhydrous aluminium chloride is employed as Lewis acid catalyst. Almost quantitative yield of



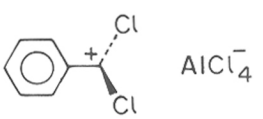
[1] [2]



SYSTRAL

[2] [3]

SCHEME 4·1: SYNTHESIS OF SYSTRAL FROM CHLOROBENZENE THROUGH CHLOROBENZOPHENONE



STRUCTURE OF STABLE PHENYLDICHLOROCARBENIUM TETRACHLOROALUMINATE COMPLEX

4-chlorobenzophenone is possible if molar ratio of the Lewis acid catalyst and chlorobenzene is 3:1. Scheme 4.1 represents the synthesis of *para* chlorobenzophenone [2] from chlorobenzene [1] and conversion of [2] into the final drug, systral [3].[7,8]

It has been observed that when benzoyl chloride was used as benzoylating agent, negligible amount of 4-chlorobenzophenone was formed. Exactly the same procedure was followed as given in Section 4.1.2, the only difference being the use of benzoyl chloride (7.05 gms, 0.05 moles) as the benzoylating agent. Under the same reaction conditions a quantitative yield of [2] was obtained when (trichloromethyl) benzene was used. Various molar proportions of $AlCl_3$ with respect to chlorobenzene were tried to get the quantitative yield of 4-chlorobenzophenone (Table 4.1). The results clearly show that when the molar ratio was 3, there was practically no formation of *ortho* and *meta* chlorobenzophenone. The percentage of 2-chlorobenzophenone formed has been plotted against the molar concentration of $AlCl_3$ with respect to chlorobenzene (Fig. 4.1). The analysis of 2-, 3- and 4-chlorobenzophenone was carried out by gas-liquid chromatography, the details of which are presented in Section 4.2.

4.1.1 Experimental

Melting points were obtained in capillary tubes. IR spectra were recorded using a Perkin-Elmer 221 spectrophotometer and ^1H NMR spectra were obtained using a Varian FT-80 spectrometer using TMS as the internal reference. Mass spectra were recorded on a Finnigan Mat 1020 automated GC/MS spectrometer. Gas chromatographic analysis was carried out using a Hewlett-Packard Model 5880A gas chromatograph equipped with a level 4 integrator and a flame ionization detector.

4.1.2 Procedure

To a cooled solution (0-5°C) of anhydrous aluminium chloride (20g, 0.15 mole) in ethylene dichloride (125 ml), benzotrichloride (9.8 g, 0.05 mole) was added over 15 minutes. This was followed by an addition of chlorobenzene (5.6 g, 0.05 mole) over 0.5 hr. The reaction mixture was stirred for 5 hrs. Thin layer chromatographic analysis was carried out on silica gel plates using petroleum ether and ethyl acetate (5:1) as eluent. After completion the reaction mixture was poured

TABLE 4.1

YIELD OF CHLOROBENZOPHENONE ISOMERS WITH RESPECT TO MOLAR RATIO OF ALUMINIUM CHLORIDE TO CHLOROBENZENE

	Benzolylating Agent (BA)	Molar ratio $AlCl_3$: BA	Molar ratio $AlCl_3$:Chloro-benzene	Percentage Yield of Chlorobenzophenones (CBP)			Total Yield
				2-CBP*	3-CBP	4-CBP	
1)	Benzoylchloride	3 : 1	3 : 1	3.15	1.01	95.82	8.87
2)	Benzotrichloride	0.75 : 1	0.75 : 1	10.96	0.3	88.74	40
3)	Benzotrichloride	1 : 1	1 : 1	7.94	0.09	91.97	52
4)	Benzotrichloride	2 : 1	2 : 1	4.75	-	95.25	94
5)	Benzotrichloride	3 : 1	3 : 1	2.15	0.09	97.76	98
6)	Benzotrichloride#	3 : 1	3 : 1	0.76	-	99.24	98

* This percetage was found by gas chromatography using internal standard method which is described in section 4.2.

\# Product crystallized from hexane.

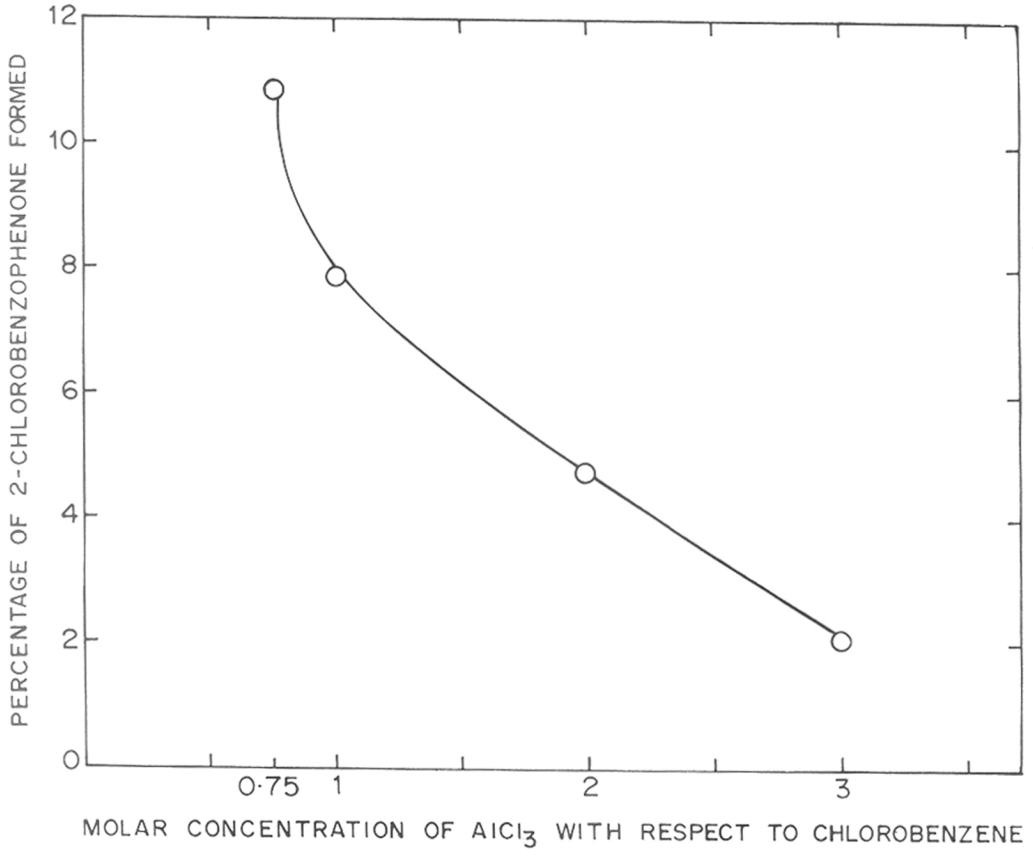


FIG. 4.1 : **GRAPH OF PERCENTAGE OF 2-CHLOROBENZOPHENONE FORMED AGAINST THE MOLAR CONCENTRATION OF $AlCl_3$ WITH RESPECT TO CHLOROBENZENE**

into crushed ice (250 g) and concentrated hydrochloric acid. It was stirred at 75°C for 0.5 hr. The organic layer was separated, washed with water (2 x 150 ml) and dried. Ethylene dichloride was removed by distillation and the product [2] was distilled under vacuum (4 mm) at 165°C.

M.P. 75-76°C, lit. M.P.[1] 75-76°C, Yield: 10.6 g 98%, IR (Nujol): 1640, 1580, 1440, 1360, 1300, 1280, 1140, 1080, 1000, 920, 840, 790, 720, 690. ^{1}H NMR (80 MHz, $CDCl_3$) : δ 7.33-7.82 (9H, m, Ar-H). MS : M/e (rel. int. %) : 218 $(M + 2)^+$ (18), 216 M^+, (58), 181 (21), 152 (10), 139 (92), 105 (100), 77 (47).

Anal. Calcd. for $C_{13}H_9C10$: C, 72.22; H, 4.16; Cl, 16.20.

Found : C, 72.14; H, 3.98; Cl, 15.92.

4.2 Gas Chromatographic Method for the Analysis of Chlorobenzophenone Isomers

In order to optimize the conditions for the reaction described in Section 4.1, it was necessary to find out an analytical method which can estimate the amounts of *ortho* and *meta* isomers in *para* chlorobenzophenone (PCBP).

In the literature, separation of dichlorobenzophenone isomers has been reported on 2.5% Carbowax 20M which takes more than 40 minutes[9]. Gas-liquid chromatography of substituted benzils and benzophenones has also been reported where retention of benzophenones with different substituents such as *para* methyl, *para* bromo, *para* chloro and *para* methoxy is compared.[10] It is pointed out that the *meta* methoxy benzophenone and the *meta* methoxy benzil have shorter retention times than their *para* counterparts. Bishara and Smith[11] have reported separation of dichloro and chlorofluorobenzophenone isomers by high performance liquid chromatography (HPLC) using Zorbax-ODS column and water-tetrahydrofuran (55:45) as mobile phase. Recently a collection of analytical data for benzodiazepines and benzophenones has been published which gives GC, HPLC and TLC methods for the analysis of benzophenones formed after the hydrolysis of benzodiazepines.[12,13] Analysis of chlorophenylphenols has been done on 15% butenediol succinate at 216°C[14]. Chlorinated products of ketones and alcohols such as methyl benzyl ketone are analyzed on various columns, such as diisodecylphthalate, dimethyl siloxane polymer, Carbowax 20M, diethyleneglycol succinate "Craig", didecyl phthalate and silicon (GE-SF-96)[15]. Chromatographic properties of methyl ethers of chlorinated 2-phenoxyphenols are reported[16]. However, reference to the

separation of *ortho*, *meta* and *para* isomers of chlorobenzophenone is not found in the literature. In this section we describe a rapid and an efficient GC method for the quantitative analysis of *para* chlorobenzophenone.

4.2.1 Experimental

The analysis was performed on a Hewlett-Packard 5880A gas chromatograph equipped with level 4 integrator, computing system and a flame ionization detector. Cross linked methyl silicon gum (25 m x 0.2 mm x 0.33 μm) fused silica column was purchased from Hewlett-Packard. Carbowax 20M -5%, OV-210 - 3% and OV-17 - 5% columns were purchased from Chromato-Pak Enterprises while the following columns were purchased from Alltech Associates : OV-351-10%, Dexil 300 - 3%, KG-02 on Uniport HP (10ft x 2 mm, glass lined SS (GLT)), Alltech CS-10 - 10% (6ft x 2mm, (GLT)), 0.3% Carbowax 20M + 0.1% H_3PO_4 on Graphpac GC, and Apiezon-L - 10% + 2% KOH on Chromosorb WAW 80/100 (GLT) - 2m Column A.

Apiezon L was coated on Chromosorb WAW 80/100 at the concentration of 2% and packed in a 2 mm x 3m SS column - Column B. SE-30 - 5% and 10% Columns as well as tri-[4-(2'-phenylisopropyl) phenyl] phosphate[17] columns were packed in the laboratory.

Complete separation of all the isomers of chlorobenzophenone was achieved on Column B at 160°C. Quantitative estimation and minimum detectable amount of *meta* isomer in *para* chlorobenzophenone was found out on Column B at 200°C. Quantitative estimation of *ortho* isomer in *para* chlorobenzophenone was done at 200°C on Column A by an internal standard method. Benzophenone was used as an internal standard. The chemicals used were of Aldrich or Merck grade and their purity was checked by gas chromatography. Nitrogen was used as the carrier gas at the flow rate of 30ml/min.

Preparation of the Standard and Unknown Mixtures

I. Two hundred and fifty milligrams of *ortho* chlorobenzophenone (OCBP) was weighed accurately in a 25ml volumetric flask and diluted to 25 ml with acetone. Similarly 250 mg of benzophenone (ISTD) was weighed accurately, transferred in a 25 ml volumetric flask and diluted upto the mark with acetone. In a series of 10ml volumetric flasks,

aliquots of 1, 2, 3, 4, 5, 0.1, 0.2, 0.3, 1.4 and 0.14 ml stock solution of OCBP isomer were taken. Two ml ISTD solution was added to each of these and the volume was made upto 10 ml with acetone.

After instrument stabilization 2.0 µl of each standard solution was injected thrice. Areas of individual peaks were obtained from the integrator. The ratios of peak areas of OCBP to ISTD were plotted against the concentration (mg ml^{-1}). Separate graphs were plotted for two different concentrations : i) 1 to 5 mg ml^{-1}, and ii) 0.1 to 0.3 mg ml^{-1}. Both the graphs as can be seen are linear.

Samples (P-1, P-2, P-1 crude, P-4 and P-5) were weighed accurately and 2 ml ISTD stock solution was added to each product before dilution upto 10 ml with acetone.

