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THE DYNAMIC MODIFICATION OF GAS CHROMATOGRAPHIC STATIONARY PHASES BY VAPOURS

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The dynamic modification of gas chromatographic stationary phases by vapours

by

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Submitted in partial fulfilment of the requirements for the degree

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To my parents who have enough love for me regardless of the results; and

to my elder brother who has always enough faith in me to keep me working hard.

.

Luck is not chance.

Luck is when opportunity meets preparation.

Accountability

This is a story about People named Everybody, Somebody, Anybody and Nobody. There was an important job to be done and Everybody was sure that Somebody would do it. Anybody could have done it but Nobody did it. Somebody got angry about that because it was Everybody's job. Everyone thought Anybody could do it but Nobody realized that Everybody would not do it. It ended up that Everyone blamed Somebody when Nobody did what Anybody could have done.

The dynamic modification of gas chromatographic stationary phases by vapours

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Summary

A general method for the modification of stationary phase selectivity by vapours in capillary gas chromatography is presented. The modification is temporary and reversible. There are two approaches. The first procedure involves injection of a modifier of low volatility, as a large vapour plug, onto a capillary column at an elevated temperature. At subsequent lower analysis temperatures, the elution of the modifier is so slow as to enable the normal use of the flame ionization detector(FID). A silicone phase was modified to achieve chiral separation and the polarity of a polyethylene glycol column was significantly increased by this dynamic procedure. The reversible nature of the process presents a convenient way to study intermolecular interactions and potential selectors for novel GC stationary phases.

In the second approach, a volatile modifier is continuously added to the carrier gas. Water vapour, to which the FID shows no response, was used

to alter the retention properties of a polyethylene glycol coated capillary GC column. A dramatic increase in hydrogen bonding interactions was observed towards alcohols and carboxylic acids. The Kovats index of methanol was found to increase by 353 units. The application of the dynamically modified stationary phase to the analysis of alcohol additives in petrol was investigated.

Dynamic modification of stationary phases can be performed with minimum alteration to existing equipment. Special selectivity can be obtained without the need for expensive custom-made columns.

Samevatting

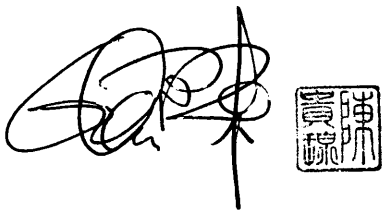
'n Algemene metode vir die modifisering van die stasionêre fase in kapillêre gas chromatografie word voorgestel. Die modifikasie is tydelik en omkeerbaar. Daar is twee benaderings. Die eerste procedure behels die inspuit van 'n groot damp-sone op 'n kapillêre kolom by hoë temperatuur. By die opvolgende, laer analise-temperatuur word die modifiseerder vasgevang in die stasionêre fase en nie deur die vlam-ionisasie-detektor (FID) waargeneem nie. So is 'n silikoon-fase gemodifiseer om chirale skeiding te bewerkstellig en die polariteit van 'n poli-etileenglikol-kolom aansienlik verhoog. Die omkeerbare aard van die proses vergemaklik die studie van intermolekulêre interaksies en die soeke na nuwe selektors vir stasionêre fases.

Met die tweede benadering word 'n vlugtige modifiseerder kontinu toegevoeg tot die draer gas. Waterdamp, wat geen FID respons gee nie, is gebruik om die retensie-eienskappe van 'n poli-etileenglikol bedekte kapillêre kolom te verander. 'n Dramatiese toename in waterstof-binding met alkohole en organiese sure is waargeneem. Die Kovats-indeks vir metanol het toegeneem met 351 eenhede. Praktiese toepassing van die dinamies-gemodifiseerde kolom ondersoek in die veld van petrol analise.

Dinamiese modifisering van stasionere fases vereis minimale veranderings bestaande toerusting. Spesifieke selektiwiteit kan verkry word sonder duur, spesiaal-vervaardigde gaschromatografiese kolomme.

DECLARATION

I declare that the thesis, which I summited with this degree of Doctor of Philosophy at the University of Pretoria, is my own work and has not previously been summitted by me for a degree at other university.



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This thesis is the synergistic product of a team of people. It is impossible for me to represent the real debts of gratitude which I owe to those people who have been involved in one way or another with production of this thesis.

The world of chromatography was brought to me by the late Professor Victor Pretorius, and I grew in the same scientific field with my supervisor, Professor Egmont Rohwer. I wish to express my first appreciation to Professor Egmont Rohwer, for affording me the opportunity to understand and master the use of gas chromatographs.

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Although it's not possible to acknowledge all the sources and influences that have contributed to the writing of this thesis, I would also like to express my appreciation to

1. all other authors whose books or articles added to my knowledge
2. those for listening to my ramblings, anxieties and joys
3. those for helping me in living and life

Finally, I want to thank my family and friends for all their support.

Thank You --- Guay-Chuan

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CHAPTER 1

INTRODUCTION

1.1 Background

Capillary gas chromatography has developed into such a powerful and robust technique that most separations can be achieved by using only a few stationary phases. Problems are however encountered when very polar separations are required and are also frequently experienced with the separation of chiral mixtures.

Polar phases are difficult to coat on smooth glass or fused silica due to their tendency to form droplets which results in loss of column efficiency. Increases in the surface tension of polar liquid phases necessitates the use of very polar support surfaces to ensure even coating of the capillary walls. The lack of very high polarity stationary phases in capillary GC is often lamented, especially those that offer selectivity on the basis of hydrogen bonding interactions with analytes.

The mechanism of separation for chiral mixtures is still poorly understood. It is often impossible to predict how an enantiomeric separation pair will behave on a specific chiral selective stationary phase. Many different phases are required to analyse a broad range of chiral compounds.

A number of cases have been reported where sample overloading causes unwanted retention time shifts in capillary gas chromatography. Similar

retention time shifts have been reported for minor peaks following or preceding major peaks [1,2].

Berezkin and Korolev [3] have done some studies with capillary columns and the effect of water vapour on retention. They studied the effect of smaller amounts of water on retention time reproducibility in capillary GC systems. Carrier gas saturated with water at room temperature was the maximum concentration of modifier used. At a column temperature of 70°C the maximum Kovats retention index change of 13.74 units was reported for n-propanol on polyethylene glycol (PEG) 20M.

Yet, the deliberate modification of the stationary phase has not been thoroughly studied for capillary columns. Volatile modifiers have indeed been studied extensively in packed column GC [4~13], where the procedure was termed 'vapour phase chromatography' when the modifier was undiluted with another gas. Saturated water vapour was used in one case to provide both the stationary and mobile phase in a packed column, resulting in such a high polarity as to elute the heavier alcohols before the lighter ones [13].

In liquid chromatography the term 'dynamic modification' is used to describe a very similar technique [14,15] where cationic and anionic detergents are used on silica to create columns that temporary work like chemically bonded ODS silica columns.

The success of these modification systems depends on the non-interference of the detection system by the modifier. This problem is dealt with in two ways in this thesis:

1) Through the use of modifiers of low volatility that can withstand high loading temperatures and show negligible bleed-rate at the subsequent much lower analysis temperature.
2) Continuous introduction of volatile modifiers that are invisible to the (selective) GC detector.

1.2 Approach

1.2.1 Modifiers of low volatility

Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (hereafter permethylated β-cyclodextrin), is a chiral modifier of low volatility commonly introduced in the conventional static coating method as a mixture with silicone phases [16]. The reported GC analysis of permethylated β-cyclodextrin) [17,18], and the availability of high temperature capillary columns led to the idea that such compounds could be injected in large amounts onto conventional columns to temporarily and reversibly alter their selectivity. The procedure would involve the volume and concentration overloading of robust stationary phases by the modifier at high temperature. This procedure is similar to that routinely performed when large amounts of solvent is injected with on-column injection techniques at low temperature. After uniformly filling the column with the vapour phase, the temperature would be reduced to effectively 'immobilize' the modifier during subsequent gas chromatographic analysis. This loading procedure could be undone by simply flushing out the modifier at high temperature. The potential advantages of such a strategy would clearly be to avoid purchasing the required number of expensive columns for enantiomeric analysis of a broad range of substances [16]. One conventional column could simply be used with a range of modifiers to be tried for different classes of chiral compounds.

For the uniform injection of a long vapour plug, a high capacity injector insert was designed to prevent the liquid modifier from moving into the column and to simultaneously allow carrier gas to equilibrate with modifier present at the injector temperature. This temperature is kept just below the temperature of the column oven in order to obtain the maximum vapour concentration without danger of pure modifier condensing downstream in the column.

Disappointing results with loading of the derivitized cyclodextrin on a high temperature dimethyl silicone phase, PS-086, prompted us to focus our attention on other modifiers that do not require the excessively high temperatures for vapour phase introduction. Thus two further systems are reported on to demonstrate the viability of the general principle of this novel method of dynamic modification in capillary GC:

1. Modification of a Carbowax column with glycerol to provide a very polar column that offers selectivity through hydrogen bonding interaction with analytes.

2. Modification of the SE-30 column with a chiral selector, N-lauroyl-L-valine-butylamide, a diamide known for its high enantiomeric selectivity towards derivitized amino acids [19~21] as used in the commercial Chirasil-Val® (Alltech) capillary column where the selector is covalently bonded to a silicone polymer backbone.

1.2.2 Volatile modifiers

An important feature of this approach is the use of a detector that does not respond to the modifier used, as for example electron capture when alkanes or alcohols are used in the carrier gas [10,11] or UV detection for organic modifiers as carrier gas [8]. Water and formic acid have frequently been used in packed column GC due to their lack of response in the flame ionization

ionization detector(FID). In packed columns the effect of vapours in the carrier gas stationary phase selectivity is complicated by the simultaneous deactivation effect normally observed due to the covering of active sites on the solid support.

Berezkin and Korolev [3] studied the effect of smaller amounts of water vapour on retention time reproducibility with capillary columns. However even split injection of different volumes of aqueous samples on Carbowax capillary columns [22] seemed to indicate more drastic effects on the retention behaviour of some analytes.
It was thus decided to study the effect of higher concentrations of water vapour in a capillary GC column coated with Carbowax 20M. This would serve as an example of the general case of controlling capillary GC selectivity by dynamic modification without detector interference from the modifier. The choice of this modifier would furthermore enable a comparison with the hydrogen bonding behaviour introduced by glycerol in the Carbowax column as mentioned in 1.2.1.

For the continuous introduction of various concentrations of water vapour, a simple, non-permanent, modification was made to the GC carrier gas system of a gas chromatograph.

1.3 Organisation of the thesis

After setting the stage in this introductory chapter, Chapter 2 provides relevant background material. The role of the GC stationary phase in chromatographic separations is stressed with emphasis on the various retention mechanisms and how these effect selectivity. Various aspects of column manufacturing are

defined including theoretical aspects of wettability and inertness. Column pre-treatment and coating procedures are discussed. The process of dynamic modification is explained.

In Chapter 3 the manufacturing of PS-086, OV-1701 and OV-1701-OH columns using the static coating procedure is described. A chiral selector (Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin) is added to the coating solutions prior to coating and chiral columns are manufactured.

Two modes of dynamic modification are discussed in this thesis. Chapter 4 describes the first mode where modifiers of low volatility are loaded at high temperature and immobilised during subsequent lower analysis temperatures. Chapter 5 describes a mode of dynamic modification where continuous introduction of a volatile, invisible modifier changes the polarity of the stationary phase during analysis.

CHAPTER 2

GLASS OPEN TUBULAR GC COLUMNS AND LIQUID STATIONARY PHASES

In gas chromatography samples are separated by distribution of molecules between a stationary phase and an inert mobile phase by adsorption and/or partition. In gas chromatography the interaction between the analytes and the stationary phase is essential for separation.

2.1 Chromatographic Theory and the Role of the Liquid Stationary Phase

The function of the liquid stationary phase, which should have reasonable chemical and thermal stability, is to separate the sample components into discrete peaks. The chromatographic separation is well defined, as expressed in terms of column efficiency (N), selectivity factor (separation factor or relative retention)(α) and retention factor (or capacity factor) (*k).* The influence of the three factors on resolution is discussed below and illustrated by examples taken from capillary gas chromatography [23].