II. Eleven milligrams of *meta* chlorobenzophenone (MCBP) was weighed accurately in a 10 ml volumetric flask and diluted upto the mark with acetone. Similarly 2.5 gms of PCBP was weighed in a 25 ml volumetric flask and diluted upto the mark with acetone. In a series of 10 ml volumetric flasks aliquots of 500, 250, 200, 150 and 100 µl of MCBP stock solution were taken. PCBP stock solution (5 ml) was added to each of these and the volume was made upto 10 ml with acetone.

After instrument stabilization, 1µl of each standard solution was inejcted thrice on Column B at 200°C. Solutions of our products were also injected at the same temperature.

4.2.2 Results and Discussion

Separation of chlorobenzophenone isomers was tried on the following liquid stationary phases coated on chromosorb WAW or HP DMCS (80/100 mesh) at various loadings packed in SS or glass lined SS columns (1.8 x 2 mm): Carbowax 20M, SE-30, OV-17, OV-351, Dexil, OV-210, Alltech CS-10, KG-02 on Uniport, TPIPP, Apiezon L and Graphatized carbon. Most of the stationary phases separate either *ortho* and *para* or *meta* and *para* isomers. Dexil 300 shows a good separation between *ortho* and *para*, but *meta* and *ortho* isomers donot separate while KG-02 separates *ortho* isomer but *meta* and *para* isomers show overlapping or single peak. Graphatized carbon modified with carbowax 20M or SP1000 retains all the isomers for a long time and peaks are very broad. On TPIPP all the three isomers are resolved partially. Analysis was also tried on crosslinked methyl silicon gum (25m x 0.2mm x 0.33 µm film thickness) at 200°C. Carrier gas was

nitrogen at a flow rate of 1.5 ml min^{-1} and splitting ratio was 1:135. A good separation between *ortho* and *para* isomers was obtained but *meta* and *para* isomers were only partially resolved. The separation did not improve further even at lower temperature. Apiezon L-10% coated on 2% KOH treated chromosorb WAW glass lined stainless steel column gave a good separation of all the three benzophenone isomers at 160°C and benzophenone (ISTD), OCBP and PCBP at 200°C (Fig. 4.2). Hence this column was used for the ISTD method. Figures 4.3 and 4.4 show calibration curves for two concentration ranges : 1 to 5 mg ml^{-1} and 0.1 to 0.3 mg ml^{-1}. Statistical evaluation of the method is given in Table 4.2 while Table 4.3 gives the results obtained for our products. In order to find out the minimum detectable amount of MCBP in PCBP, a standard solution of 0.1% MCBP in PCBP was injected on this Column A. Since MCBP was not detected on this column, a longer column with less percentage loading was prepared (Column B). It gives complete separation of all the three isomers (Fig. 4.5) and can detect MCBP upto 0.04% in PCBP even at 200°C. Table 4.4 gives the retention times of chlorobenzophenone isomers and ISTD on Column A as well as Column B. When our products were injected on Column B, the presence of MCBP was seen in the range of 0.08 to 0.3% . An important point to be noted is that PCBP synthesized by our method contains 0.09% of the *meta* isomer at the crude stage. It completely disappears after recrystallization of PCBP from hexane. The recrystallized PCBP does not show presence of *meta* isomer impurity. This suggests that even if it is present it must be in negligible amount (less than the minimum detectable limit or < 0.04%).

4.2.3 Conclusion

We have described an efficient and rapid gas chromatographic method for the quantitative estimation of *ortho* chlorobenzophenone in *para* chlorobenzophenone using Apiezon L as stationary phase and benzophenone as an internal standard. The total analysis time is only 8 minutes and the standard deviation ranges from 8.15×10^{-3} to 1.27×10^{-2}. Complete separation of *ortho*, *meta* and *para* isomers of chlorobenzophenone has been achieved using 2% Apiezon L coated on chromosorb WAW DMCS (80/100 mesh) packed in a 3m x 2mm stainless steel column. The minimum detectable amount of *meta* isomer in the *para* isomer is 0.04% .

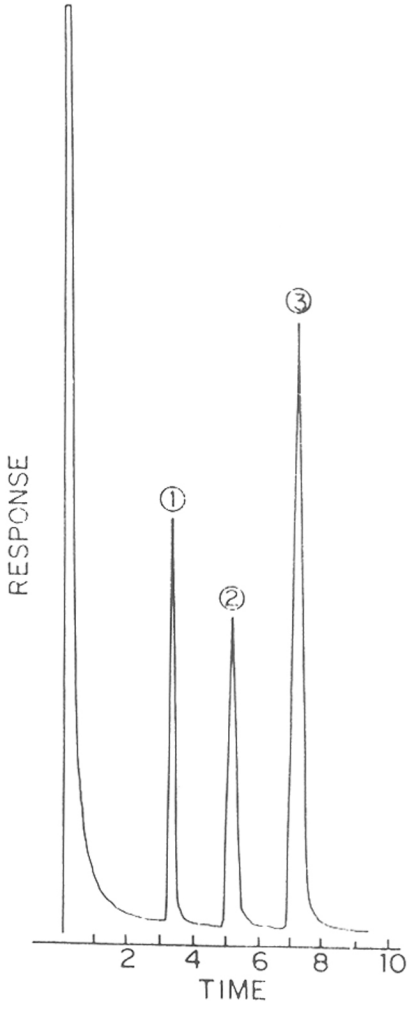


FIG. 4.2 : **SEPARATION OF BENZOPHENONE (ISTD), 2-CHLOROBENZOPHENONE AND 4-CHLOROBENZOPHENONE ON COLUMN A**
Oven temp. 200°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 240°C, Detector temp. 300°C.
Peaks: 1) benzophenone, 2) 2-chlorobenzophenone
3) 4-chlorobenzophenone

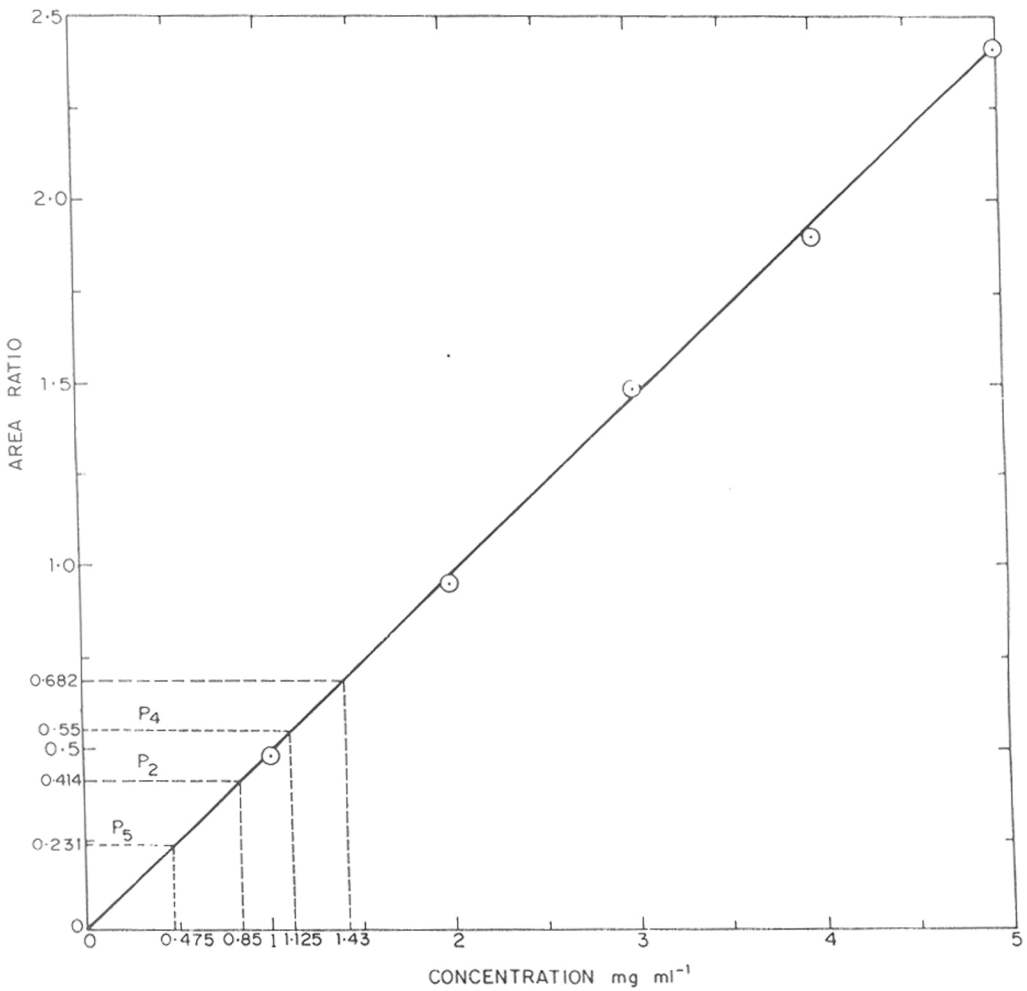


FIG. 4.3 : CALIBRATION PLOT FOR 2-CHLOROBENZOPHENONE IN THE RANGE 1-5 mg/ml

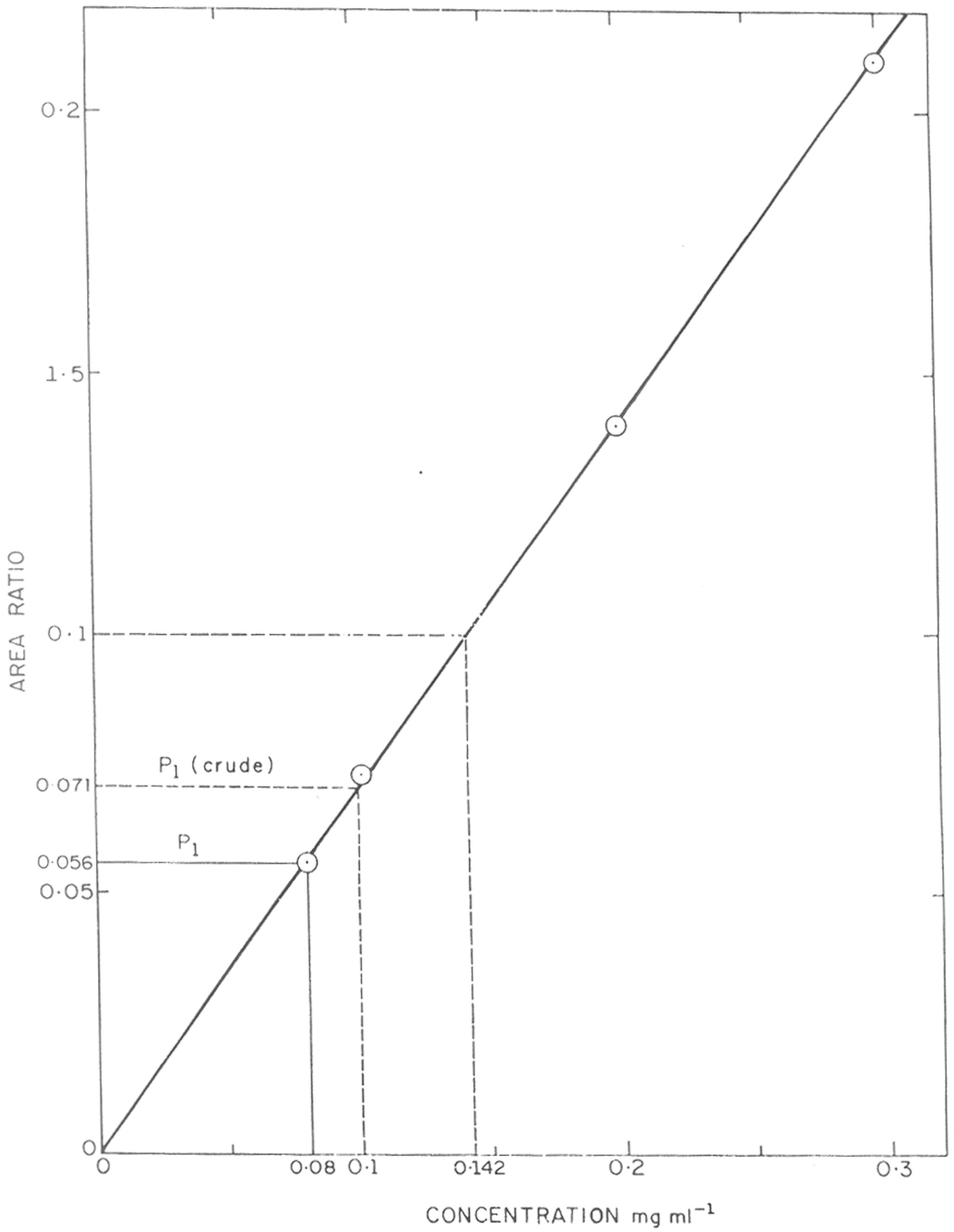


FIG. 4.4 : **CALIBRATION PLOT FOR 2-CHLOROBENZOPHENONE IN THE RANGE 0.1-0.3 mg/ml**

TABLE 4.2

STATISTICAL EVALUATION OF THE ISTD METHOD

Set	Amount taken	Amount found*	Standard deviation	Percentage error
I	0.14 mg	0.142 mg	1.2×10^{-2}	+ 1.4
II	14 mg	14.3 mg	1.09×10^{-2}	+ 2.1

* Average of 3 determinations

TABLE 4.3

RESULTS OBTAINED FROM GRAPH FOR TECHNICAL SAMPLES

	Area ratio*	Amount of OCBP mg/ml	Percentage composition	Standard deviation
P-1#	0.056	0.08	0.76	1.27×10^{-2}
P-1 (crude)	0.071	0.1	0.95	2.14×10^{-3}
P-2	0.414	0.85	7.94	8.15×10^{-3}
P-4	0.55	1.125	10.96	1.23×10^{-2}
P-5	0.231	0.475	4.75	4.134×10^{-3}

* Average of 3 determinations; # recrystallized from hexane

TABLE 4.4

RETENTION TIMES (min) OF CHLOROBENZOPHENONE ISOMERS AND BENZOPHENONE (ISTD)

Column	Temperature	Benzophenone	OCBP	MCBP	PCBP
A	200°C	3.46	5.29	6.34	7.34
B	160°C	12.43	20.61	26.75	28.95
B	200°C	5.39	8.00	9.94	10.92

OCBP = *ortho* Chlorobenzophenone

MCBP = *meta* Chlorobenzophenone

PCBP = *para* Chlorobenzophenone

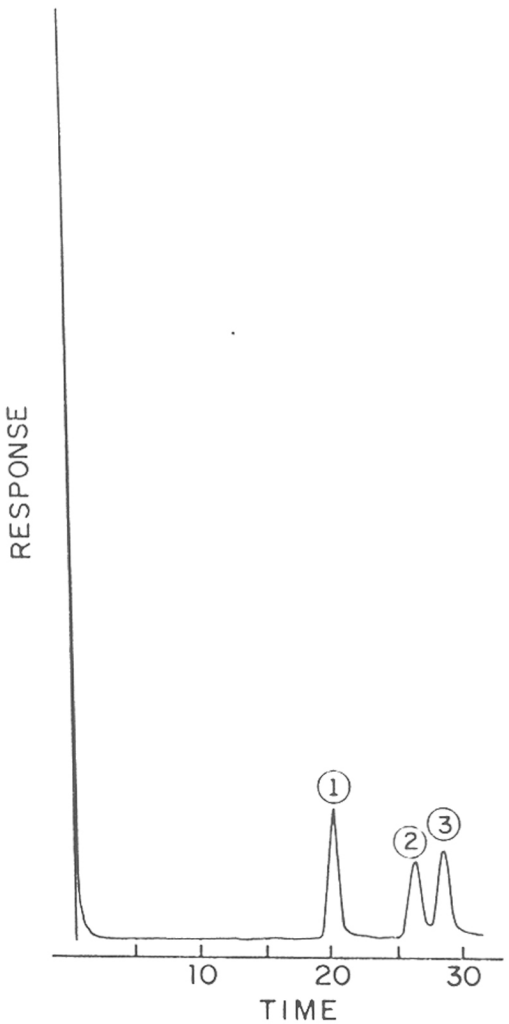


FIG. 4.5 : **SEPARATION OF CHLOROBENZOPHENONE (CBP) ISOMERS ON COLUMN B**
Oven temp. 160°C, Carrier gas nitrogen flow rate - 30 ml/min, Injector temp. 200°C, Detector temp. 250°C.
Peaks: 1) 2-CBP, 2) 3-CBP, 3) 4-CBP

REFERENCES

1. J.O. Morley, *Synthesis*, (1977) 54.
2. J.O. Morley, *J. Chem. Soc. Perkin Trans. II*, (1977) 601.
3. E. Franz, E. Gerhard, H. Klaus, *Ger. Offen*, 2, 204,973, Aug. 9, 1973, Appl. 3 Feb. 1972. CA 79 : 115315e.
4. H. Seifertand, B. Wenh, *Ger.* 876, 690 (1953) CA 52:8200d.
5. U.S.Racherla, T. Daniel, P.R. Rajmohanan, N.R. Ayyangar, *J. Am. Chem. Soc.* **111** (1989) 7659.
6. N.R. Ayyangar, R.J. Lahoti, K.V. Srinivasan, T. Daniel, *Synthesis* (1991) 322.
7. H. Arnold, N. Brock, E. Kuhas, *U.S. Patent* 2, 785, 202, Mar. 12, 1957, CA 51:6698d.
8. B.K. Wasson, *Brit.* 743, 043 Jan 4, 1956, CA 50:15589g.
9. M.H. Abraham, D. Huq, R.U. Koenigsberger, J.B. Rose, *J. Chromatogr.*, **206** (1981) 147.
10. W.F. Brubakar, M.A. Ogliaruso, *J. Chromatogr.* **324** (1985) 450.
11. R.H. Bishara, S.L. Smith, *J. Chromatogr.* **234** (1982) 261.
12. M. Japp, K. Garthwaite, A.V. Geeson, M.D. Osselton, *J. Chromatogr.*, **439** (1988) 317.
13. S.I. Weston, M. Japp, J. Partridge, M.D. Osselton, *J. Chromatogr.*, **538** (1991) 277.
14. B.J. Gudzinowicz, *Anal. Chem.*, **34** (1962) 1032.
15. C. Walling, A. Padwa, *J. Am. Chem. Soc.*, **85** (1963) 1593.
16. C.A. Nilsson, K. Andersson, *Chemosphere*, **6** (1977) 263.
17. N.R. Ayyangar, A.S. Tambe and S.S. Biswas, *J. Chromatogr.*, **483** (1989) 33.

CHAPTER 5

ENANTIOMERIC SEPARATIONS

CHAPTER 5

ENANTIOMERIC SEPARATIONS

5.1 Separation of Enantiomers by Gas Chromatography on Chiral Stationary Phases

A substance that can rotate the plane of polarized light is known as optically active or chiral substance and the property is called as optical activity or chirality. The two different non-superimposible forms of a chiral compound are called optical isomers or enantiomers . They show similar chemical composition and constitution but different sterical configuration. The biological or pharmacological activity and effectiveness of chiral molecules depend largely on their configuration. One of the enantiomers shows pharmacological activity while the other may be inactive or even toxic. This has led to the concept of stereoselective syntheses. Determination of optical purity and separation of enantiomers is a challenging task. Resolution of enantiomeric mixtures by gas chromatography is an attractive method for the analysis of asymmetric reactions. High resolution, precision, and reproducibility are the merits of gas chromatography for enantiomeric resolution.

The resolution of enantiomeric mixtures by GC can be performed in two modes:

a) Conversion of the enantiomers into diastereomeric derivation by chemical reaction with an auxiliary, enantiomerically pure chiral resolving agent and subsequent GC separation of the resulting diastereomers on an achiral stationary phase.

b) Direct resolution of the enantiomers on a chiral stationary phase containing an auxiliary resolving agent of high enantiomeric purity.

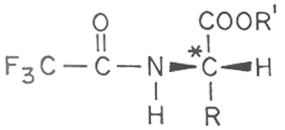
The first method suffers from many drawbacks. For example, to prepare diastereomeric derivatives substrate must possess at least one reactive function. Also, racemization may take place during the reaction. The enantiomeric purity of the resolving agent will affect the accuracy of the percentage of enantiomeric excess determination for highly enriched mixtures. The second method is more reliable since the enantiomeric ratio is independent of enantiomeric purity of the chiral stationary phase. There are four major types of optically active stationary phases in gas chromatography.

1. Low molecular weight stationary phases where resolution of enantiomers is obtained *via* hydrogen bonding,
2. Optically active polysiloxane phases,
3. Chiral metal chelates as stationary phases in complexation gas chromatography, and
4. Cyclodextrin type stationary phases.

The details of each one of these are given below.

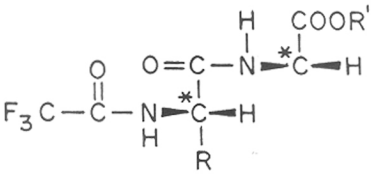
Low Molecular Weight Stationary Phases

Gil-Av *et al.*[1] reported the first quantitative and fully reproducible GC separation of enantiomers in 1966. They separated 18 racemic amino acids as their N-trifluoroacetyl (TFA) derivatives in glass capillaries coated with optically active N-TFA-L-isoleucine dodecyl ester (amino acid phase). In order to improve the thermal stability, dipeptide[2-4], diamide,[5-7] ureide[8] and amide[9] phases were developed. Their structures can be seen as [1] to [5] in Scheme 5.1. In the dipeptide phases, it was observed that only the N-terminal amino acid contributed to high separation factors. This observation led to the diamide phases which contain two thermostable amide groups but only one asymmetric centre. The ureide phase is particularly suitable for the separation of racemic N-TFA amines. Further, a chiral acyl component such as (1R, 3R)-*trans* crysanthemic acid and N-lauroylproline was introduced in the amide phases[10,11]. These could separate underivatized menthol, racemic nitriles, *cis* and *trans* crysanthemic acid,[12] and macrolide lactones[13]. Oi *et al.*[14] improved the temperature stability by incorporating the chiral structural elements into a triazine skeleton *via* N-terminus. Gil-Av *et al.*[15] briefly reviewed the applications of chiral hydrogen bonding gas chromatographic phases to the resolution of various classes of optical isomers. Proline is one of the most difficult amino acids to resolve and an amide function has to be introduced through the carboxylic group to form N-TFA-Pro-NH (tert. Bu) to obtain the resolution[14,16]. With the use of isocynate reagents, carbamate (-$NHCO_2$) or ureide (-NHCONH) derivatives can be formed which can be resolved into enantiomers[17]. Ketones can be resolved on these type of phases when converted into oximes.[18]



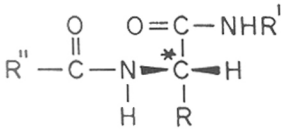
R = sec-butyl
R' = dodecyl

(1)
Amino acid phase



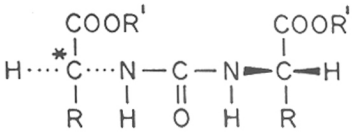
R = isopropyl
R' = cyclohexyl

(2)
Dipeptide phase



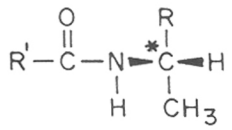
R = isopropyl
R' = tert-butyl
R'' = undecyl ($C_{11}H_{23}$)
$C_{21}H_{43}$

(3)
Diamide phase



R = R' = isopropyl

(4)
Ureide phase



R = 1-naphthyl
R = undecyl

(5)
Amide phase

SCHEME 5·1: REPRESENTATIVE STRUCTURES OF OPTICALLY ACTIVE STATIONARY PHASES FOR GC ENANTIOMER RESOLUTION VIA HYDROGEN BONDING

Optically Active Polysiloxane Phases

Frank *et al.*[19] were the first to make an optically active polysiloxane phase. In this phase the selector is attached to the carboxyl group of a polymeric matrix *via* an amide linkage. Thus the enantio-specificity of the selector L-valine-t-butylamide is combined with the high thermal stabily, viscosity and involatility of organic polysiloxanes. The commercially available optically active polysiloxane Chirasil-Val can be used between 30°C and 230°C. Other than amino acids, a number of other classes of compounds may be separated into enantiomers on this phase after appropriate derivatization.[20,21] Direct enantiomeric resolution of primary, secondary, and tertiary alcohols as well as carbonyl compounds, and even diketones is reported.[22-24].

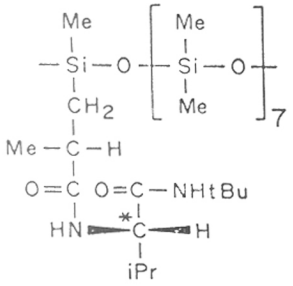
Chirasil-Val stationary phase has proved useful for many polar, and less volatile compounds. Frank and Bayer[25] reported gas chromatography-mass spectrometric analysis of optically active metabolites and drugs on Chirasil-Val. Bayer *et al.*[26] also reported separation of enantiomeric sulphur compounds on Chirasil-Val. Schurig[27] reviewed gas chromatographic separation of enantiomers on optically active, metal complex free stationary phases.