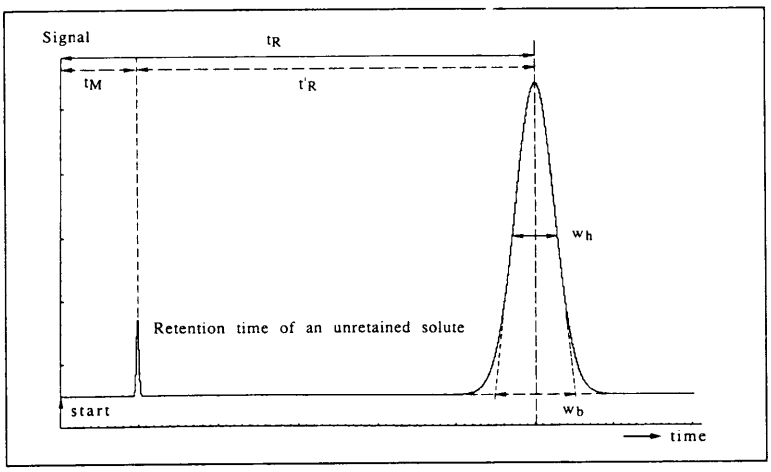


Figure 2-1. Schematic chromatogram

Figure 2-1 is a typical chromatogram for a sample that contains a single analyte. The time it takes after sample injection for the analyte peak to reach the detector is called the retention time, t_R. The peak is characterized by the retention time t_R and the peak width (w_b). The small peak on the left is unretained by the column and elutes at the speed of the mobile phase. The retention time of an unretained solute(t_m) is also called the hold-up or dead time. The retention factor (k) is defined by the time the solute spends in the stationary phase relative to the time it spends in the mobile phase (the relative migration rate of the analyte on the column):

$$k = \frac{t_R - t_M}{t_M} = \frac{t_R'}{t_M} \quad \text{---------- (1)}$$

Where t'_R is the adjusted retention time or the time the solute spends in the stationary phase. An advantage of using k rather than retention time to characterize a component is that it is a dimensionless quantity and independent of the mobile phase velocity.

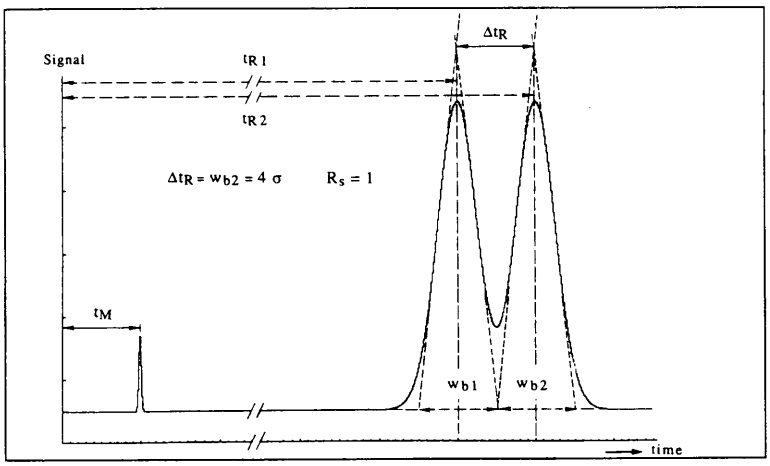


Figure 2-2. Peak resolution, R_S=1.

Peak resolution (R_S) is defined as the separation of two peaks ($t_{R2} > t_{R1}$) in terms of their average peak width at base (Figure 2-2):

$$R_S = \frac{t_{R2} - t_{R1}}{\frac{1}{2}(w_{b1} + w_{b2})} \quad \text{----------} \quad (2)$$

t_{R1}: retention time peak 1
t_{R2}: retention time peak 2
w_{b1}: width of peak 1 at base
w_{b2}: width of peak 2 at base

For two adjacent peaks it is assumed that $w_{b1}=w_{b2}$ and the average value may be substituted by the width of the last eluting peak:

$$R_S = \frac{t_{R2} - t_{R1}}{w_{b2}} = \frac{\Delta t_R}{w_{b2}} \quad \text{----------} \quad (3)$$

A resolution of 1.5 gives an essentially complete separation of the two components, where a resolution of 0.75 does not. At a resolution of 1.0, the separation is 95.4 % complete [23]. Equation (2) and (3) follow directly from the graphic representation of two adjacent Gaussian peaks (Figure 2-2). Translated into chromatographic terms Δt_R is related to the interaction of the solutes with the stationary phase (column selectivity). The peak width (w_{b2}) is related to band broadening mechanisms (column efficiency), in turn, a function of the residence time (retention time) of the solutes in the column. The resolution equation is based on the separation of two components only.

A chromatographic separation is optimized by varying experimental conditions until the components of a mixture are sufficiently separated with minimum expenditure of time. Optimization experiments are aimed at either:

(1) reducing zone broadening or

(2) altering relative migration rates of the components.

Zone broadening is increased by the kinetic variables that increase the plate height of a column. Migration rates are varied by changing the variables that affect the retention and selectivity factors. There are several forms of the chromatographic resolution equation.

From *Equation 3* the following equation can be derived

$$R_S = \frac{\sqrt{N}}{4} \frac{\alpha - 1}{\alpha} \frac{k_2}{1 + k_2} \quad \text{---------- (4)}$$

where the three terms relate to the following:

$\frac{\sqrt{N}}{4}$ Column efficiency

$\frac{\alpha - 1}{\alpha}$ Selectivity

$\frac{k_2}{1 + k_2}$ Retention

Equation 4 is preferred in high efficiency capillary chromatography because the peak widths of two adjacent peaks (the critical pair) are taken to be equal [23]. *Equation 4* is the key equation for separation optimization and can be put to practice. It is useful to consider the three terms of *Equation 4* as independent functions in order to investigate the effect these terms have on resolution.

Column efficiency

$$N = \frac{L}{H} = 16\left(\frac{t_{R2}}{w_{b2}}\right)^2 \quad \text{----------} \quad (5)$$

Where L is the length of the column, H is the plate height.

Peak resolution is proportional to the square root of the plate number which, in term of the plate theory of chromatography, reflects the number of times the solute partitions between the two phases during its passage through the column. Increasing the plate number by a factor 4, doubles the peak resolution. However, the analysis time will also be doubled if there is a two-fold increase in column length. It is expensive in terms of time required to complete the separation unless the increase in N is brought about by reduction in H rather than by lengthening the column. *k* and α do not change with increasing N and L.

Selectivity

$$\alpha = \frac{t'_{R2}}{t'_{R1}} = \frac{t_{R2} - t_M}{t_{R1} - t_M} = \frac{k_2}{k_1} \quad \text{----------} \quad (6)$$

In gas chromatography selectivity between compounds of the mixture is attained through interaction with the stationary phase. Selectivity is measured by the selectivity factor, α. Solute pairs with large α values can be separated with ease even on low-resolution columns. As α approaches unity, columns with an increasingly larger number of theoretical plates are required to achieve separation as illustrated by Figure 2-3. Often optimizing *k* and increasing N are not sufficient to give a satisfactory separation of two solutes in a reasonable time. The main factor governing α is the nature of

the stationary phase. Optimization of the selectivity factor α has great impact on peak resolution. Increasing α from 1.05 to 1.1 nearly doubles the peak resolution (with typical N and *k* values), while a slight decrease, α = 1.03, causes a drastic loss of resolution. The selectivity factor is only temperature dependent for compounds of a different chemical class. This fact may be utilized in high efficiency columns for the separation of closely related components [24]. In general, however, increasing the selectivity factor in capillary gas chromatography is cumbersome as it almost always entails the changing of separation columns.

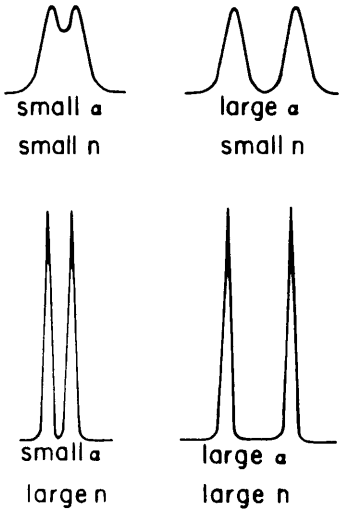


Figure 2-3. Relationship of column efficiency, as denoted by the theoretical plate number N, to the selectivity factor α in component separation.

Retention

$$k_2 = \frac{t_{R2} - t_M}{t_M} \quad \text{---------- (7)}$$

The optimum values of *k* are in the range $3 < k < 5$. For values above 5, the influence of *k* on R_s is small. Increasing k_2 from 5 to 20 increases

resolution by only 14%. But increasing the k_2 value from 0.25 to 5 corresponds to a resolution gain of 378%. Increasing the retention factor can be achieved by using columns with a thicker film or by lowering the column temperature. The latter also has an influence on the selectivity factor.

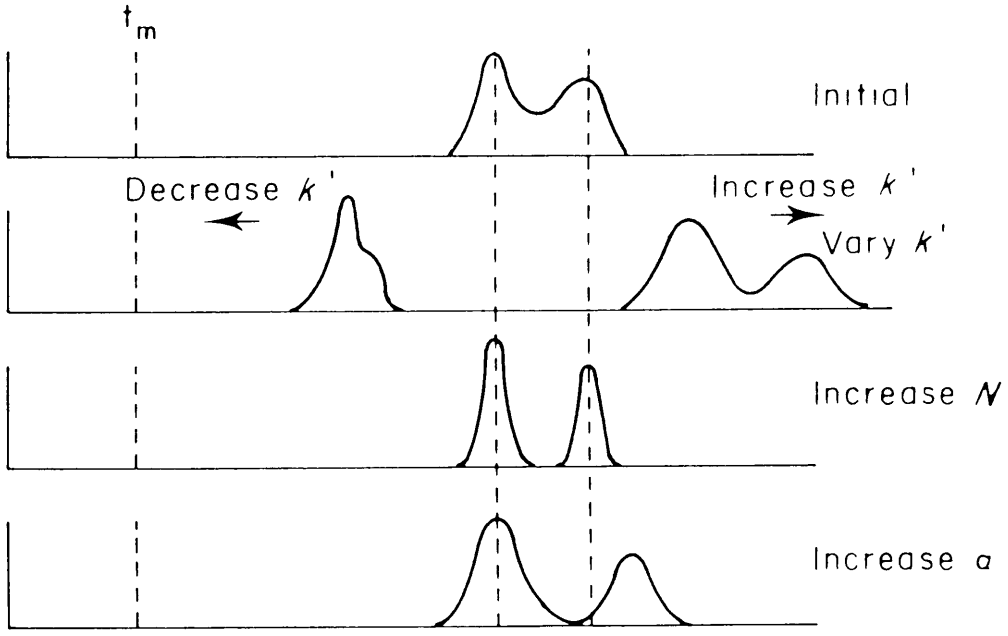


Figure 2-4. Effect on R_S of change in k, N and α

No other change in separation conditions will give as large a change in R_S with as little effort as a change in k. Conditions should first be adjusted so that k falls in the optimum range of $3 < k < 5$. An increase in N will produce narrow, higher peaks with concomitant increase in Rs, but the retention times will not be affected if the change in N has been brought about by a decrease in H. Changes in α will shift the band centre of one peak relative to another and may lead to a re-ordering of the peaks without the overall resolution of the mixture being affected. However, if α is very close to1.0 a change in α will produce a large change in resolution. This approach should be considered for solutes with low α values.

2.2 Selectivity of the Liquid Stationary Phase

To have a reasonable residence time in the column , a species must show some degree of solubility with the liquid stationary phase. The liquid stationary phase must be a good solvent for the mixture. "Like dissolves like" or the liquid stationary phase and the component to be dissolved in it should be chemically similar. The solubility increases as the intermolecular forces increases. The selectivity in capillary gas chromatography is based on differences in intermolecular forces between the molecules to be separated and the molecules of the stationary phase.