Saeed *et al.*[28,29] prepared another optically active polysiloxane phase. The cyano group of OV-225 was hydrolyzed and the carboxyl groups were coupled with L-valine-t-butylamide *via* the amino group. Konig *et al.*[30-32] prepared a phase analogous to Chirasil-Val containing (R)- or (S)-1-phenylethylamine as an additional chiral component in the amide function of the L-valine selector. For the first time the enantiomer separation of carbohydrates was achieved on XE-60-L-Valine-δ-α-phenylethylamide.[32]

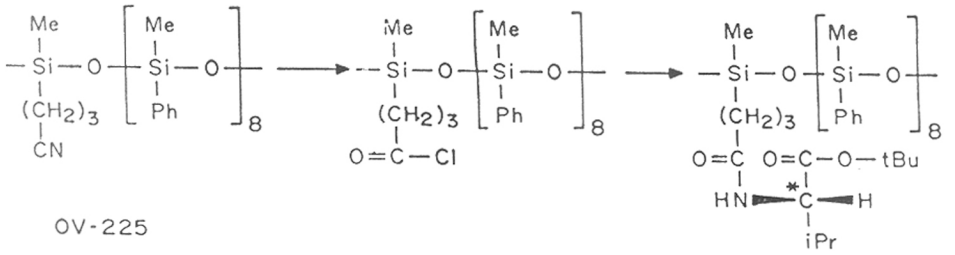
The reduction of the cyano groups of OV-225 to the methyl amine and coupling with N-acyl-L-valine *via* the carboxyl group leads to another optically active polysiloxane[33]. This phase is particularly suitable for the separation of racemic N, O-*bis*-acylamino alcohols and N-acylamines. The structures of all the four polysiloxanes phases are given in Scheme 5.2. Schurig[34,35] reviewed gas chromatography on chiral stationary phases for determination of enantiomeric compositions.

Chiral Metal Chelates in Complexation Gas Chromatography

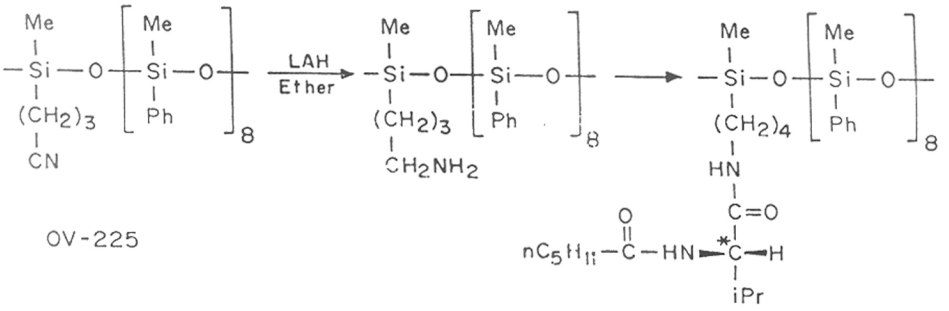
In complexation GC, an electronically and coordinatively unsaturated transition metal compound is added to the liquid stationary phase. Chiral metal coordination compounds are employed as additives to the stationary phase and optical isomer separation is achieved.



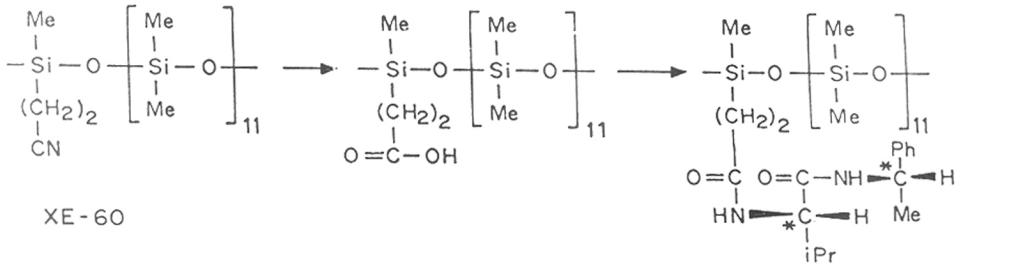
Chirasil-Val



OV-225



OV-225



XE-60

XE-60-S-Valine-S-Phenylethylamide

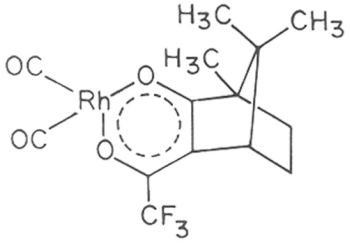
SCHEME 5·2: POLYMERIC OPTICALLY ACTIVE STATIONARY PHASES FOR GC ENANTIOMER RESOLUTION

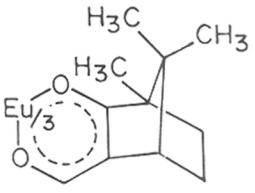
The first chiral metal chelate phase dicarbonylrhodium (I)-3-trifluoroacetyl-(1R)-camphorate was synthesized by Schurig and Gil-Av in 1971-72.[36,37] The separation of racemic 3-methylcyclopentene was obtained on this phase in 1977.[38] Schurig[39] reported quantitative resolution of methyloxirane and *trans* 2,3-dimethyloxirane on nickel (II) *bis* (3-trifluoroacetyl-(1R)-camphorate dissolved in squalane. Corresponding heptafluorobutanoyl derivative gives better separation factors. Nickel (II) can also be replaced by manganese (II) in the complex.[40] Underivatized alcohols as well as ketones can be separated by complexation chromatography[41]. Complexation gas chromatography can be used to separate isotopomers, e.g., deutorated solutes differing in only one mass unit.[42,43] A serious limitation to the method is the requirement of solute volatility, thermal stability and quantitative resolvability. Scheme 5.3 gives the structures of chiral metal chelates for enantiomer resolution by complexation gas chromatography. The scope, merits and limitations of enantiomer analysis by complexation chromatography has been reviewed by Schurig[44].

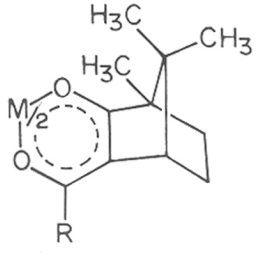
Cyclodextrin Stationary Phases

Cyclodextrins are a homologous series of nonreducing cyclic oligosaccharides made up of six or more (α)-D-glucopyranose units linked together by α-1,4-glycoside bonds (Fig. 5.1). The conformation and complexing ability of cyclodextrins can be tailored by chemical modification.

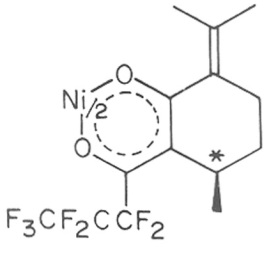
The separation of enantiomers on stationary phases containing cyclodextrins (CDS) by liquid and thin layer chromatography is well established.[45-47] The application of acylated CDS in GC for the separation of fatty esters was reported[48] in 1961 while separation of hydrocarbons was achieved by employing permethylated CDS in polysiloxane as column packings[49]. The greater retentions for *iso* alkanes than for *n*-alkanes were attributed to the formation of inclusion complexes. There are few more references in which gas chromatographic separation of achiral compounds was obtained on columns packed with CD phases.[50-54] The first gas chromatographic separations of enantiomers on columns packed with CD phases were observed for pinenes and pinanes[55,56]. These packed columns had very low efficiencies and the peaks were broad. Therefore it was proposed to develope thermally stable CD phases for use in high resolution capillary columns. Underivatized cyclodextrins are not suitable for coating capillary columns. Dilution of peralkylated CDS in moderately polar polysiloxanes offer many advantages regarding temperature, efficiency, polarity, amount of CD required and time.



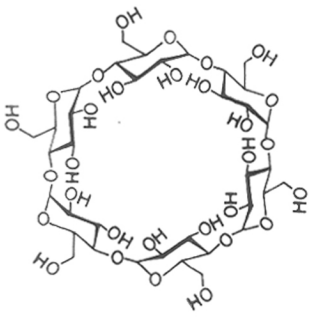




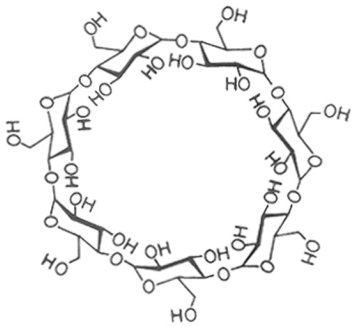
M=Ni, R = CF_3, C_3F_7
M=Mn, R = C_3F_7



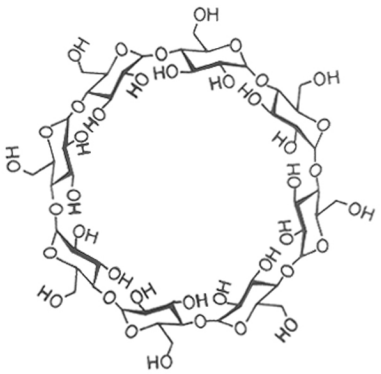
SCHEME 5·3: CHIRAL METAL CHELATES FOR ENANTIOMER RESOLUTION BY COMPLEXATION GAS CHROMATOGRAPHY



α-CYCLODEXTRIN



β-CYCLODEXTRIN



γ-CYCLODEXTRIN

Fig. 5.1 : α - CYCLODEXTRIN (CYCLOHEXAAMYLOSE), β - CYCLODEXTRIN (CYCLOHEPTAAMYLOSE), AND γ - CYCLODEXTRIN (CYCLOOCTYLAMYLOSE).

The cyclodextrin derivatives that have successfully been used in solution as stationary phases for gas chromatographic separation of enantiomers are (2,3,6-tri-O-methyl) -α-, -β- and -γ-CD derivatives, (2,6-di-O-methyl-3-O-trifluoroacetyl)-β-, and -γ-CD and (3,O-heptafluorobutanoyl-2,6-di-O-methyl)-β-CD[72,73]. Heptakis (2,6-di-O-methyl)-, tri-O-ethyl, tri-O-n-propyl-, tri-O-n-butyl-β-CD derivatives can also be used but the separation factors are low.

Venema and Tolsma[57] separated enantiomers of 2-substituted propionic acid esters and some lower alcohols on heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin. Konig *et al.*[58-69] employed (2,3,6-tri-O-n-pentyl)-α-, -β-, -γ-CD, and also, (3-O-acetyl-2,6-di-O-pentyl)-α-, -β- and (3-O-butanoyl- 2,6-di-O-n-pentyl)-γ-CD for separating enantiomers of racemic mixtures.

Armstrong *et al.*[70,71] used partially alkylated liquid CD derivatives as stationary phases on untreated fused silica capillary columns for GC enantiomer separations, e.g., (O-(s)-2-hydroxypropyl-per-O-methyl)-α- , -β-CD, (2,6-di-O-n-pentyl)-α-, -β-CD, and heptakis (2,6-di-O-n-pentyl-3-O-trifluoroacetyl)-β-CD. All these derivatives have free hydroxyl groups which make them hydrophilic. Table 5.1 presents the various derivatives of CD used as chiral stationary phases in GC and different types of compounds which can be separated into their enantiomers on these derivatives alongwith the reference numbers.