2.2.1 Intermolecular forces

The intermolecular forces can be divided into two general types: Van der Waals forces and donor-acceptor forces. Van der Waals forces can be subdivided into dipole-dipole, dipole-induced dipole and London dispersion forces. In addition, there is a specific intermolecular force that affects only certain molecules: the hydrogen bond. All these forces result from the mutual attraction of unlike charges or the mutual repulsion of like charges.

2.2.1.1 Dipole-dipole forces

Neutral molecules that have a dipole moment can experience dipole-dipole forces, as the result of electrical interactions with dipoles on neighbouring molecules. Molecules are orientated in definite directions. This type of interaction is sometimes called *orientation forces*. However, the molecules are in continuous motion which tends to break up any preferred alignment. This thermal motion increases as the temperature is raised and the orientation of the molecules becomes more disturbed. Since dipole-dipole forces decrease as the temperature is increased, selectivity due to dipole-dipole interactions is more pronounced at low temperatures.

2.2.1.2 Dipole-induced dipole forces

A charge or strong dipole on one molecule can cause a temporary charge separation in a second molecule and hence a dipole moment. This is also called induced dipole, Debye, or induction forces [25] between two molecules result in and may lead to an interaction similar to dipole-dipole attractions but of weaker intensity.

2.2.1.3 London dispersion forces

All molecules (including the noble gases), regardless of their polarity, experience London dispersion forces, which result from the motion of electrons around atoms. At any given instant the electron distribution in an atom may be unsymmetrical, giving the atom a short-lived dipole moment. This instantaneous dipole on one atom can affect the electron distributions in neighbouring atoms and induce temporary dipoles. As a result, weak attractive forces develop [26].

2.2.1.4 Hydrogen bonds

This is a special case of a dipole-dipole force. A hydrogen bond is an attractive interaction between a hydrogen atom bonded to an electronegative O, N, or F atom and an unshared electron pair on another nearby electronegative atom. Thus, the hydrogen bond is a bridging bond. It is of variable strength, determined by the particular electronegative elements involved. Water itself is strongly hydrogen bonded. All the hydrogen atoms having a partial positive charge and the oxygen atoms bearing a partial negative charge. When water and alcohol are mixed the alcohol, which itself is associated, must be able to break some of the water hydrogen bonds and replace the water molecules by itself [27, p.62]. As the number of carbon atoms in the alcohol molecules increases the solubility in water decreases [28] . There is a general tendency for water solubility to decrease with an increase in chain length. This effect can be explained

most simply in terms of the large number of water hydrogen bonds that must be broken if a long-chain molecule is to be inserted into the water structure. A considerable amount of energy must be absorbed to break these bonds, making the process less spontaneous and decreasing the solubility.

2.2.1.5 Donor-acceptor forces [29, p.83]

These forces are distinguished form Van der Waals forces because electrons of one atom are physically shared by another. If one of two partners has a high electron affinity (acceptor) and the other a π system with a low ionization energy (donor), a partial transfer from the loaded orbital of the donor to the vacant orbital of the acceptor is possible. Typical acceptors applied in gas chromatography include nitro aromatics, nitrile ethers and tetrachlorophthalate. They selectively retain appropriate donors such as aromatics and olefins.

2.2.3 Classification of stationary phases

The selectivity dictates the suitability of a stationary phase for a specific separation. There are various means of classifying liquid stationary phases [29, p.84]. The Kovats retention indices (RI) is a systematic method for expressing retention data. This system indicates where compounds will appear on a chromatogram with respect to a homologous series of straight-chain alkanes.

$$I = 100z + 100\left[\frac{\log t'_{R(x)} - \log t'_{R(z)}}{\log t'_{R(z+1)} - \log t'_{R(z)}}\right]$$

Where :

I = retention index

x = substance of interest

z = normal alkane with z carbon atoms emerging before the substance of interest.

z+1= normal alkane with z+1 carbon atoms emerging after the substance of interest

Since the chromatographer is interested in the selectivity of a column for a variety of functional groups, it is important to classify each of the stationary phases by its ability to retard specific functionalities. For this purpose the McReynolds system of phase constants has become the most widely used systematic approach. Virtually all popular phases have been characterized by this method. In spite of its popularity the approach is fundamentally flawed and the phase constants are an unreliable indication of phase properties. The basic approach, however, has influenced the development of other methods of selectivity characterization. Unfortunately many of these methods have inherited the deficiencies of their parent. The McReynolds approach is formulated on the assumption that intermolecular forces are additive and is based on earlier theoretical considerations proposed by Rohrschneider. Their individual contributions to retention can be evaluated from differences between the retention index values for a series of test solutes measured on the liquid phase to be characterized and squalane at a fixed temperature of 120°C [30].

The rule of thumb to follow when selecting liquid phases is "like dissolves like" *e.g.* for the separation of alcohols use a polyglycol as a liquid phase; for the separation of hydrocarbons use a hydrocarbon liquid phase, and so on. The polarities of solute and stationary phase should be somewhat alike. Other factors also influence the choice of the stationary phase [29] :
-the concentration and/or type of terminal groups,
-differences in the ratio of comonomers in a copolymer,

- the concentrations of the respective functional group,
- the type of backbone to which the functional group is attached

Table 2-1 lists the most widely used stationary phases for both packed and open tubular gas chromatographic columns in order of increasing polarity. These six liquids are estimated to provide satisfactory separation for 90% or more of the samples encountered by scientists [31].

Table 2-1. Some Common Stationary Phases for Gas-Liquid Chromatography

Stationary Phase	Common Trade Name	Maximum Temperature, °C	Common Applications
Polydimethyl siloxane	OV-1, SE-30	350	General-purpose nonpolar phase; hydrocarbons; polynuclear aromatics; drugs; steroids; PCBs
Poly(phenylmethyldimethyl) siloxane (10% phenyl)	OV-3, SE-52	350	Fatty acid methyl esters; alkaloids; drugs; halogenated compounds
Poly(phenylmethyl) siloxane (50% phenyl)	OV-17	250	Drugs; steroids; pesticides; glycols
Poly(trifluoropropyldimethyl) siloxane	OV-210	200	Chlorinated aromatics; nitroaromatics; alkyl-substituted benzenes
Polyethylene glycol	Carbowax 20M	250	Free acids; alcohols; ethers; essential oils; glycols
Poly(dicyanoallyldimethyl) siloxane	OV-275	240	Polyunsaturated fatty acids; rosin acids; free acids; alcohols

2.2.4 Polar stationary phases

With polar stationary phases polar solute molecules are retarded more than non-polar molecules because these undergo dipole-dipole and induction forces in addition to dispersion forces. With polar stationary phases in capillary gas chromatography retention is often caused by a combination of boiling-point and polar mechanisms.

2.2.5 Chiral stationary phases for enantiomeric separation

The analytical separation of enantiomers by capillary gas chromatography on chiral stationary phases was reviewed by Schurig [32]. The separation of enantiomers by gas chromatography can be performed in two modes:

(1) indirect method: off-column conversion of enantiomers into diastereomeric derivatives by complete chemical reaction with an enantiomerically pure resolving agent and subsequent gas chromatographic separation of the diastereomers on a conventional non-chiral stationary phase, and

(2) direct method: gas chromatographic separation of the enantiomers on a chiral stationary phase containing a resolving agent of high (but not necessary complete) enantiomeric purity.

Diastereomers display different physical properties and this is the basis of separation of both methods. While *method 1* involves the formation of diastereomers before separation, *method 2* involves the rapid and reversible diastereomeric association between the chiral stationary phase (also termed chiral selector) and the racemic or non-racemic analyte (selectand). This causes the stationary phase to selectively retain the one enantiomer more than the other.

In *method 1* discrimination by incomplete recovery, decomposition and losses may occur during work-up, isolation and sample handling. In gas chromatography erroneous results may arise from split injection techniques or from differing detector responses to the diastereomers. Consequently, the *method 2* is preferred.

The theory of chiral chromatography is still rather rudimentary. A number of chiral recognition models have been proposed to account for optical resolutions by GC and LC. These are often based on the " three-point interaction " theory advanced by Dalgliesh [33]. According to this postulate, a minimum of three simultaneous interactions between an enantiomer and the stationary phase are required for chiral discrimination. The enantioselective situation is visualized in Figure 2-5.

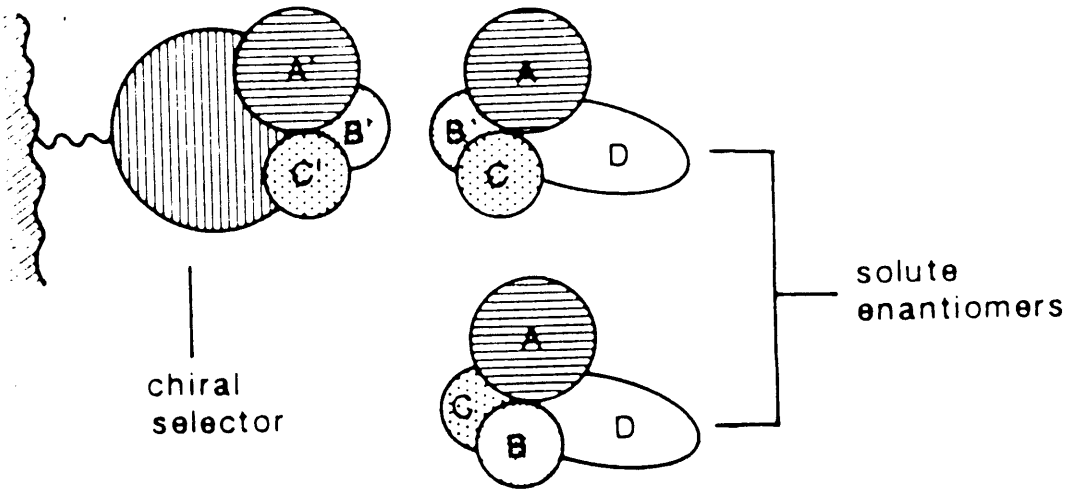


Figure 2-5. The three-point interaction model advanced by Dalgliesh.

Enantiomeric separation by gas chromatography is mainly performed on three types of chiral stationary phases:

(I) on chiral amino derivatives *via* hydrogen bonding
(II) on chiral metal coordination compounds *via* complexation
(III) on cyclodextrin derivatives *via* inclusion

Chiral selectors are used as non-volatile neat liquids (*undiluted*) or as solutions in polysiloxanes (*diluted*). Cyclodextrin derivatives have proved to be the most versatile and universal chiral stationary phases in gas chromatography. More information on cyclodextrin derivatives and their applications can be found in a book by König [34]. Anchoring the chiral selectors to a polysiloxane backbone leads to Chirasil-type stationary phases with improved temperature stability, efficiency and lifetimes.

2.3 Manufacturing Glass Open Tubular Columns

2.3.1 Wettability and inertness [35,36]

The two basic criteria for a good open tubular column are maximum separation efficiency and the absence of isolated active sites that can give rise to peak tailing.

The *wettability* of the support surface by the liquid phase determines how evenly the liquid phase will coat the capillary wall. Uneven stationary phase thickness leads to the reduction of separation efficiency as measured by a loss of plate number (N). In other words, separation efficiency constitutes a direct measure of film homogeneity. The wettability is a thermodynamic function of the equilibrium between the cohesion forces inside the liquid and the surface energy of the solid surface. The contact angle depends on the specific surface free energy of the solid and liquid. Spreading generally occurs when the specific surface free energy of the liquid is less than that of the solid support [35, p.57].

Intermolecular forces in liquids increase with increasing population of groups easily polarized or polar functional groups such as phenyl, cyano and hydroxyl groups. Liquids with increasing polarity show increasing

surface tension. Consequently, wettability by more polar liquids requires more polar support surfaces.

The stationary phase should act as a solvent for analytes in gas chromatography. Neither the liquid phase nor the support material should cause additional physical or chemical effects. An inert column will elute all components of a sample without delay due to adsorption and cause no chemical or catalytic effects. Inertness is often selective in that it is excellent for one class of compound but unacceptable for another [36]. More polar stationary phases are often less inert because the support material has to be more polar in order to stabilize the liquid layer. The polar moieties are expected to cause adsorption of the sample. This problem is best solved when wettability is based on structural similarity.

The coating procedure entails various steps to improve inertness and ensure adequate wettability of the support surface by the liquid phase. Surface pretreatment, coating and immobilization procedures will be discussed in the next section.

2.3.2 Column materials

A number of column materials have been used for the fabrication of open tubular columns. These include plastics, nickel, copper, stainless steel, glass and fused silica. Each of these has advantages and disadvantages [29,30,35]. Assessing the relative suitability is difficult, since essential subjective arguments play an important role. The general criteria as listed by Grob [36] are price, transparency, availability and convenience of manipulation. Borosilicate glass and fused silica are the most often used.

Fused silica is the column material preferred for commercial use. Polyimide-coated fused silica columns tend to break after repeated use at temperatures above 300°C [37~39] and rapidly above 360°C [40]. For in-house column production borosilicate glass is preferred. Borosilicate glass is also preferred for high temperature GC work. Improved inertness and thermostability is obtained with intensively leached borosicate glass silica surfaces. These columns exhibit good inertness after prolonged routine use at 330°C (OV-1701-OH) or 380°C (PS-086) [41]. It has been demonstrated that borosilicate glass capillary columns can be coated with OH-terminated high temperature polysiloxane phases of moderate polarity [42,43]. Thus the use of borosilicate glass capillaries will be beneficial for the loading of low volatility modifiers to be used for dynamic modification as explained in Chapter 1.2.

2.3.3 Surface pre-treatments

Pre-treatment steps are necessary for deactivation of the glass capillary column wall prior to stationary phase coating. Proper deactivation determines column inertness and lifetime. Deactivation is generally attained after leaching followed by rinsing, dehydration and persilylation. The surface pre-treatments have two primary purposes [35,p.60]:

1. Deactivation of the active surface sites, and
2. Chemical and/or physical modification to enhance surface wettability.

Unfortunately, it is difficult to accomplish both goals simultaneously. Different preparation procedures are used for different stationary phases to maximize surface wettability.

Leaching

All types of glass contain metal ions. Some types of glass also contain covalently bonded boron. These act as adsorptive and chemically active sites and reduce the inertness of the support surface [36]. The process of leaching with hydrochloric acid removes the metallic cations from the glass matrix and increases the density of hydroxyl (silanol) groups on the surface. The silanol rich surface serves as a good starting point for further surface modification.

Rinsing and dehydration

The metal ions leached from the column are removed by rinsing with a dilute acid. The column is then dehydrated to eliminate adsorbed water after rinsing.

Persilylation

The last step before coating is a modification of the surface to eliminate the surface hydroxyl groups. These groups are chemically replaced with inert groups. Care has to be taken that the silanol groups are removed, hence, the term persilylation. The surface wettability can be enhanced by choosing modifying groups which are compatible with the desired stationary phase. Inert columns are produced when the maximum coverage density is obtained [44].

2.3.4 Coating procedures [35,36]

The ideal coating procedure produces a uniform stationary phase film throughout the length of the column. Stationary phases are classified into apolar, moderately polar and polar phases. Coating with a polar phase presents serious preparation problems. Due to high surface tension the phases spread only on strongly active surfaces. Since it is very difficult to achieve sufficient activity by silylation, inorganic surface structures are normally used. The columns consequently possess significant residual activity.

Column coating can be accomplished by two general methods called static and dynamic coating. There may be a need for the introduction of immobilization with coupling agents after column coating. The static coating method has generally been considered to be superior to the dynamic method [35].

2.3.4.1 Static coating

A column is completely filled with a dilute solution of liquid phase in a suitable low-boiling solvent. One end is carefully sealed. It is critically important that the column be completely filled with coating solution and that no air or vapour bubbles exist in the column. The column is then kept under vacuum and at a constant temperature to ensure uniform film deposition as the solvent evaporates from the open end of the column.

The advantages of this method are that

(I) The amount of stationary phase in the column can be measured and the film thickness can easily be calculated;

(ii) Viscous liquids can be introduced for the production of the thick film columns;

(iii) The film thickness is very uniform throughout the length of the column.

The disadvantages are that the static coating is time consuming and the end sealing required some skill.

2.3.4.2 Dynamic Coating [35]

2 ~ 15 coils of the column is filled with a solution of the stationary phase. The solution is forced through the column at a velocity of approximately 1 ~ 2 $cm.sec^{-1}$ with gas pressure. A thin film of this solution is left behind on the column wall. Continuous flushing with helium after coating evaporates the remaining solvent and leaves a thin coating of stationary

phase. Often, non-uniform films are obtained by this method. The dynamic coating method has been improved in several ways. Probably the most significant development in dynamic coating was introduction of the "mercury plug" method. This method involves adding a mercury plug between the solvent plug and the driving gas which, because of its high surface tension, wipes most of the coating solution off the surface as the plug moves through the column to yield a thinner, uniform film. Less time is required for the solvent to evaporate from the column. Less time is therefore available for the solution to run into droplets. The end result is a more uniform layer of stationary phase.

The advantage of this method is that it is easy and quick.
The disadvantages are that:

(I) The amount of the stationary phase in the column cannot be determined exactly;

(ii) Deviations of the inside diameter of the tube result in variations of the film thickness; and

(iii) the column efficiency is lower than with static procedures [29].

2.3.5 Immobilization

Immobilization is effected by:

1. surface bonding --- bonds between the support surface and the stationary phase,
2. cross-linking --- bonds among the stationary phase molecules
3. a combination of both.

The advantages of the immobilization is listed below [36]

1. No phase stripping for large amounts of injected solvents.
2. Wider practical temperature range
3. Stabilization of very thick coatings

4. Washability
5. Thermostability, durability, ruggedness

For glass columns, the major disadvantage of immobilization is with thermal deformation of permanently coated glass. End straightening the capillary for connection with the injector and detector causes damage to the cross linked surface. This problem was solved by Rohwer [39] who used flexible fused silica as end sections, press fitted to the glass capillary column. If the above listed advantages are not essential then there is no need to immobilize a coated column. In most of the immobilization reactions, cross-linking is the predominant process. Surface bonding plays an equivalent role only with OH-terminated or Carbowax phases. Grob [36] has observed that in addition to cross-linking, surface bonding becomes increasingly important with increasing polarity of the phase, and with increasing film thickness.

2.4 Permanent Modification with Low-volatility Selectors

Modifiers can be used to enhance or decrease retention of solutes [6~8]. Modifiers of low volatility have been added to stationary phases before coating [16,33,34,45-62]. The modifier is dissolved in a stationary phase to form a binary stationary phase

The use of mixed stationary phases in gas-liquid chromatography became less significant due to the increasing application of open tubular columns [29]. For some special compounds such as enantiomers, there is a need for adding particular compounds as modifiers to the stationary phases for enantiomeric gas chromatographic analysis.

Gas chromatography using enantioselective stationary phases represents a powerful tool for enantiomeric analysis. There are two approaches: Schurig and Nowotny [47] first described the use of diluted peralkylated cyclodextrins for enantiomeric separation for high resolution capillary columns. Undiluted derivatized cyclodextrins that are in the liquid state at the temperature of analysis were also used [63,64]. These approaches were reviewed by Schurig and Nowotny [16] and König [65]. A large number of works on the study of cyclodextrins and their derivatives have been published [16,33,34,45~60]. Enantiomeric separation can also be achieved with cyclodextrin derivatives covalently bonded to polysiloxanes [66,67].

Feibush [20] studied an optically active diamide stationary phase, N-lauroyl-L-valine-*t*-butylamide, for enantiomeric resolution of the N-trifluoroacetyl-(±)-methyl ester of α-amino acids. Problems were encountered with the separation of the D and L isomers of aspartic acid and proline. Charles *et al.* [21] prepared a series of optically active diamide stationary phases having high separation factors which allowed for the separation of D and L isomers with a packed column. Takeichi *et al.* [68] investigated a chiral stationary phase prepared by bonding L-valine-*t*-butylamide to XE-60 coated on glass and metal capillaries for the separation of amino acid derivatives.

Complex formation between fluorinated and methylated β-diketonates of praseodymium and europium, dissolved in a polydimethylsiloxane stationary phase, and the analysis of aliphatic alcohols, methyl ketones, methyl ethers and carboxylic acid methyl ester were studied by Kowalski [61]. The author concluded that gas chromatographic stationary phases containing adduct forming lanthanide chelates are particularly important for selective separation of mixtures of nucleophilic solutes.

Vapour modifiers used in gas chromatography can be volatile or less volatile. Volatile modifiers have been used directly as carrier gases or have been mixed with inert gases.

2.5 Temporary Vapour Phase Modification of GC Columns

Parcher [5] reviewed vapour phase chromatography concerning the applications of adsorbable vapours as carrier gases for gas-solid and gas-liquid chromatography. The term " Vapour phase chromatography " was initially used for the method known today as gas-liquid chromatography. However, for a number of reasons, the actual use of vapours as carrier gases never become popular. Permanent gases, such as helium, nitrogen and hydrogen are the most commonly used today and insoluble in the common liquid stationary phases. A vapour carrier gas can be absorbed in a liquid stationary phase as a volatile modifier and produce a binary liquid phase that may have radically different solvent properties from the original stationary phase.

The column polarity can be increased or a specific selectivity can be achieved by dynamic modification of the stationary phase. This type of modifiers has changed the patterns of the chromatographic analyses. They affect peak shapes, alter retention of analytes or offer specific separations.

2.5.1 Unintentional shifting of retention times due to large amounts of solvent injected

Peak shifting was thought by Dietz [69] to be due to the same solvent effect described by Grob for splitless injections to focus chromatographic peaks. Solvent effects are also known to occur with split mode injections [1]. The

retention shifting was traced to changes made in the injection split ratios due to solvent effects from sample components and split ratio.

Grob and Habich [22] have shown that a small amount of water in the carrier gas can have a dramatic effect on the retention times of polar solutes in a Carbowax column. This phenomenon was also shown by Berezkin [3] on analysing samples containing water as a main component on the PEG-20M column.

2.5.2 Deliberate vapour modification of stationary phases

The use of organic gases and vapours, or their mixtures with ordinary carrier gases as the mobile phase in gas chromatography was studied by Garusov and Vigdergauz [9]. Many different carrier gas mixtures were studied with a variety of analytes and detectors. The first studies, using organic gases blended with inert carrier gases in gas chromatography, were published as early as 1956-1959. The chromatographic separation pattern of the analytes were dramatically changed by using the above mixtures.

It was demonstrated that the chromatographic process can be carried out in ethanol vapour. This significantly influenced the nature of the sorption of the analytes in the column changing the retention and shape of the concentration bands [9]. The separation of phenol, cresols and other compounds with water vapour was also described [10].

It was shown that the continuous introduction of strongly adsorbed material with helium carrier gas reduces the requirement for inertness of the solid support, since the adsorbed material blocks the active centres. This gives more symmetrical peaks with polar sorbates [70].

Tsuda *et al.* [7,8] used a variety of organic vapours such as ethanol, 10% water in ethanol, benzene, carbon tetrachloride, *etc.* to intentionally alter the chromatographic properties of dioctylphthalate, silicone oil and polyethylene glycol stationary phases. These authors observed the effects of volatile modifiers on the peak shapes, retention time and efficiency (HETP) of different solutes.

Rudenko and co-workers [10] used water, formic acid and methanol as mobile phases with several different liquid stationary phases to improve the separation of free fatty acids, amines and haloalkanes on both flame ionization(FID) and electron capture detectors(ECD). Siu and Aue [11] used an electron capture detector with non-polar dodecane as the vapour modifier to change the separating power of polar liquid stationary phases (Surfynol 485 and OV-275) *in situ*. The ECD is useful for dynamic modification since many possible modifiers such as alkanes do not give a response with this detector.

Some theoretical and practical aspects of the controlled modification of a stationary phase by vapour was recognized by Kyazimov *et al.* [12]. Ethanol, acetone and hexane were used as vapour modifiers on Apiezon L and polyethylene glycol adipate liquid phases with thermal conductivity detection. It was shown that the stationary phase is modified temporarily and acquires a different polarity.

Parcher and Hyver-LoCoco [6] investigated the use of ethanol on Carbowax 1500. Ethanol as a component of the carrier gas allows continuous control of the amount of modifier adsorbed and alters the chromatographic selectivity by means of the carrier gas composition and pressure. This polar modifier enhanced the retention and improved the peak symmetry of polar solutes.