Konig[74] reported that enantioselectivity was strongly influenced by the size of the macrocylic cavity. Still the question whether a substrate must intrude into the cavity or the overall chirality of the CD matrix is sufficient for enantioselective interactions remains unanswered. Fischer *et al.*[75] and Schurig *et al.*[76] have recently reported Chirasil-Dex stationary phases in which the CD derivatives are chemically bound to polysiloxanes. Schurig[77] also reported immobilization of cyclodextrin phases which then become suitable for the separation of enantiomers by SFC and has reviewed[78] GC separation of enantiomers on CD derivatives. Grob and co-workers reported that the dilution of CD derivatives in normal phase causes a loss in the selectivity of CD derivative[79]. Armstrong and Han reviewed enantiomeric separations in chromatography.[80]

The low separation factors (α) observed till now make the CD phases unsuitable for the preparative scale GC enantiomer separations. At present there is no universal enantioselective CD phase available. Therefore invention of stationary phase based on CD with a wide range of applications is awaited. However, these CD phases are not expected to entirely replace the other chiral

TABLE 5.1

VARIOUS DERIVATIVES OF CYCLODEXTRINS (CD) USED AS CHIRAL STATIONARY PHASES IN GC AND DIFFERENT TYPES OF COMPOUNDS THAT CAN BE SEPARATED ON THESE DERIVATIVES

Name of the CD derivative used as chiral stationary phase	Types of solutes separated into enantiomers
Undiluted liquid cyclodextrin derivatives	
1. Hexakis (2,3,6-tri-O-n-pentyl-α-CD	trifluoroacetylated alcohols[58], epoxy alcohols[59], diols, triols carbohydrates[60], alkyl halides, spiroacetals, glycerol derivatives[61], N-alkylated barbiturates[62], 4-methyl-3-heptanone[63].
2. Hexakis (3-O-acetyl-2,6-di-O-n-pentyl) -α-CD	γ-lactones, cyclic carbonates of 1,2-diols and succinimides[64], trifluoroacetylated carbohydrates and hydroxy compounds[63].
3. Heptakis (2,3,6-tri-O-n-pentyl)-β-CD	trifluoroacetylated alcohols, carbohydrates[65]. acyclic monocyclic and bicyclic olefins and dienes[65].
4. Heptakis (3-O-acetyl 2,6-di-O-n-pentyl)-β-CD	trifluoroacetylated amines, amino alcohols, cyclic trans diols[67], methyl ester of lipoic acid[68].
5. Octakis (2,3,6-tri-O-n-pentyl)-γ-CD	non polar substrates e.g. 2,4-dimethyl-1-heptene, planer-chiral metal carbonyl π complexes[68].
6. Octakis (3-O-butyryl-2,6-di-O-n-pentyl)-γ-CD	N-trifluoroacetylated -O-methyl derivatives of α- and β-amino acids[69], O-trifluoroacetylated (TFA) α- and β- hydroxy acids, racemic alcohols, diols as TFA esters, terpene ketones, hexachlorocyclohexane, γ- and δ-ketones[69].
7. Heptakis (O-(S)-2-hydroxypropyl -per-O-methyl)-β-CD	trifluoroacetylated secondary amines, amino alcohols, epoxides, β- and γ-lactones, norbornene, norbornone[70].
8. Heptakis(2,6-di-O-n-pentyl)-α-CD and β-CD	compound types similar to that for n-pentylated CDS.
9. Heptakis (2,6-di-O-n-pentyl-3-O- trifluoroacetyl)-β-CD	trifluoroacetylated carbohydrates[71].

....Cont.

Cyclodextrin derivatives diluted with OV-1701

10. Heptakis (2,3,6-tri-O-methyl)β-CD in OV-1701	cyclic ethers, cyclic and acyclic ketones, γ-lactones, terpene lactones, underivtized secondary aliphatic alcohols, aromatic alcohols, terpene alcohols, underivatized aliphatic diols oxiranes, bicycloalkanes[51, 72] neomenthol, menthol and isomenthol[73].
11. Hexakis(2,3,6-tri-O-methyl)-α-CD in OV-1701	phenyl oxiranes[72].
12. Octakis(2,3,6-tri-O-methyl)-γ-CD in OV-1701	spiroketals
13. Heptakis (2,6-di-O-methyl-3-O-trifluoroacetyl) -β-CD in OV-1701	γ-lactones[73]
14. Octakis (2,6-di-O-methyl-3-O-trifluoroacetyl) -γ-CD in OV-1701	δ-lactones
15. Heptakis (2,6-di-O-methyl-3-O-heptafluorobutry) -β-CD in OV-1701	cyclic and acyclic ketones

stationary phases but they represent a major extension to the range of enantiomer separation techniques. According to Gil-Av[81]. "Every phase, whether low or high molecular, will be recognized by suiting best a certain niche of applications".

5.2 Enantiomer Separations of β-γ Unsaturated Esters and Various Analogues of Butenolides on Known Chiral Stationary Phase

Kolbb[82] for the first time in 1898 used the term 'butenolide' for an α-β unsaturated γ lactone. The butenolide moiety is present in numerous biologically active natural products especially, insect sex harmones. In our laboratory a new approach has been developed for the synthesis of butenolides from β-γ unsaturated esters. This section describes a gas chromatographic method for the resolution of butenolide enantiomers on a known chiral stationary phase.

Enantiomer separation of α-substituted-γ-butyrolactones[83] and α-methylene-γ-butyrolactones[84] is reported by liquid chromatography on chiral stationary phase. Preparative resolution of γ-phenyl and γ-ethyl -γ-butyrolactone by liquid chromatography on cellulose triacetate has been achieved.[85] Cyclodextrin derivatives are used for enantiomer separation by normal phase liquid chromatography.[86]

Gas chromatographic resolution of dicarbamate derivatives of γ-lactones is reported.[87] Engel *et al.*[88] reported GC separation of carbamide derivatives of β- and γ- lactones. Stereochemical differentiation of γ-lactones by diastereomeric derivatization with (S)-O-acetyllactyl chloride is done.[89] Koenig *et al.*[64] reported that hexakis (3-O-acetyl - 2,6-di-O-n-pentyl) -α-cyclodextrin stationary phase is particularly effective for separating racemic five membered heterocycles such as γ-lactones. There are few references in which enantiomers separation of γ-lactones using cyclodextrin derivatives as stationary phase has been described[70,71,73 76,90]. However, enantiomer separations of α-β unsaturated γ lactones, to our knowledge, have not been reported in the literature.

Derivatization free enantiomer resolution of dihydroxy compounds, hydroxy lactones, and even diketones on Chirasil-Val stationary phase is reported. Chirasil-Val is a highly versatile enantiospecific stationary phase developed by Frank *et al.*[19] in which the enantiospecificity of the selector L-Valine tertiary butylamide is combined with the high thermal stability of organic polysiloxane. The dimethylsiloxane units keep L-Valinamide units at a distance and prevent formation of intermolecular hydrogen bonds. The range of compounds separated by Chirasil-Val has been

covered by Koppenhoefer and Bayer.[21] Direct enantiomeric resolution of primary, secondary, and tertiary alcohols on Chirasil-Val indicates that one strong attraction may be sufficient to produce significant chiral recognition. Since separation of pantolactone has been reported on Chirasil-Val at 80°C with high separation factor (1.158), we tried separation of butenolides on Chirasil-Val. Almost all of these β-γ-esters and butenolides are new compounds and not reported in the literature.

5.2.1 Experimental

A Hewlett Packard 5880A model with a level 4 integrator and flame ionization detector was used. It was modified for the use of capillary columns by fitting a split injector and detector gas make-up valve. Adapters were used to fit capillary columns at the injector and detector ends. Capillary fused silica columns (25 m X 0.25mm X 0.12 μm) coated with D and L Chirasil-Val were purchased from Chrompack Netherlands. IOLAR grade nitrogen was used as the carrier gas with velocity 2ml min^{-1}. The split ratio was 1 : 100. Detector temperature at 250°C and injector temperature at 40°C above the oven temperature were maintained. Nitrogen flow was measured with soap bubble flow meter. Structures of the analyzed β-γ esters and butenolides are given in Fig. 5.2. Separation of diastereomers of β-γ esters and lactones was tried on packed columns : 3% SE-30 on chromosorb WAW DMCS (1/8" X 6ft), and 2% Apiezon L on chromosorb WAW DMCS (1/8" X 10ft) SS columns. Carrier gas nitrogen at the flow rate of 30 ml min^{-1} was used. The oven temperatures employed during the analysis are listed in Tables 5.2 and 5.3.

5.2.2 Results and discussion

Recently, from our laboratory a shorter route for the synthesis of Heritol has been discribed.[91] In order to generalize the new methodology, eleven analogues of Heritionin (methyl ether of Heritol) were prepared. The butenolide moiety was prepared from β-γ unsaturated ester by dihydroxylation with OSO_4 and lactonization was achieved in presence of *para* toluene sulfonic acid. The analysis of these esters and lactones was carried out by gas chromatography.

Beta-gamma esters:

The esters showing presence of diastereomers in nuclear magnetic resonance spectrum were injected on packed columns to check the diastereomer separations. All the esters separate well into their diastereomers when injected on SE-30 as well as on Apiezon-L column. The ester - 10 shows three peaks when injected on 3% SE-30 column at 220°C, while two sharp peaks are obtained

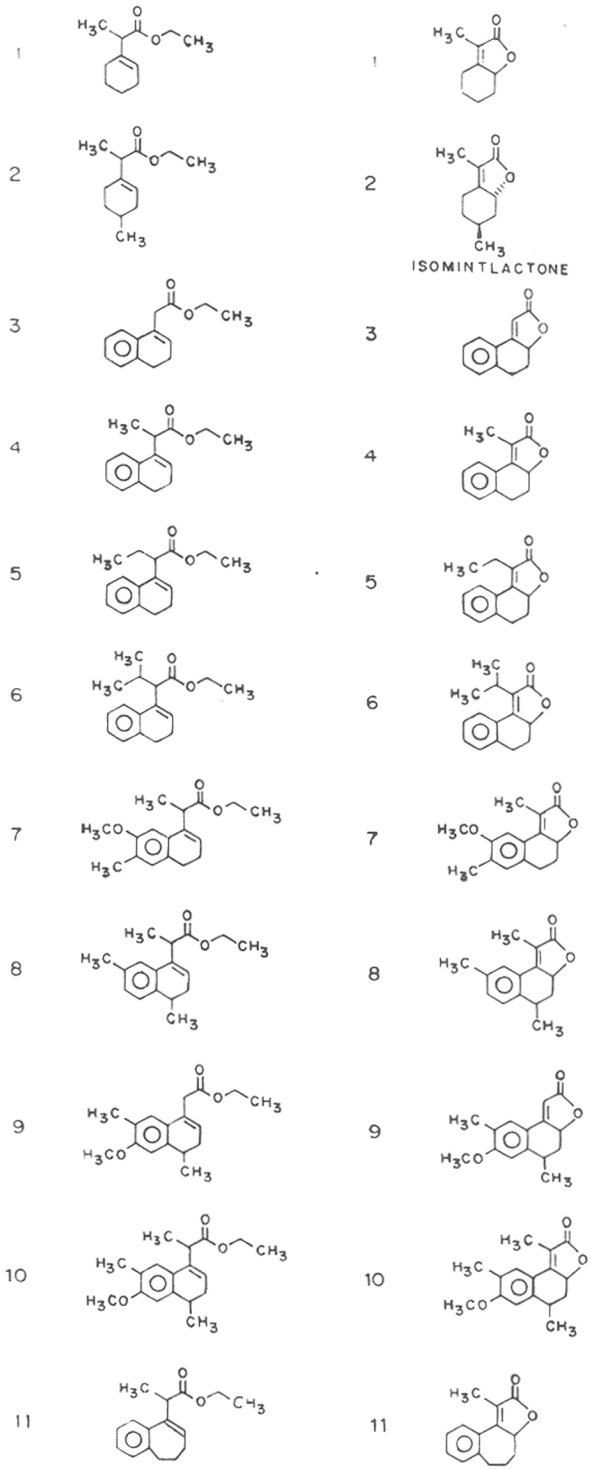


Fig. 5.2 : STRUCTURES OF THE β − γ ESTERS AND BUTENOLIDES ANALYZED BY GAS CHROMATOGRAPHY.