Berezkin and Korolev [3] studied the influence of water contained in samples as a main component. This study was extended to include the effect of water added deliberately to the carrier gas. *This study is to the best of the author's knowledge the only study on deliberate modification of a capillary column.* A major influence on retention time reproducibility of compounds, using non-polar SE-30 and polar PEG-20M, 25 m × 0.2 mm was observed. The carrier gas was saturated with water vapour at ambient temperature. This work was of interest for the accuracy of the retention value measurements in capillary chromatography when aqueous samples were analysed. It was recommended that the water vapour be added to the carrier gas to reduce tailing and obtain reproducible and quantitative results [7]. Phier and Plummer [13] studied the GC separation of alcohols with selectivity based on polarity. It was achieved by using water as a liquid support and a mixture of nitrogen and steam as the carrier gas with flame ionization detection. One of the experiments described the separation of C_1 and C_5 straight-chain alcohols eluted in reverse order.

CHAPTER 3

PREPARATION OF GLASS OPEN TUBULAR GC COLUMNS

In this chapter a chiral modifier of low volatility will be added to various polysiloxanes. These mixtures will then be coated by the static coating method [36] onto borosilicate capillary columns. The chapter serves to demonstrate practical aspects of column manufacturing. The columns should be useful to compare with the dynamically modified columns prepared in Chapter 4.

10% (m/m) heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin will be diluted in OV-1701, OV-1701-OH and PS-086 and coated onto capillaries drawn from borosilicate glass. These columns will be designated with the prefix Chiral.

Differences in the liquid phases will require slight differences in the preparation for column coating.
OV-1701 and OV-1701-OH contain:

7% phenyl, 5~7% cyanopropyl and 86~88% methyl polysiloxane

whereas PS-086 contains :

12~15% phenyl and 85~88% methyl with OH-termination of the polysiloxane backbone.

3.1 Preparation of Open Tubular columns

3.1.1 Glass capillary drawing

Pyrex glass tubing was the raw material used for drawing capillaries. The Shimadzu glass capillary drawing machine was used. No cleaning or chemical purification of the glass was necessary before drawing. The glass tubes were drawn to capillaries of 0.3 mm i. d.

3.1.2 Glass surface pre-treatments

Columns were prepared from 25 metre lengths of 0.3 mm i. d. glass capillaries.

3.1.2.1 Leaching

Capillaries were filled with 20% HCl by means of compressed gas. The ends were flame-sealed under vacuum when 3 ~ 3.5% of the capillary length at each side was still empty. The leaching conditions were:

stationary phase	temperature	time
OV-1701	150°C	overnight
Chiral OV-1701	150°C	overnight
PS-086	170°C	12 hours
Chiral PS-086	160°C	8 hours
OV-1701-OH	180°C	12 hours
Chiral OV-1701-OH	180°C	12 hours

Care should be taken to ensure that capillaries do not touch heated parts of the oven.

3.1.2.2 Rinsing

The leached and hydrochloric acid-filled capillaries (which can be stored without damage) were broken into pieces longer than the required 25m final length. The capillaries were rinsed with twice the capillary volume of 1% HCl solution at a rate of 1 $cm.sec^{-1}$. The rinsing procedure must be followed immediately by dehydration.

3.1.2.3 Dehydration

The wet capillaries were placed in an oven with both ends accessible outside the oven.

For the OV-1701 and Chiral OV-1701 stationary phases, the wet capillary was placed in an oven with both ends accessible outside the oven. The capillary was kept at *250*°C for 2 hours and *one end* was connected to vacuum. The vacuum connection for the two ends was alternated roughly every 20 minutes.

For the OH-terminated OV-1701-OH, PS-086 and their chiral counterparts, the wet capillary was also placed in an oven with both ends accessible outside the oven but the capillary was kept at *280*°C for 2 hours with *both ends* connected to vacuum.

The leached and dehydrated capillaries can be stored .

3.1.2.4 Persilylation

Silylating agents:

(I) For OV-1701 and Chiral OV-1701 the silylating agent 1,3-diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS) is used. A 1:1 (v/v) solution in pentane was prepared.

(II) For the OH-terminated OV-1701-OH, Chiral OV-1701-OH, PS-086 and Chiral PS-086 phases the silylating agent consists of 1 volume 1,3,5-trimethyl-1,3,5-triphenylcyclotrisiloxane (TMTPCTS) and 1 volume 1,3-diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS). 1 volume of the above mixture was diluted with 4 volumes of methylene chloride [41].

In each case the solution was sucked into the capillary to fill about 10% of the capillary length. The liquid was moved through the capillary at a rate of 1 $cm.sec^{-1}$ by means of a dry inert gas. When the solution left the capillary, both ends of the capillary were then connected to the vacuum and evacuated for about 15 minutes. (Complete evaporation of methylene chloride is assured by flushing the capillary for about 10 minutes with nitrogen at room temperature for the agent (II).) Both ends were washed with ethanol and carefully flame-

sealed. The capillary was placed in the oven of a gas chromatograph. After heating at full power to 200°C, the temperature was increased at a rate of 2°C.min^{-1}. All columns were kept overnight at 400°C (410°C for the PS-086 phase; 8 hours at 380°C for Chiral PS-086 phase). The capillary was then cooled slowly in the closed oven. Both ends were cut and the capillary was rinsed with hexane, methanol and ether, each solvent amounted to 10%, 20% and 10% (respectively) of the capillary volume.

3.1.3 Column coating by the static method [36]

3.1.3.1 Preparation of coating solutions

A fresh solution of each coating material was prepared in pentane and methylene chloride (1:1 v/v) with a concentration within the range of 0.3 ~ 0.4%. This will give a film thickness of 0.25 µm. The film thickness was calculated as follows:

Film thickness (µm) = 2.5 x capillary i.d. (mm) x stationary phase concentration (%)

The correct calculation would require the concentration of the coating solution to be expressed in volume of the phase per volume of the solution. For simplicity, mass of the phase per volume of the solution as a concentration was used (density ca. 1 g.cm^{-3}).

Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin was prepared according to the literature [62] (Appendix A1). A 10%(m/m) Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin solution was employed (Appendix A2) in OV-1701, OV-1701-OH or PS-086 as a chiral stationary phase for enantiomeric separation. A solution of 0.03 ~ 0.04% heptakis(2,3,6-tri-*O*-methyl)-β-Cyclodextrin and 0.3 ~ 0.4% stationary phase in pentane and methylene chloride (1:1, v/v) was prepared.

3.1.3.2 The coating procedure

The water bath was stabilized at 35°C. The coating solution was forced into the capillary by pressurizing the container with nitrogen. The nitrogen was switched off when the coating solution started to bleed from the capillary exit. 2,2,4-trimethylpentane was loaded and the end of the capillary blocked with molten wax. Care was taken that no air gap existed between the coating solution, 2,2,4-trimethylpentane and wax in this closed end. The open end of the capillary was connected to vacuum. The capillary was placed into the water bath. The vacuum line to the capillary was then opened. Then, the coated capillary was removed from the water bath, the wax closure was cut off and the column was left to dry at room temperature for 5 minutes. The vacuum was switched off and one coil was cut from the other end. The column was mounted with deactivated fused silica ends as a hybrid fused silica/glass capillary column [39].

3.1.4 Column conditioning

A freshly deposited liquid phase will retain residual traces of solvent, along with lower molecular mass liquid phase fractions. These products progress through the column and emerge at the detector as column bleed to yield a baseline offset and extraneous peaks. Therefore, programmed conditioning of the column is necessary.

The freshly prepared columns were mounted in a gas chromatograph with flame ionization detection. They were flushed with a head pressure of about 0.3 $kg.cm^{-2}$ carrier gas using the following temperature conditions:

Stationary phase	Initial temperature	Ramp rate	Final temperature (over night)
OV-1701	40°C	2°C/min	250°C
Chiral OV-1701	40°C	2°C/min	190°C
OV-1701-OH	40°C	1°C/min	300°C
Chiral OV1701-OH	40°C	1°C/min	300°C
PS-086	40°C	1°C/min	300°C
Chiral PS-086	40°C	4°C/min	230°C

3.1.5 Column tests

Whether using homemade or commercial columns, the assessment of column quality is important to all chromatographers. Periodic column testing is the best method of monitoring changes in column properties.

The Grob test was performed on all the columns prepared. A chiral test was performed on the three chiral modified columns.

3.1.5.1 Instrumentation and conditions

After column conditioning, the tests were carried out with a Carlo Erba Fratovap Series 4200 gas chromatograph with a flame ionization detector and a split injector without septum purge. Hydrogen was used as carrier gas for all experiments. The other GC working conditions were as follows:

Injector temperature	250°C
Detector temperature	250°C
GC Attenuation	Input: 10 ; Output: 4
Make-up gas	No

A chart recorder and the Hewlett-Packard 3350 data system were used to record all of chromatograms.

3.1.5.2 Procedure

(1) The GC oven was set to 70°C.

(2) A suitable carrier gas pressure was set by injecting butane and measuring the time between injection and the first peak signal with a stopwatch. The linear flow rate was set to 50 cm.sec^{-1} at a GC oven temperature of 100°C for hydrogen. Changing the split flow after setting this value may significantly affect the carrier gas pressure.

(3) The temperature program was set to 190°C at 3°C.min^{-1}.

(4) 1 µl of the test mixture was injected with splitting ratio of 1:20 to 1:50 depending on injector design.

(5) The temperature program was started immediately after the injection.

3.1.5.3 Grob test mixture

The overall column quality was tested with the Grob test mixture: 2,3-butanediol, n-decane, 1-octanol, n-dodecane, 2,6-dimethylphenol, 2,6-dimethylaniline, 2-ethylhexanoic acid, methyl decanoate, dicyclohexylamine, methyl undecanoate, methyl dodecanoate.

3.1.5.4 The chiral test mixture

The enantiomeric separation characteristics of the chiral columns were tested using part of the Schurig test mixture [71]: 2,3-butanediol (racemic), 2,3-butanediol (meso), γ-valerolactone, 1-phenylethylamine, 1-phenylethanol and 2-ethylhexanoic acid in Figure 3-1, which are all polar racemates.

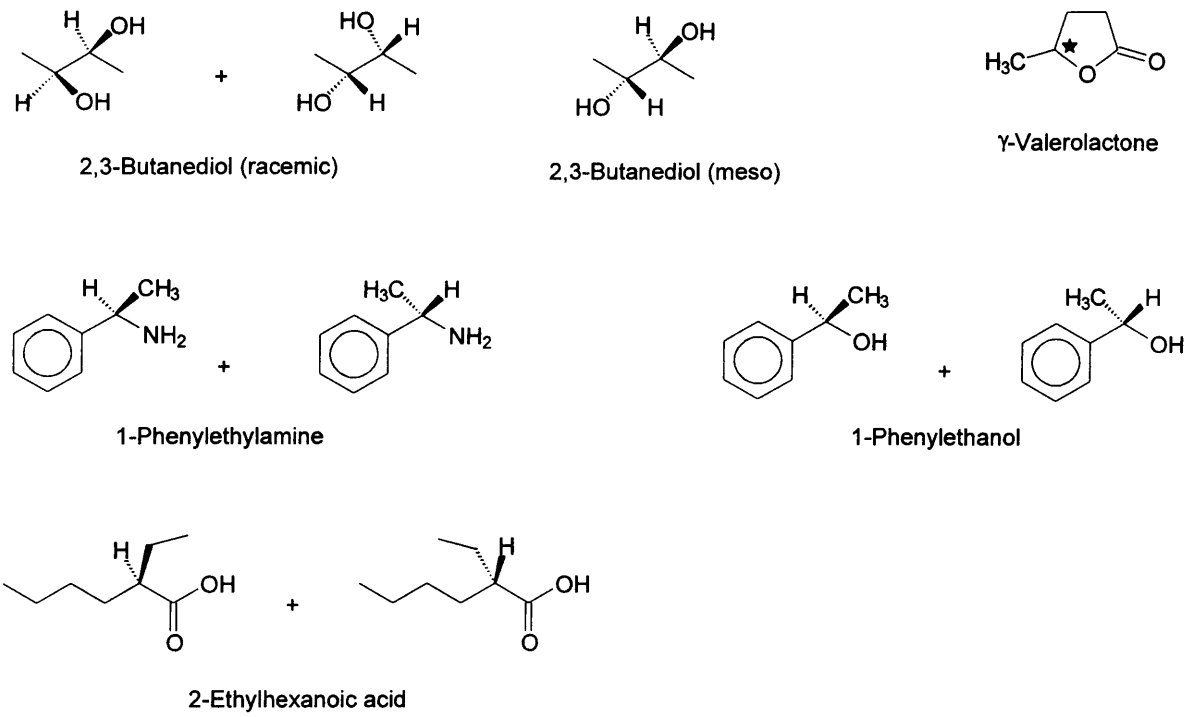


Figure 3-1. Structural formulas of the racemates of the chiral test mixture

3.2 Results

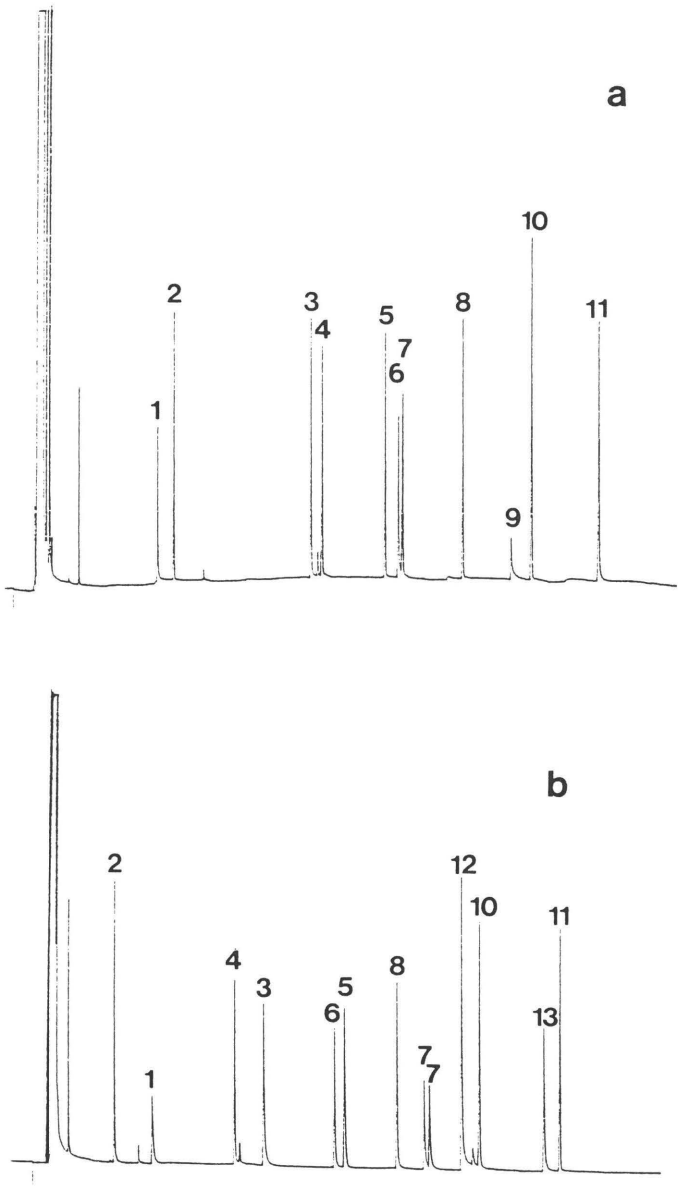


Figure 3-2 . Chromatograms showing the separation of the Grob test mixture on two columns (a) without and (b) with 10% (w/w) heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin in OV-1701, under identical conditions. Columns: 25 m x 0.3 mm i.d., temperature programs: (a)40°C to 190°C at 5°C.min^{-1}; (b)70°C to 190°C at 3°C.min^{-1}. Peaks: 1= 2,3-butanediol; 2= n-decane; 3= 1-octanol; 4= n-dodecane; 5= 2,6-dimethylphenol; 6= 2,6-dimethylaniline; 7= 2-ethylhexanoic acid; 8= methyl decanoate; 9 = dicyclohexylamine; 10= methyl undecanoate; 11 = methyl dodecanoate; 12,13 = unknown.

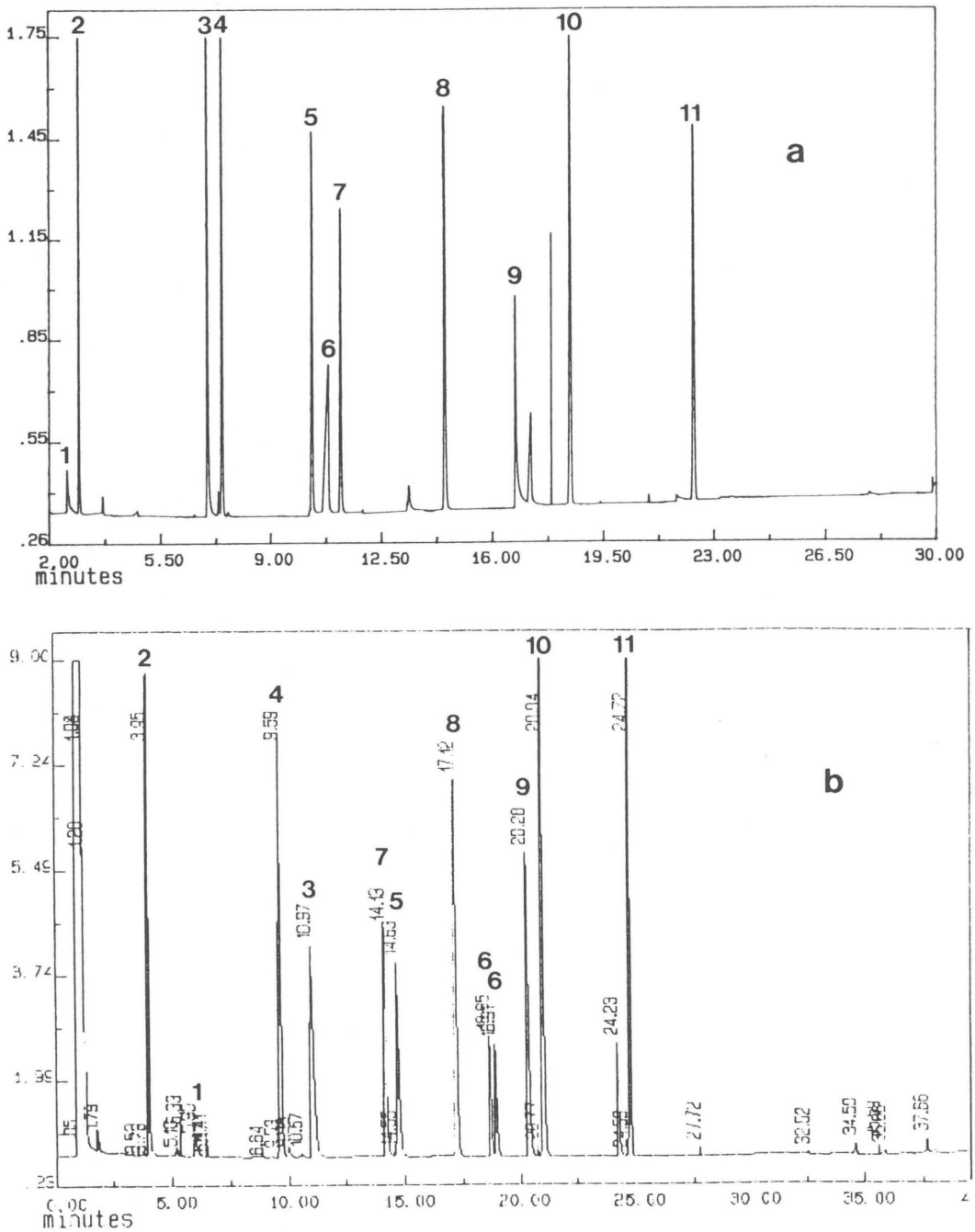


Figure 3-3 . Chromatograms showing the separation of the Grob test mixture on two columns (a) without and (b) with 10% (w/w) heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin in OV-1701-OH, under identical conditions. Columns: 25 m x 0.3 mm i.d., temperature program: 70°C to 190°C at 3°C.min^{-1}. Peaks: 1= 2,3-butanediol; 2= n-decane; 3= 1-octanol; 4= n-dodecane; 5= 2,6-dimethylphenol; 6= 2-ethylhexanoic acid; 7= 2,6-dimethylaniline; 8= methyl decanoate; 9 = dicyclohexylamine; 10= methyl undecanoate; 11 = methyl dodecanoate.