TABLE 5.2

RETENTION DATA OF THE DIASTEREOMERS OF LACTONES AND THE β-γ ESTERS

(For structures, refer Figure 5.2)

Injection temperature = Oven temperature + 40°C,
Detector temperature = 300°C, N_2 : 30 ml min^{-1}

Compound number and Percentage composition	Lactones				β-γ-esters			
	3% SE-30		2% Apiezon L		3% SE-30		2% Apiezon L	
	temp.	t_R	temp.	t_R	temp.	t_R	temp.	t_R
2 (80 : 20)	120°C	6.58	160°C	8.66, 9.77	120°C	2.89, 3.95	160°C	3.28, 4.48
8 (60 : 40)	220°C	2.17	200°C	32.67, 33.94	220°C	1.06, 1.43	200°C	9.55, 16.97
9 (60 : 40)	220°C	4.22	220°C	35.25[a]	220°C	1.59, 2.49[b]	220°C	8.36, 14.95, 18.03
10 (60 : 40)	220°C	4.48	220°C	34.32, 36.08	220°C	1.66, 2.36, 3.38	220°C	8.22, 14.63
A-2[c] (80 : 20)	-	-	-	-	120°C	5.00	160°C	5.95, 6.48

a: Lactone-9 shows a broad peak with hump.
b: Second peak is very broad indicating there are two peaks. Ester - 9 shows anomalous behaviour.

c:A-2 is

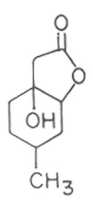


TABLE 5.3

RETENTION TIMES (MIN),RELATIVE RETENTION VALUES OF ENANTIOMERS OF LACTONES AND β-γ ESTERS ON CHIRASIL-VAL-D

(For structures, refer Figure 5.2)

Injection temperature = Oven temperature + 40°C,
Detector temperature = 300°C, N_2 : 2 ml min^{-1}, Split ratio = 1 : 100

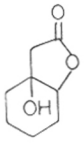
Compound number	Lactones				β-γ-esters			
	temp.	retention time		α	temp.	retention time		α
1	120°C	5.95	6.07	1.02	100°C	-	-	-
2 *cis*	120°C	7.50	7.67	1.02	100°C	4.74	4.78	1.01
trans		8.39	8.65	1.03		6.38	-	1.00
3	170°C	8.95	1.00	1.00	140°C[a]	7.16	-	-
4	170°C	8.78	8.88	1.01	140°C	7.04	-	1.00
5	170°C	9.57	9.72	1.02	140°C	9.35	-	1.00
6	170°C	9.80	9.92	1.01	140°C	9.26	-	1.00
7	200°C	9.60	9.70	1.01	170°C	7.15	-	1.00
8 *cis*	170°C	14.96	15.15	1.01	170°C	4.36	4.41	1.01
trans		15.42	15.65	1.02		6.67	-	1.00
9[b]	200°C	14.02	14.12	1.01	170°C[c]	8.42	-	1.00
						14.14	-	-
						15.53	-	-
10[b]	200°C	13.17	13.28	1.01	170°C	8.45	8.56	1.013
						13.97	-	1.00
11	170°C	9.02	9.12	1.01	170°C	3.38	-	1.00
A-1					100°C	7.67	7.70	1.004
A-2[d]					120°C	5.54	5.10	1.12

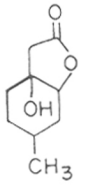
a. There is no asymmetric centre in 3rd β-γ-ester.

b. Number 9 and number 10 lactones diasteomers are not seen

c. Number 9 β-γ-ester shows anomalous behaviour.

d. Only diasteromers of the alcohol separate

A-1 is 

A-2 is 

for the same ester with expected percentage composition on 2% Apiezon-L. This indicates that some reaction may be taking place on SE-30 column. The ester 9 shows three peaks on SE-30 as well as on Apiezon-L column. The ester with free -OH group (A-2) could not be separated into the diastereomers on SE-30 column but Apiezon-L gave base line separation.

Table 5.3 presents retention times and relative retention values of enantiomers of lactones and esters on Chirasil-Val. The esters 2, 8, 9, 10, and A-1 show presence of enantiomers but the α values are small. The *cis* isomer of ester-2 shows splitting while the *trans* isomer does not separate into enantiomers. In case of the ester-10 *trans* isomer does not give separation of enantiomers while *cis* isomer is partially resolved. The enanantiomer separation of ester-10 can be seen in Fig. 5.3. The third peak coming after the *trans* isomer may be some impurity. If it was one of the enantiomers then it should have shown peak of the same size. This was confirmed by injecting the ester-10 on the column with opposite configuration. The sequence of elution of the last two peaks is the same. Since ester-9 was showing three peaks on packed column it was injected on cross-linked methyl silicon gum fused silica capillary column (25m x 0.2mm x 0.33 μm). The first peak was ~ 60% and it was followed by 2 peaks having 20% area percentage composition. When the same ester-9 was injected on the chiral column it showed the identical pattern of elution (Fig. 5.4). This is possible only if some kind of reaction is taking place on the column at 170°C. Since three peaks are seen on chiral as well as achiral column, we conclude that these can not be enantiomers and diastereomers can be formed only if some reaction is taking place on the column at such a high temperature. The alcohol A-1 shows splitting while in the case of A-2 only diastereomer separation is achieved.

α-β unsaturated γ lactones:

The lactones showing presence of diastereomers in nuclear magnetic resonance spectrum were injected on packed columns. While SE-30 column is unable to separate diastereomers, Apiezon-L column gives very good separation of the diastereomers of all the other lactones except lactone 9 and 10. The lactone 9 gives a broad peak with slight hump. In the case of lactone 10 two peaks are seen but the second peak is only 2.7% which is not matching with the NMR analysis. This suggests that a minor impurity which is not seen in NMR spectrum must be present and the diastereomers are not separating. The retention data can be seen in Table 5.2.

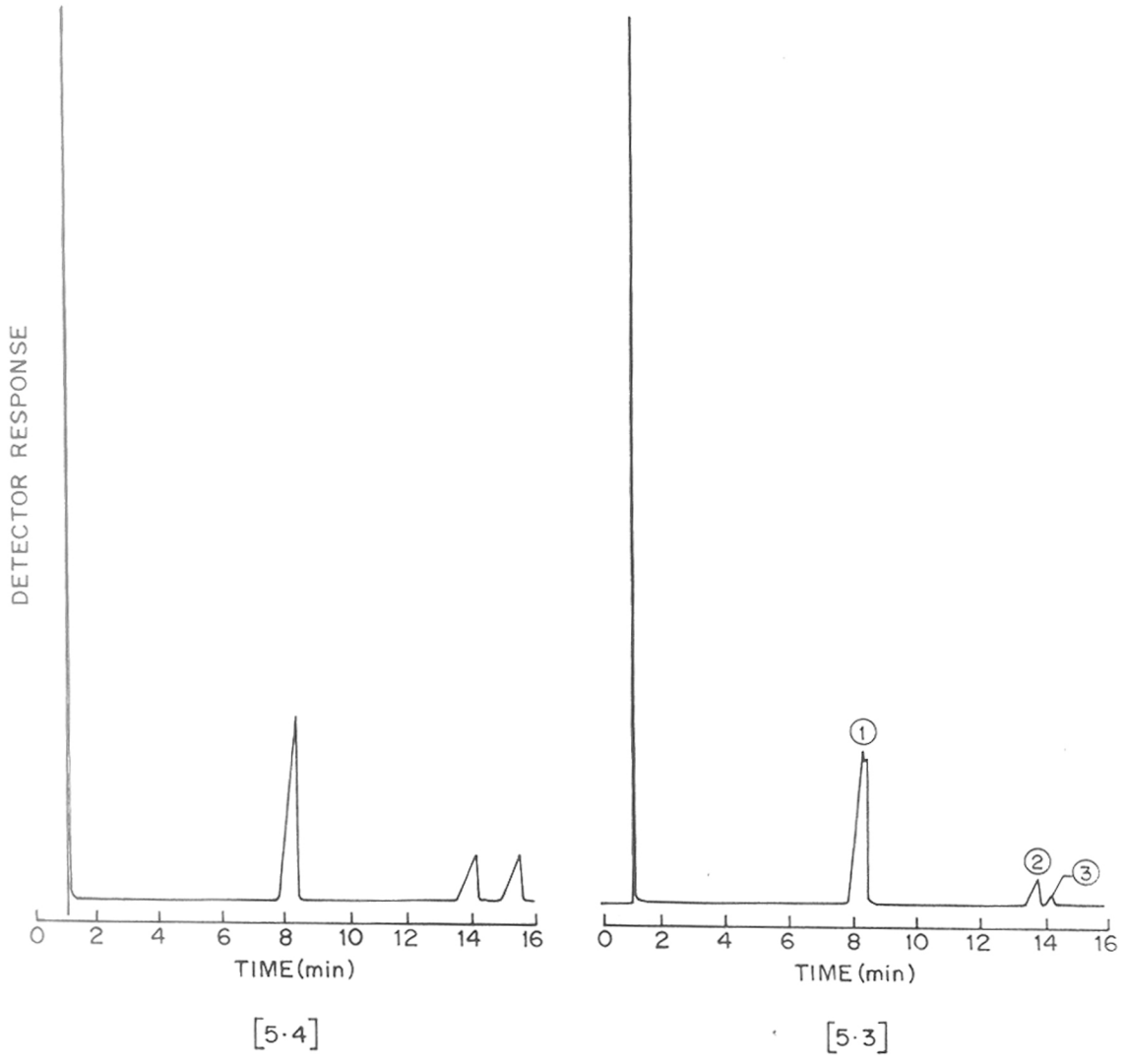


Fig. 5.3 : THE ENANTIOMER SEPARATION OF ESTER - 10 ON CHIRASIL-VAL-D.
Oven Temp. 170°C, Injector Temp. 210°C, Detector Temp. 250°C, Carrier gas nitrogen flow rate - 2 ml/min, Split ratio - 1:100.
Peaks : 1) cis isomer, 2) trans isomer, 3) impurity

Fig. 5.4 : GAS CHROMATOGRAPHIC ANALYSIS OF ESTER-9.
Oven Temp. 170°C, Injector Temp. 210°C, Detector Temp. 250°C, Carrier gas nitrogen flow rate - 2 ml/min, Split ratio - 1:100.

All the lactones show enantiomer separation at least to some extent as can be observed from the α values in Table 5.3. A very good separation of diastereomers of lactone-2 (mintlactone and *iso*-mintlactone) into their enantiomers can be seen in Figure 5.5. Lactone 8 also gets separated very well into the diastereomers and each one again shows 2 peaks, clearly indicating 50:50 enantiomeric composition (Figure 5.6).

Lactone 9 and lactone 10 (Heritionin) is splitting into 2 peaks and it is not clear whether these are diastereomers or enantiomers. Even on packed columns these are showing only one peak and the diastereomers are not separated.

5.2.3 Absolute Configuration

In our laboratory 97% optically pure (-) mintlactone ($[\alpha]_D^{20} = -53.0, C = 3.5, EtOH$) was prepared from isopulegol. Gas chromatographic analysis shows that it contains *iso* mintlactone impurity. When this 97% pure (-) mintlactone was mixed with lactone-2 and injected on Chirasil-Val-D, the first peak of mintlactone and the second peak of *iso* mintlactone increased. The absolute configuration assigned to (-) mintlactone is 6R 7aR[92]. Therefore we can infer that RR isomer elutes first on Chirasil-Val D column followed by the SS isomer. Second peak of *iso* mintlactone may be assigned RS configuration as this is coming as an impurity in mintlactone prepared from isopulegol (one centre is fixed). Hence the sequence of elution of isomers is identified as RR, SS, SR and RS. This was also confirmed by injecting pure *iso* mintlactone (RS) prepared from *iso*-limolene. When a mixture of lactone-2 and *iso* mintlactone (RS) is injected, the second peak of *iso* mintlactone increases. The order of elution of lactone-2 on the column with L configuration is SS, RR, RS and SR.

5.2.4 General Remarks

The assignment of absolute configuration to all the esters and lactones could not be achieved since we did not have authentic samples of enantiomerically pure or enriched compounds. As we have used flame ionization detector, it was not possible to collect the sample and take optical rotation. Optically pure β-γ esters and butenolides are being synthesized and the work will continue.

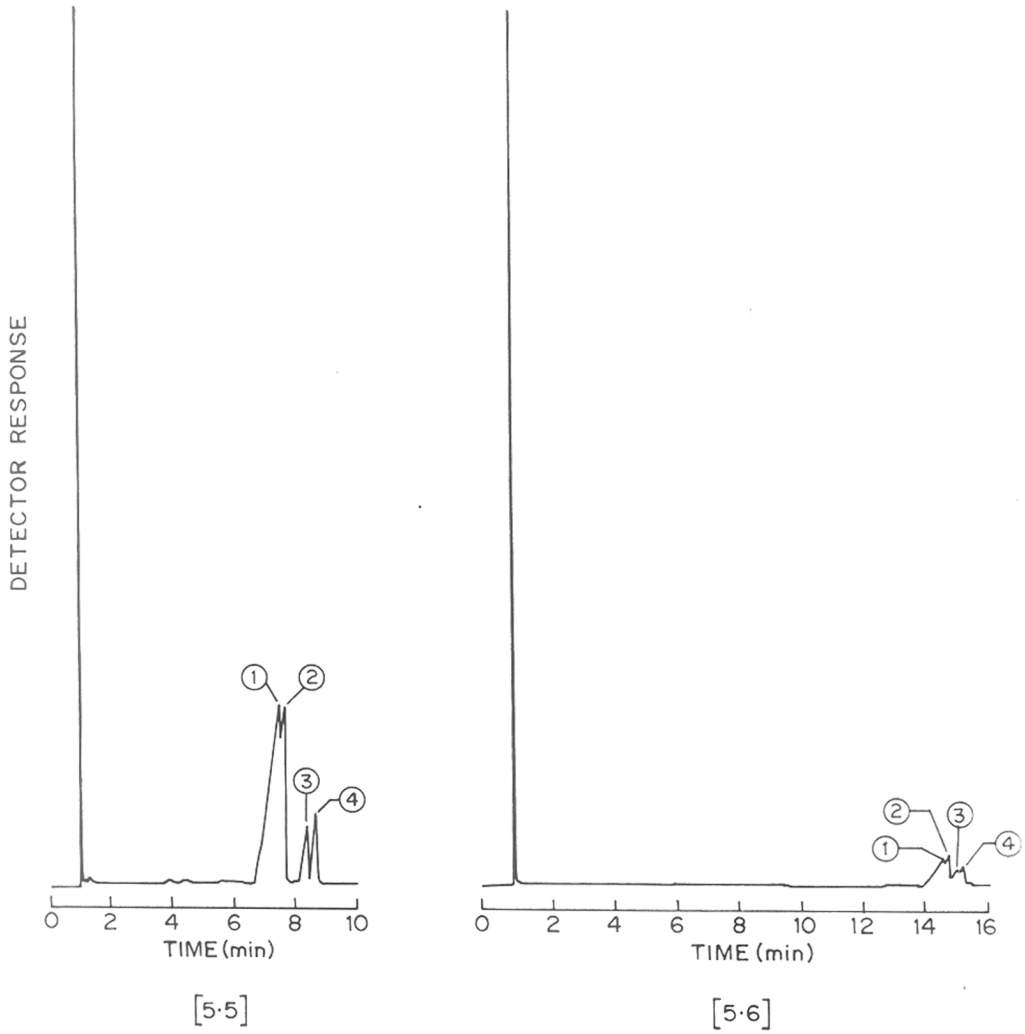


Fig. 5.5 : THE ENANTIOMER SEPARATION OF LACTONE 2 (MINTLACTONE AND ISO MINTLACTONE) ON CHIRASIL-VAL-D.
Oven Temp. 120°C, Injector Temp. 210°C, Detector Temp. 250°C, Carrier gas nitrogen flow rate - 2 ml/min, Split ratio - 1:100.