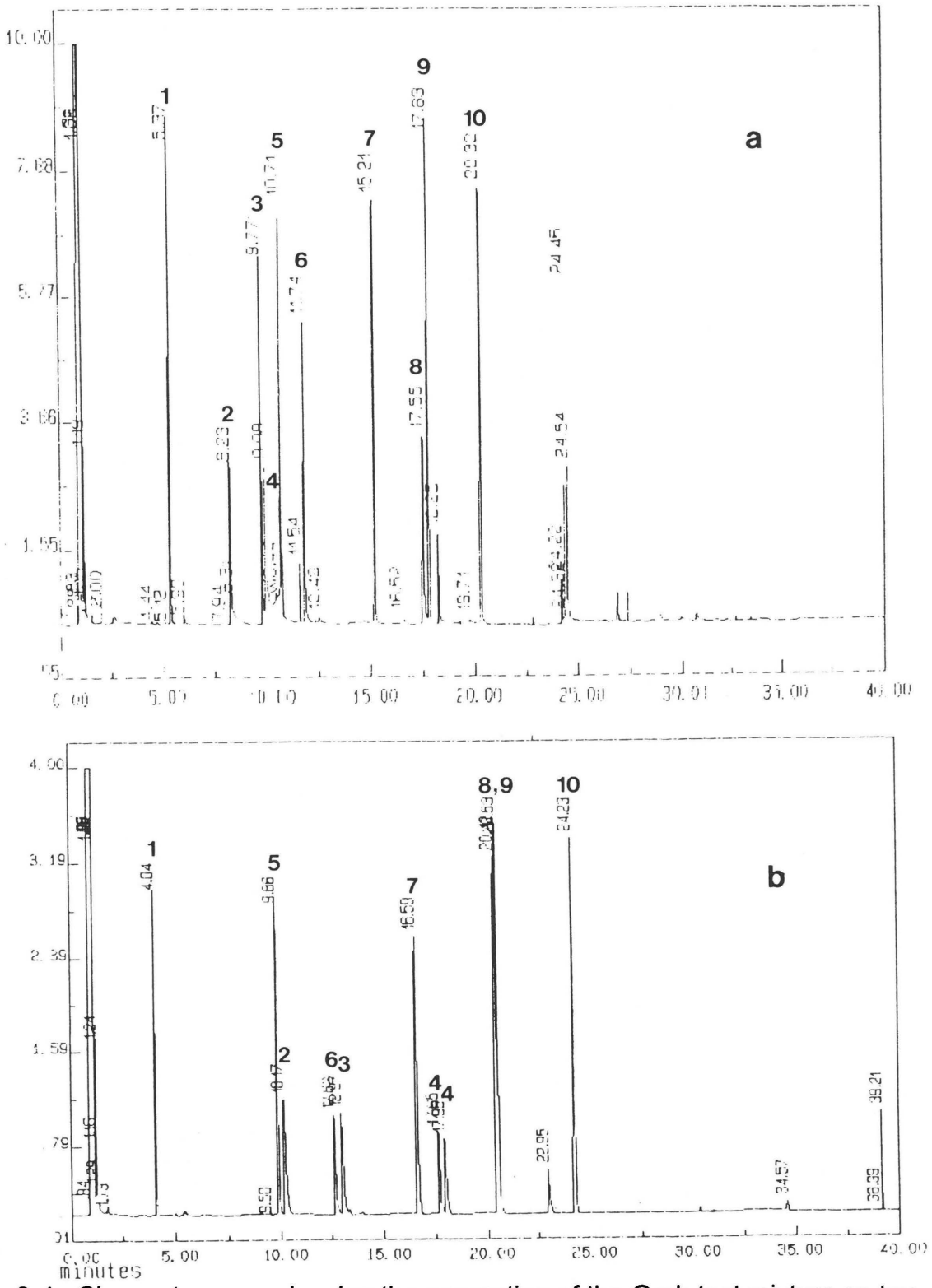
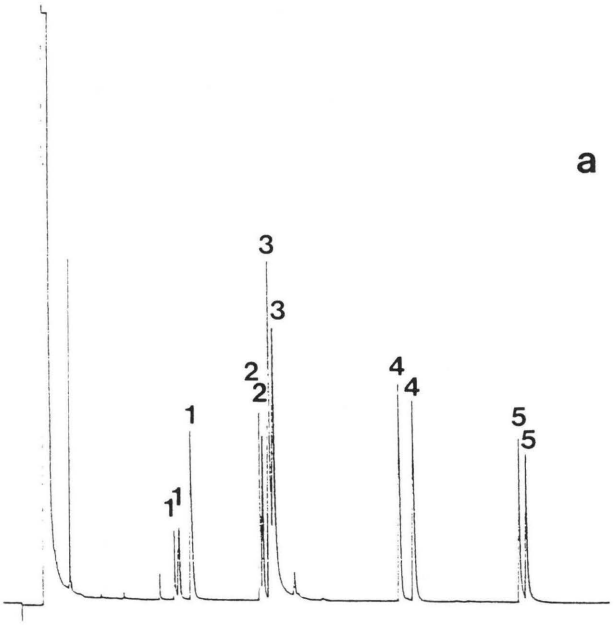
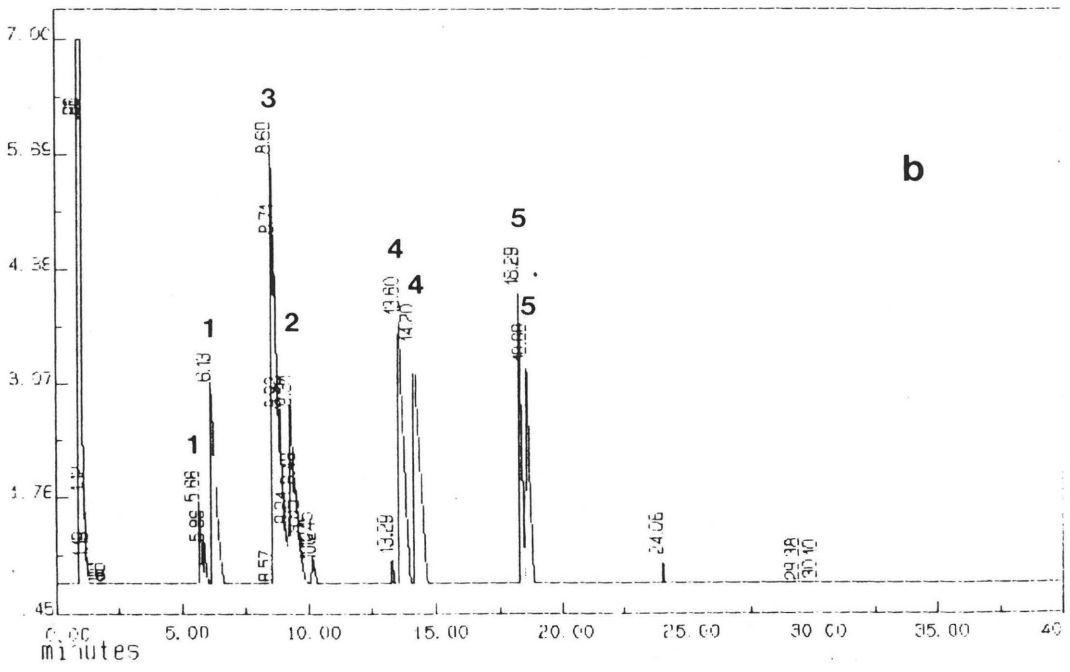


Figure 3-4 . Chromatograms showing the separation of the Grob test mixture on two columns (a) without and (b) with 10% (w/w) heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin in PS-086, under identical conditions. Columns: 25 m x 0.3 mm i.d., temperature program: 70°C to 190°C at 3°C.min^{-1}. Peaks: 1= n-decane; 2= 1-octanol; 3= 2,6-dimethylphenol; 4= 2-ethylhexanoic acid; 5= n-dodecane; 6= 2,6-dimethylaniline; 7= methyl decanoate; 8 = dicyclohexylamine; 9= methyl undecanoate; 10 = methyl dodecanoate.





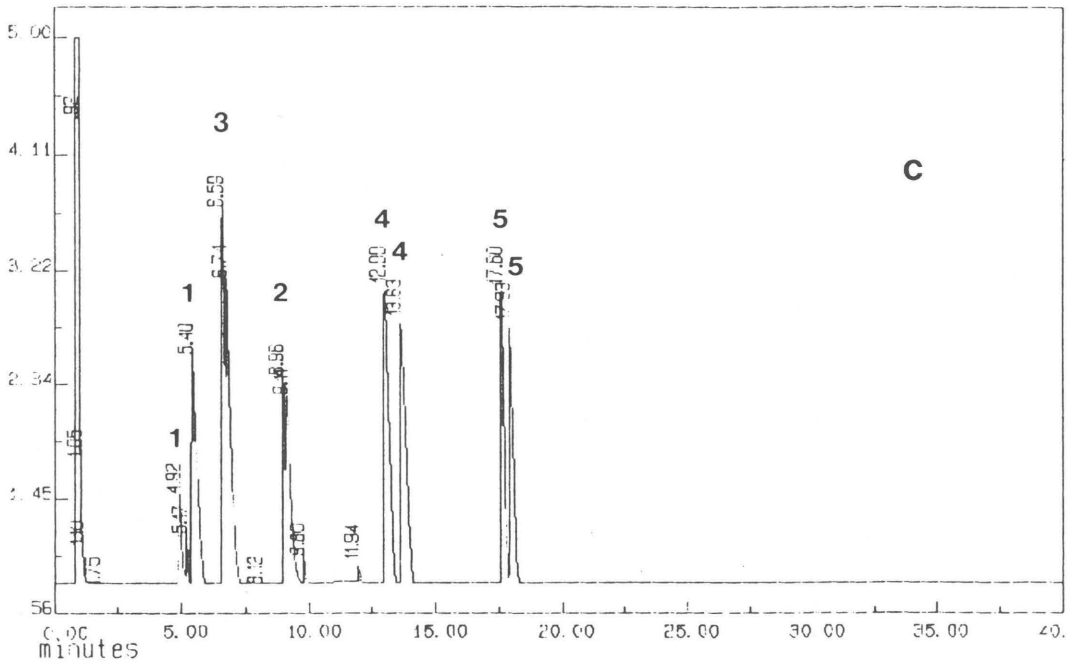


Figure 3-5. Enantiomeric separation of 2,3-butanediol (racemic + meso) (1), γ-valerolactone (2), 1-phenylethylamine (3), 1-phenylethanol (4) and 2-ethylhexanoic acid (5) on a 25 m x 0.3 mm i.d. glass open tubular column, coated with 10% (w/w) heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin in (a) OV-1701, (b) OV-1701-OH and © PS-086. Temperature program:70°C to 190° C at 3°C.min^{-1}.

3.3 Discussion

Grob [36] developed a test mixture to evaluate the overall performance of capillary columns coated with liquid stationary phases. The 'Grob test mixture' determines the character of the liquid phase in one run. Some components verify the acid/base behaviour, while other compounds will show tailing peaks and reduced peak heights if the phase has active (non-inert) characteristics. These critical components form an integral part of the test mixture for quality control of open tubular columns.

Since the Grob test mixture contains only two enantiomers (2-ethylhexanoic acid) additional testing is required for the characterization of chiral columns. The Schurig test mixture for a superior evaluation of the inertness of the cyclodextin column [71] contains meso and racemic underivatized diol, a lactone, an underivatized amine, an underivatized alcohol and a free carboxylic acid.

The analytical separation of enantiomers by capillary gas chromatography on chiral stationary phases has been reviewed by Schurig [32].
Even if, to the best of my knowledge, an entirely satisfactory theoretical explanation of the mechanisms of GC separations with derivatived cyclodextrins which interprets a racemate resolution has yet to be found. In chiral gas chromatography, the stationary phase itself is an optically active compound. Diastereomeric interactions with solute enantiomers are responsible for differences in retention. With regard to the nature of the host-guest interaction in CDs, a number of different contributions have been considered, described by Schurig and Nowotny [16]

1. Steric fit by conformational change of the guest molecule and/or of the CD molecule (induced fit) during the molecular inclusion process,
2. Hydrogen bonding,
3. Van der Waals interactions (London dispersion forces and dipole-induced-dipole interactions),
4. Hydrophobic interactions,
5. Dipole-dipole interactions,
6. Charge-transfer interactions,
7. Electrostatic interactions,
8. Release of " high-enthalpy" water molecules from the CD cavity,
9. Release of solvent molecules from the CD cavity with a gain in entropy,
10. Relief of the ring strain of the macrocycle.

The two most import aspects of the separation mechanism of cyclodextrin columns are the cyclodextrin cavity size available for analyte inclusion and analyte bonding to the cyclodextrin methoxy groups. Generally the hydrophobic portion of the analyte enters the cyclodextrin cavity while analyte polar groups bond to the cyclodextrin methoxy groups (Figure 3-6).

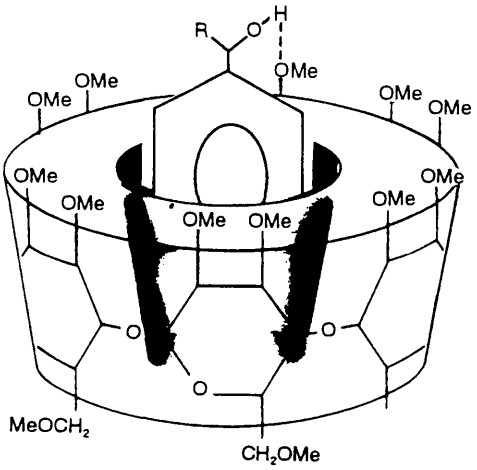


Figure 3-6. Analyte Inclusion into Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin Cavity and Hydrogen bonding

Figure 3-2 to Figure 3-4 compare the performance of columns coated with 0.25 µm films of moderately polar phases (OV-1701, OV-1701-OH and PS-086) with that of the chiral modified columns. The chiral columns containing 10%(m/m) heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin exhibit a considerable increase in polarity. This is demonstrated by the peak shifts of 1-octanol, 2,3-butanediol, 2.6-dimethylaniline, 2,6-dimethylphenol and 2-ethylhexanoic acid. Baseline resolution of the enantiomeric pair 2-ethylhexanoic acid was obtained.

Figure 3-5 demonstrates that the column coated with Chiral OV-1701 shows the most efficient enantioselective separation of the chiral test mixture. Very good separation of the enantiomeric pairs of 2,3-butanediol (racemic and meso), γ-valerolactone, 1-phenylethylamine were obtained. These enantiomeric pairs are only partially resolved on the Chiral OV-1701-OH and Chiral PS-086 stationary phases. And they display less polarity and less enantioselective separation of the chiral mixture. 1-phenylethylamine is even eluted earlier on the Chiral PS-086 phase than on the Chiral OV-1701-OH phase.

Bicchi [55] evaluated the effect of the ratio of CD derivative to polysiloxane, column conditioning, the volatility of the racemates, column length, film thickness and analysis temperature on column performance. According to Mosandl *et al.* [50], the enantioselectivity of heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin is influenced by the polysiloxane used as solvent. This is perhaps a result of the polarity of the solvent and interaction between the cyclodextrin and function groups of the polysiloxane (*e.g.* cyanopropyl in OV-1701).