Peaks: 1) RR mintlactone, 2) SS mintlactone,
3) SR iso mintlactone, 4) RS iso mintlactone,

Fig. 5.6 : THE ENANTIOMER SEPARATION OF LACTONE 8 ON CHIRASIL-VAL-D.
Oven Temp. 170°C, Injector Temp. 210°C, Detector Temp. 250°C, Carrier gas nitrogen flow rate - 2 ml/min, Split ratio - 1:100.

Peaks: 1 and 2 - enantiomers of cis isomer
3 and 4 - enantiomers of trans isomer.

The ester-6 shows presence of diastereomers in NMR spectrum. Also, in GC studies, we found one small peak (~ 15%) eluting after the major peak on Apiezon-L as well as Chirasil-Val-D column. This may be attributed to the fact that the isopropyl group may be on either side of the carbon atom and these are positional isomers (regioisomers).

REFERENCES

1. E.Gil-Av, B. Feibush, R. Charles-Sigler, *Tetrahedron Lett.*, **6** (1966) 1009.
2. J.H. Liu, W.W. Ku, *J. Chromatogr.*, **271** (1983) 309.
3. W.A. Konig, G.J. Nicholson, *Anal. Chem.*, **47** (1975) 951.
4. W.A. Konig, W. Parr, H.A. Lichtenstein, E. Bayer, J. Oro., *J. Chromatogr. Sci.*, **8** (1970) 183.
5. U. Beitler, B. Feibush, *J. Chromatogr.*, **123** (1976) 149.
6. B. Feibush, *J. Chem. Soc. Chem. Commun.*, (1971) 544.
7. S.C. Chang, R. Charles, E. Gil-Av, *J. Chromatogr.*, **235** (1982) 87.
8. B. Feibush, E.Gil-Av, *J. Gas Chromatogr.*, **5** (1967) 257.
9. S. Weinstein, B. Feibush, E.Gil-Av, *J. Chromatogr.*, **126** (1976) 97.
10. N. Oi, H. Kithara, T. Doi, *J. Chromatogr.*, **213** (1981) 137.
11. N. Oi, H. Kithara, Y. Inda, *J. Chromatogr.*, **237** (1982) 297.
12. N. Oi, H. Kithara, T. Doi, *J. Chromatogr.*, **254** (1983) 282.
13. N. Oi, R. Takai, H. Kithara, *J. Chromatogr.*, **256** (1983) 154.
14. N. Oi, M. Horiba, H. Kithara, *J. Chromatogr.*, **202** (1980) 299.
15. Shu-Cheng Chang, E. Gil-Av, R. Charles, *J. Chromatogr.*, **289** (1984) 53.
16. Shu-Cheng Chang, R. Charles, E.Gil-Av, *J. Chromatogr.*, **202** (1980) 247.
17. W.A. Konig, I. Benecke, N. Lucht, E.S. Schmidt, J. Schulz, S. Sivers, *J. Chromatogr.*, **279** (1983) 555.

18. W.A. Konig, I. Benecke, K. Earnst, *J. Chromatogr.*, **253** (1982) 267.

19. H. Frank, G.J. Nicholson, E. Bayer, *J. Chromatogr. Sci.*, **15** (1977) 174.

20. B. Koppenhoefer, E. Bayer, *Chromatographia*, **19** (1984) 123.

21. B. Koppenhoefer, E. Bayer, *J. Chromatogr.*, Library **32** (1985) 1.

22. H. Frank, G.J. Nicholson, E. Bayer, *Angew. Chem. Int. Ed. Engl.*, **17** (1978) 363.

23. B. Koppenhoefer, H. Allmendinger, *Chromatographia*, **21** (1986) 503.

24. B. Koppenhoefer, H. Allmendinger, G. Nicholson, *Angew. Chem. Int. Ed. Engl.*, **24** (1985) 48.

25. H. Frank, E. Bayer, *J. Chromatogr.*, **146** (1978) 197.

26. E. Bayer, E. Kuster, G.J. Nicholson, H. Frank, *J. Chromatogr.*, **320** (1985) 393.

27. V. Schurig, *Angew. Chem. Int. Ed. Engl.* **23** (1984) 747.

28. T. Saeed, P. Sandra, M. Verzele, *J. Chromatogr.*, **186** (1980) 611.

29. T. Saeed, P. Sandra, M. Verzele, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, 3 (1980) 35.

30. W.A. Konig, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, **5** (1982) 588.

31. W.A. Konig, I. Benecke, H. Breting, *Angew. Chem. Int. Ed. Engl.*, **20** (1981) 693.

32. W.A. Konig, I. Benecke, S. Silvers, *J. Chromatogr.*, **217** (1981) 71.

33. W.A. Konig, I. Benecke, *J. Chromatogr.*, **209** (1981) 91.

34. V. Schurig, *Kontakte (Darmstadt)* **1** (1986) 3.

35. V. Schurig, *Asymmetric Synthesis, 1* (1983) 59.

36. V. Schurig, E. Gil-Av, *J. Chem. Soc., Chem. Commun.*, (1971) 650.

37. E. Gil-Av, V. Schurig, *Anal. Chem.*, **43** (1971) 2030.

38. V. Schurig, *Angew. Chem. Int. Ed. Engl.*, **16** (1977) 110.

39. V. Schurig, W. Burkle, *Angew. Chem. Int. Ed. Engl.*, **17** (1978) 132.

40. V. Schurig, R. Webber, *J. Chromatogr.*, **217** (1981) 51.

41. V. Schurig, W. Burkle, *J. Am. Chem. Soc.*, **104** (1982) 7573.

42. V. Schurig, *Angew. Chem. Int. Ed. Engl.*, **15** (1976) 304.

43. V. Schurig, D. Wistuba, *Angew. Chem. Int. Ed. Engl.*, **22** (1983) 772.

44. V. Schurig, *J. Chromatogr.*, **441** (1988) 135.

45. T.J. Ward, D.W. Armstrong, *J. Liq. Chromatogr.*, **9** (1986) 407.

46. D. Sybilska, J. Zukowski, J. Bojarski, *J. Liq. Chromatogr.*, **9** (1986) 591.

47. K. Fujimura, M. Kitagawa, H. Takayanagi, T. Ando, *J. Liq. Chromatogr.*, **9** (1986) 607.

48. D.M. Sand, H. Schlenk, *Anal. Chem.*, **33** (1961) 1624.

49. B. Casu, M. Reggiani, G.R. Sanderson, *Carbohydr. Res.*, **76** (1979) 59.

50. Z. Juvancz, G. Alexander, J. Szejtli, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, **10** (1987) 105.

51. V. Schurig, P. Nowothy, *J. Chromatogr.*, **441** (1988) 155.

52. M. Tanaka, S. Kawano, T. Shono, *Fresenius Z. Anal. Chem.*, **316** (1983) 54.

53. D. Sybilska, T. Koscielski, *J. Chromatogr.*, **261** (1983) 357.

54. T. Koscielski, D. Sybilska, *J. Chromatogr.*, **349** (1985) 3.

55. T. Koscielksi, D. Sybilska, J. Jurczak, *J. Chromatogr.*, **280** (1983) 131.

56. T. Koscielksi, D. Sybilska, J. Jurczak, *J. Chromatogr.*, **364** (1986) 299.

57. A. Venema, P.J.A. Tolsma, *J. High Resolut. Chromatogr.*, **12** (1989) 32.

58. W.A. Konig, S. Lutz, G. Weng, *Angew. Chem. Int. Ed. Engl.*, **27** (1988) 979.

59. W.A. Konig, S. Lutz, G. Weng, *Angew. Chem. Int. Ed. Engl.*, **28** (1989) 178.

60. W.A. Konig *et al*, *Carbohydr. Res.*, **183** (1988) 11.

61. W.A. Konig, S. Lutz, G. Weng, *Proc. Int. Symp. Cyclodextrin* 4th, 1988, 465 (c.f.p. 145).

62. W.A. Konig *et al*, *J. Chromatogr.*, **503** (1990) 256.

63. W.A. Konig, *Carbohydr. Res.*, **192** (1989) 51.

64. W.A. Konig *et al*, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, **11** (1988) 621.

65. W.A. Konig *et al*, *J. High Resolut. Chromatogr.*, **12** (1989) 35.

66. J. Ehlers, W.A. Konig *et al*, *Angew. Chem. Int. Ed. Engl.*, **27** (1988) 1556.

67. W.A. Konig *et al*, *J. High Resolut. Chromatogr., Chromatogr. Commun.*, **11** (1988) 506.

68. W.A. Konig, *et al*, *J. High Resolut. Chromatogr.*, **12** (1989) 790.

69. W.A. Konig, R. Krebber, P. Mischnick, *J. High Resolut. Chromatogr.*, **12** (1989) 732.

70. D.W. Armstrong, W.Y. Li *et al.*, *Anal. Chem.*, **62** (1990) 914.

71. W.Y. Li, H.L. Jin, D.W. Armstrong, *J. Chromatogr.*, **509** (1990) 303.

72. H.P. Nowotny, D. Schmalzing, D. Wistuba, V. Schurig, *J. High Resolut. Chromatogr.*, **12** (1989) 383.

73. V. Schurig *et al. J. High Resolut. Chromatogr.*, **13** (1990) 470.

74. W.A. Konig, R. Krebber, G. Wenz, *J. High Resolut. Chromatogr.*, **12** (1989) 641.

75. P. Fischer, R. Aichholz, U. Bolz, M. Juza, S. Krimmer, *Angew. Chem. Int. Ed. Engl.*, **29** (1990) 427.

76. V. Schurig, D. Schmalzing, U. Muhleck, M. Jung, M. Schleimer, P. Mussche, J. Duvekot, J.C. Buyten, *J. High Resolut. Chromatogr.*, **13** (1990) 713.

77. V. Schurig, D. Schmalzing, M. Schleimer, *Angew. Chem. Int. Ed. Engl.*, **30** (1991) 987.

78. V. Schurig, H.P. Nowotny, *Angew. Chem. Int. Ed. Engl.*, **29** (1990) 939.

79. H.G. Schmarr, A. Mosandl, H.P. Neukom, K. Grob, *J. High Resolut. Chromatogr.*, **14** (1991) 207.

80. D.W. Armstrong, S.M. Han, *Critical Reviews in Analytical Chemistry*, **19** (1988) 175.

81. E. Gil Av, *Preface to the practice of enantiomer separation by capillary gas chromatography*, W.A. Konig, Huthing, Heidelberg, 1987.

82. T. Kolbb, *Bull. Soc. Chim. Fr.* (1898) 389.

83. S.Hunig, N. Klaunzer, K. Gunther, *J. Chromatogr.*, **481** (1989) 387.

84. A. Tambute, M. Linne, M. Caude, R. Rosset, *J. Chromatogr.*, **448** (1988) 55.

85. E. Francotte, D. Lohmann, *Helv. Chim. Acta.*, **70** (1987) 1969.

86. D.W. Armstrong *et al.*, *Anal. Chem.*, **62** (1990) 1610.

87. K.H. Engel, R.A. Flath, W. Albrecht, R. Tressel, *J. Chromatogr.*, **479** (1989) 176.

88. K.H. Engel, W. Albrecht, J. Heidlas, *J. Agric. Food. Chem.*, **38** (1990) 244.

89. A. Mosandl *et al. J. High Resolut. Chromatogr. Chromatogr. Commun.* **10** (1987) 67.

90. K. Grob, H.P. Neukom, H.G. Schmarr, A. Mosandl, *J. High Resolut. Chromatogr.*, **13** (1990) 433.

91. P.K. Zubaidha, S.P. Chavan, U.S. Racherla, N.R. Ayyangar, *Tetrahedron*, **47** (1991) 5759.

92. K. Tarahashi, T. Someya, S. Maruki, T. Yoshida, *Agric. Biol. Chem.*, **44** (1980) 1535.

SUMMARY

The thesis deals with the gas-liquid chromatographic techniques and is divided into five chapters. The first chapter reviews various methods available for the characterization of liquid stationary phases. The merits and limitations of these methods are discussed. The most widely used method for stationary phase characterization was first proposed by Rohrschneider and later modified by McReynolds. This approach has been utilized in characterization of various stationary phases described in Chapters 2 and 3.

Chapter 2 is divided into two sections. The first section reviews various applications of crown compounds in analytical chemistry including gas-liquid and liquid-liquid chromatography. In the second section chromatographic characteristics of dibenzo-18-crown-6 (DB18C6), dibenzo-24-crown-8 (DB24C8) and dicyclohexano-24-crown-8 (DCH24C8) are compared. The phase transition studies were carried out by plotting *log* of the specific retention volume against inverse of absolute temperature. Efficiencies of various crown ethers and Carbowax 20M are compared. Comparison of McReynolds constants for these crown ethers shows that the average polarities of DB24C8 and DCH24C8 are similar to those of Carbowax 20M and tricresyl phosphate respectively. McReynolds constants for DB18C6 were determined at 180°C as it is a solid at 120°C. The crown ether DB24C8 has also shown very promising results and can be used in the separations of various positional isomers (particularly of nitro compounds).

The third chapter explores the use of (phenylisopropyl)phenol (PIP) esters as stationary phases in gas-liquid chromatography. The following PIP esters were synthesized and their properties as liquid stationary phases in gas chromatography have been evaluated.

1. Di [4-(2'-phenylisopropyl)phenyl] maleate (DPIPM)
2. n-Butyl-[4-(2'-phenylisopropyl)phenyl] maleate (BPIPM)
3. [4-(2'-Phenylisopropyl)phenyl] acetate (PIPA)
4. [4-(4'-Isopropylidene bisphenol)]diacetate (IPBPDA)
5. Tri [4-(2'-phenylisopropyl)phenyl] phosphate (TPIPP)

The synthesis of these esters has been described and NMR, IR and mass spectral analysis is presented. Two of these esters are new and to our knowledge are not reported in the literature. The polarities (McReynolds constants) of these PIP esters were determined and compared with the standard stationary phases. The phosphate ester (TPIPP), can be used upto 200°C which is a significant improvement over other phosphate esters that can be used only upto 125°C. Separations of higher boiling isomers on TPIPP have been discussed.

The first section of Chapter 4 reports synthetic route to produce 4-chlorobenzophenone in quantitative yields. This is the starting material for chlorphenoxamine or 'Systral' which is used as an anti-Parkinson agent. The second section describes an efficient and rapid gas chromatographic method for the quantitative estimation of 2-chlorobenzophenone in 4-chlorobenzophenone using Apiezon - L as stationary phase and benzophenone as an internal standard. The total analysis time is only 8 minutes and the standard deviation ranges from 8.15×10^{-3} to 1.27×10^{-2}. Complete separation of 2-, 3-, and 4-chlorobenzophenone has been achieved using 2% Apiezon-L coated on chromosorb WAW DMCS (80/100 mesh) packed in 3m X 2mm stainless steel column. The minimum detectable amount of 3-chlorobenzophenone in 4-chlorobenzophenone is 0.04%.

Various chiral stationary phases used for separation of enantiomers by gas chromatography are reviewed in the first section of Chapter 5, while the second section describes the gas chromatographic analysis of some $\beta-\gamma$ esters and lactones. Recently, from our laboratory, a shorter route for the synthesis of Heritol has been proposed. In order to generalize the new methodology, eleven analogues of Heritionine (methyl ether of Heritol) were prepared. The separation of the diasteriomers and enantiomers of these $\beta-\gamma$ esters and lactones was tried by gas chromatography on packed (SE-30 and Apiezon-L) and capillary (methyl silicon gum, Chirasil-Val-D and Chirasil-Val-L) columns. A very good separation of enantiomers of mintlactone and iso mintlactone could be achieved on Chirasil-Val-D and Chirasil-Val-L at 120°C. Carrier gas nitrogen was used at the flow rate of 2ml/min. The split ratio was 1:100. The order of elution of isomers on Chirasil-Val-D is identified as RR, SS, SR and RS. The results are discussed.

APPENDIX

```
REM .....
REM BASIC LANGUAGE PROGRAMME TO FIND OUT THE DEAD TIME
REM SN$ REPRESENTS FILE TO READ DATA FROM
REM GIVE RETENTION TIMES (MINUTES) IN YY ARRAY
REM GIVE KOVATS INDEX (100 X NUMBER OF CARBON ATOMS) IN X ARRAY
REM TM = COMPUTED DEAD TIME
REM .....
DIM X(30), Y(30), W(30), Z(30), A(30), B(30, 30), CI(30), XX(25)
DIM YY(30), YCAL(30)
CLS : PREC = 1E-12: SN$ = "DB24C8"
INPUT "WANT TO READ DATA FROM FILE (Y/N) ?"; AA$
IF AA$ = "N" OR AA$ = "n" THEN 555
PRINT "CHECK THE FILE NAME IN OPEN STATEMENT (SHOULD BE SAME AS SN$)"
OPEN SN$ FOR INPUT AS #1
FOR I = 1 TO 30: INPUT #1, YY(I), X(I): IF EOF(1) GOTO 1
NEXT I
REM .....
555 REM IF THE DATA IS NOT GIVEN IN FILE, INPUT IT AS SHOWN BELOW
REM THE VALUE OF NUMBER OF POINTS (= N) MUST BE EXPLICITLY GIVEN
REM .....
N = 7
YY(1) = 1.11: YY(2) = .75: YY(3) = .54: YY(4) = .42: YY(5) = .36
YY(6) = .32: YY(7) = .29
X(1) = 1200: X(2) = 1100: X(3) = 1000: X(4) = 900: X(5) = 800
X(6) = 700: X(7) = 600
GOTO 2
REM .....
REM INITIAL VALUE OF TM SHOULD BE LESS THAN ACTUAL DEAD TIME
REM .....
1 CLOSE #1: N = I
2 SAM = 999999: SUM = 999990: TM = .01: DTM = .001
110 FOR I = 1 TO N: Y(I) = LOG(YY(I) - TM): NEXT I
GOSUB 410
REM LPRINT A(1),A(2)
FOR I = 1 TO N: CI(I) = (Y(I) - A(1)) / A(2): NEXT I
SXX = 0
FOR I = 1 TO N: SXX = (X(I) - CI(I)) ^ 2 + SXX: NEXT I
REM LPRINT "SXX=";SXX
IF SXX > SUM THEN 230
SUM = SXX: SAM = SUM
210 TM = TM + DTM
GOTO 110
230 IF SXX > = SAM THEN 250
TM = TM - DTM: GOTO 260
250 TM = TM - 2 * DTM: SUM = SAM
260 DTM = DTM / 10
IF (DTM > PREC) THEN 210
REM IF EQN. IS (Y = mx + c) THEN A(1) = C AND m = A(2) (i.e. SLOPE )
REM .....PRINTING RESULTS.....
LPRINT : LPRINT : LPRINT
LPRINT "SET IDENTIFICATION : "; SN$: LPRINT
LPRINT "VALUE OF TM="; TM
LPRINT
LPRINT "VALUES OF REGRESSION COEFFICIENTS :"; " c= "; A(1); "m="; A(2)
LPRINT "_____"
REM
LPRINT "Given Kovats Index";"  "; "Computed Kovats Index";"  "; "Retention Times"
LPRINT "_____"
REM
FOR KA = 1 TO N: YCAL(KA) = A(1) + A(2) * X(KA): NEXT KA
FOR LL = 1 TO N
```

```
LPRINT "  "; X(LL), "        ", CI(LL), "       "; YY(LL): NEXT LL
LPRINT "_____________________________________________________________________"
END
REM
REM .....................................................................................
400 REM LINEAR REGRESSION ROUTINE
410 IIJJ = 1: NABC = 1
IJK = 1: NN = 1: M = 1: MM = M
430 LW = 2 * M + 1
LB = M + 2: LZ = M + 1
FOR J = 2 TO LW: W(J) = 0: NEXT J
W(1) = N
FOR J = 1 TO LZ: Z(J) = 0: NEXT J
FOR I = 1 TO N
P = 1: Z(1) = Z(1) + Y(I)
FOR J = 2 TO LZ: P = X(I) * P
W(J) = W(J) + P: Z(J) = Z(J) + Y(I) * P: NEXT J
FOR J = LB TO LW: P = X(I) * P
W(J) = W(J) + P: NEXT J: NEXT I
FOR I = 1 TO LZ: FOR K = 1 TO LZ
J = K + I: B(K, I) = W(J - 1): NEXT K: NEXT I
FOR K = 1 TO LZ: B(K, LB) = Z(K): NEXT K
FOR L = 1 TO LZ
DIVB = B(L, L)
FOR J = L TO LB: B(L, J) = B(L, J) / DIVB: NEXT J
IA = L + 1
IF ((IA - LB) < 0) THEN 710
IF ((IA - LB) = 0) THEN 760
IF ((IA - LB) > 0) THEN 760
710 FOR I = IA TO LZ
FMUL = B(I, L)
FOR J = L TO LB
B(I, J) = B(I, J) - B(L, J) * FMUL: NEXT J: NEXT I: NEXT L
760 A(LZ) = B(LZ, LB)
I = LZ
780 SIGMA = 0
FOR J = I TO LZ: SIGMA = SIGMA + B(I - 1, J) * A(J): NEXT J
I = I - 1: A(I) = B(I, LB) - SIGMA
IF (I > 1) THEN 780
FOR I = 1 TO 30: Z(I) = 0: NEXT I
NN = N
FOR D% = 1 TO NN
YCAL = 0: W(1) = X(D%)
FOR I = 1 TO M
W(I + 1) = W(I) * W(1): YCAL = YCAL + A(I + 1) * W(I): NEXT I
YCAL = YCAL + A(1)
DVN = Y(D%) - YCAL: DVN1 = DVN: DVNSQ = DVN * DVN
DVN = DVN * 100 / Y(D%): ABDVN = ABS(DVN)
Z(1) = Z(1) + DVN: Z(2) = Z(2) + ABDVN: Z(3) = Z(3) + DVNSQ
NEXT D%
Z(1) = Z(1) / N: Z(2) = Z(2) / 2
M = M + 1: IJK = IJK + 1
IF (M < IIJJ) THEN 430
RETURN
```

LIST OF PUBLICATIONS

1. Evaluation of 4-(2'-phenylisopropyl)phenol esters as stationary phases for gas-liquid chromatography.
N.R. Ayyangar, A.S. Tambe, S.S. Biswas, *J. Chromatogr.*, 483 (1989) 33.

2. Crown ethers as stationary phases in gas chromatography : Comparison between dibenzo-18-crown-6, dibenzo-24-crown-8 and dicyclohexano-24-crown-8 with respect to polarity, selectivity and thermal stability.
N.R. Ayyangar, A.S. Tambe, S.S. Biswas, *J. Chromatogr.*, 543 (1991) 179.

3. An improved process for the separation of 1,4-benzoquinone, catechol, hydroquinone and phenol simultaneously.
P.P. Moghe, A.S. Tambe et al., Patent application filed on June 22, 1990 no. 625/DEL/90.

4. Gas chromatographic method for analysis of chlorobenzophenone isomers.
A.S. Tambe, T. Daniel, S.S. Biswas and N.R. Ayyangar, *J. Chromatogr.* (communicated). J. Chromatogr., 628 (1993) 143-147.

5. An improved synthesis of 4-chlorobenzophenone an intermediate for 4-chlorophenoxamine.
A.S. Tambe, T. Daniel and N.R. Ayyangar, *Organic preparations and Procedures International* (Communicated).

6. Direct enantiomer resolution of mintlactone and isomintlactone by gas chromatography on Chirasil-Val and assignment of absolute configuration.
A.S. Tambe, K. Zubaidha, S.S. Biswas and N.R. Ayyangar, *J. Chromatogr.* (under preparation). 3rd international symposium on chiral discrimination Tübingen 5-8.10.92.