Grob *et al.* [72] analyzed a homologous series of α-lactone (C_9~C_{12}) on a 25 m × 0.23 mm i.d. glass column, coated with 2,6 di-O-pentyl-3-acetyl-α-cyclodextrin and OV-1701-OH (1:2) to form a film of 0.2 µm thickness, at various temperature programming rates. Grob described the influence of the average linear velocity of the carrier gas on enantiomeric separation and stated that higher resolution can be obtained by adopting much higher values of linear velocity (50 and 100 $cm.sec^{-1}$) than near those suggested by the Van Deemter equation (38 $cm.sec^{-1}$). In our opinion, the associated lower elution temperatures are probably the reason for the increased resolution, rather than flow rate itself.

The Chiral OV-1701 stationary phase gave the best enantiomeric separation of the chiral test mixture. However, this phase does not have the temperature stability to allow the gas phase introduction of the modifiers of low volatility at elevated temperatures. The high temperature stability of PS-086 make this the liquid phase of choice for dynamic modification of the stationary phase by the vapour of heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin. This will be attempted in the next chapter.

CHAPTER 4

DYNAMIC MODIFICATION OF GLASS OPEN TUBULAR COLUMNS WITH COMPOUNDS OF LOW VOLATILITY

A general method for the temporary modification of stationary phase selectivity is presented in this chapter. This is achieved by injection of a modifier of low volatility, as a large vapour plug onto a capillary column at an elevated temperature. At subsequent much lower temperatures, the elution of the modifier is so slow as to enable the normal use of the FID, with the column showing altered selectivity. This approach is reversible and more versatile than others where modifiers of low volatility are either diluted in stationary phases before coating or chemically bonded to a polysiloxane.

A dimethylsilicone phase(SE-30) was modified with N-lauroyl-L-valine-t-butylamide (used as the SP-300 stationary phase from Supelco Inc), a diamide known for its high enantiomeric selectivity towards derivitized amino acids [19~21] and which has been successfully coupled to a silicone backbone in the commercial Chirasil-Val® (Alltech) capillary column. The SE-30 column was also modified with cobalt (II) acetylacetonate for complexation chromatography.

A polyethylene glycol column was dynamically modified with glycerol and diglycerol to increase the polarity of the stationary phase. The choice of this system is based on the often lamented lack of very high polarity columns in capillary GC , especially those that offer selectivity on the basis of hydrogen bonding interactions with analytes. Polar phases are difficult to coat on smooth glass or fused silica due to their tendency to form droplets which results in the loss of column efficiency.

Finally an OH-terminated dimethylsilicone column (PS-086) was dynamically modified with heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin for enantiomeric

separation. The derivatized β-cyclodextrin is commercially available, mixed with polysiloxane (HYDRODEX® β-PM FROM Macherey-Nagel) or chemically bonded to a polysiloxane backbone (Chirasil-Dex).

All of the modifiers can be removed at high temperature to return the original elution characteristics of the column.

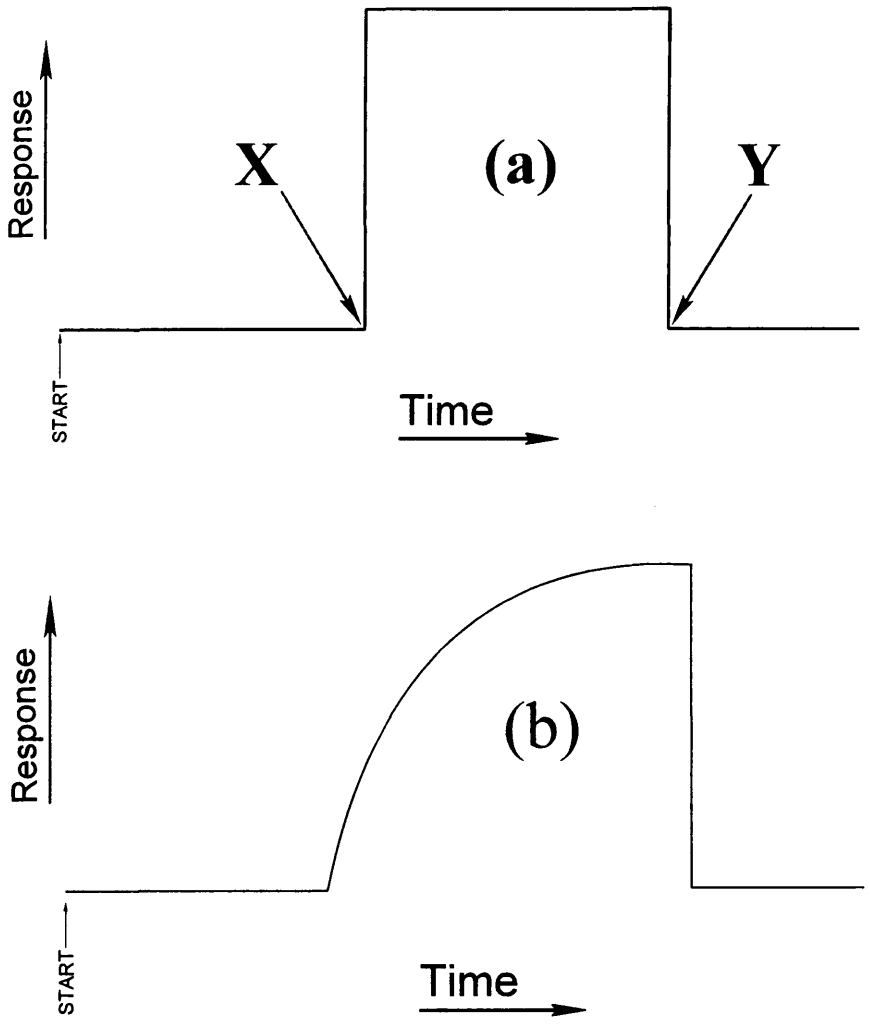


Figure 4-1. Elution of vapour plug expected under (a) linear and (b) non-linear (concentration overloading) chromatographic conditions.

In Figure 4-1, *if 2X = Y for a set of isothermal operating conditions, then X = retention time = one column volume. At time X after the peak appears, one column volume has gone through the column and therefore the column should be filled homogenously with a vapour modifier.* In practice the block pulse shape of the peak is fronting [73](Figure 4-1, *b*) thus a larger amount (Y>2X) of the modifier of low volatility needs to be introduced. To achieve high percentage loading using the overloading phenomenon, an excess of the modifier is used to ensure that column modification occurs with the flat portion of the peak, simulating the block pulse shape (Figure 4-1, *b*).

For the uniform injection of a long vapour plug, a high capacity loading insert was designed to prevent the liquid modifier from moving into the column and to simultaneously allow the carrier gas to equilibrate with modifier vapour at the preset injector temperature.

4.1 Experimental

4.1.1 Columns

Trying to reach our initial goal of loading vapour phase of permethylated β-cyclodextrin onto a column, it was clear that we should use a high temperature, low bleed column. To this end, a hydroxy-terminated dimethylsilicone (PS-086, Petrarch Systems, Inc., Bristo, PA 19007, USA) was coated into a leached and deactivated borosilicate glass capillary of 25m length and 0.3 mm inner diameter. The procedure followed (see Chapter 3) was taken from the publication of Blum [41]; the film thickness was 0.25 µm. An identical column was prepared in Chapter 3 with 10%(m/m) permethylated β-cyclodextrin in PS-086 as stationary phase for comparison purposes.

An SE-30 column of the same dimensions as above was prepared according to the method by Grob [36]. This column was selected for the loading with N-lauroyl-L-valine-*t*-butylamide and cobalt (II) acetylacetonate, as the introduction of these selectors do not require very high temperature. The result of the modification of N-lauroyl-L-valine-*t*-butylamide could be directly compared with the performance of the commercial Chirasil-Val® column.

A moderately polar column was chosed to facilitate glycerol and diglycerol solutions respectively into existing stationary phase. Therefore, chemically bonded Carbowax 20M glass capillary column, of the same dimensions as above, was prepared by the method of Grob [36] to achieve a film thickness of 0.25 µm.

4.1.2 Gas chromatographic equipment

A Varian 3700 gas chromatograph was used for the dynamic loading procedure and a Carlo Erba Fractovap Series 4200 instrument was used for the evaluation of the modified columns, both fitted with a conventional split/splitless injector and a flame ionization detector. Although the general procedure is designed for gas phase coating of the columns followed by injection of the sample on the same chromatograph, we found it convenient to use a separate GC for each step during this research project. Hydrogen was used as carrier gas throughout, at a linear flow rate of 50 $cm.s^{-1}$.

The Hewlett-Packard ChemStation (Rev. A.03.03.) was used to record most chromatograms. The software is designed to run on IBM compatible personal computers under the enhanced mode of the MS-Dos®, Microsoft® windows™ operating environment. A number of chromatograms were recorded on the much older Hewlett-Packard 3350 data system.

The only non-standard equipment used was a loading insert for the injector of the Varian GC, designed to supply a homogeneous plug of modifier vapour for the dynamic coating procedure. This part is shown in Figure 4-2 and described in 4.1.3 below.

4.1.3 High capacity loading system

This system consists of a glass loading insert (Figure 4-2) constructed by micro glass blowing techniques from a 100 µl capillary pipette, 64 mm x 2 mm o. d., containing a woven glass sleeve, of the type used for high temperature insulation of electrical conductors.

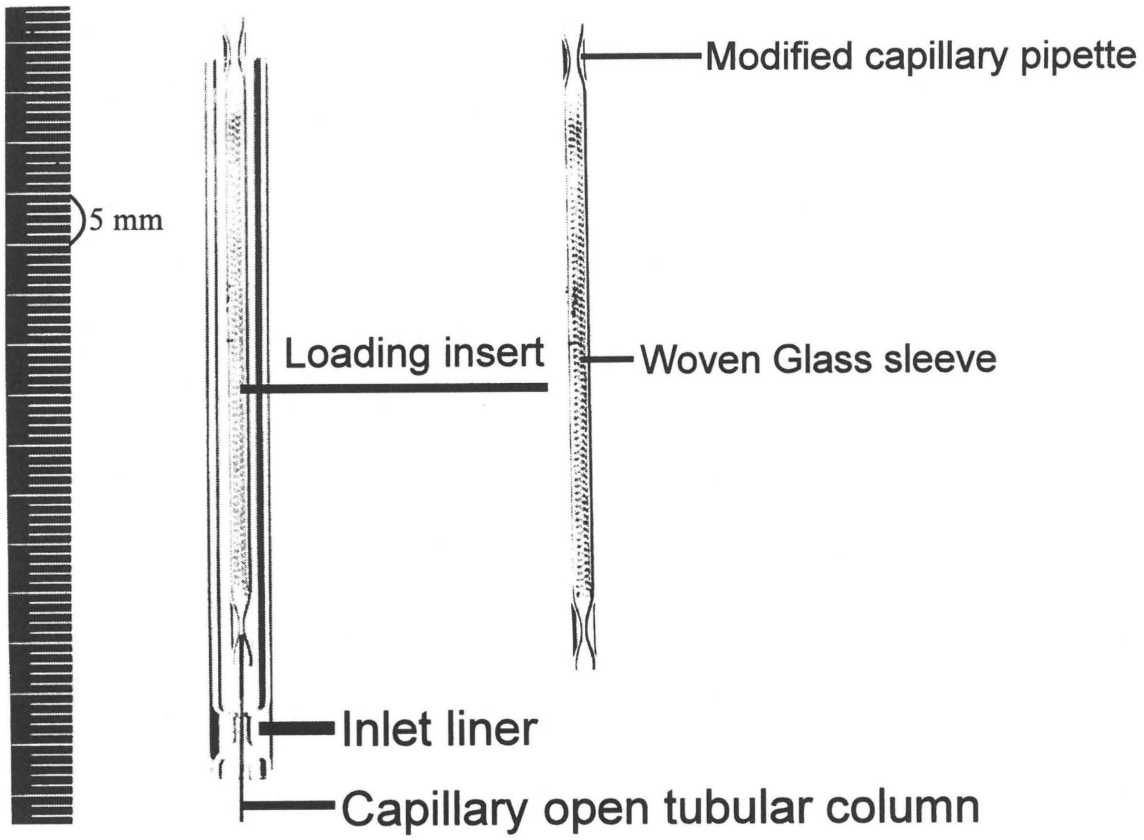


Figure 4-2. Loading insert

The carefully shaped conical section at the bottom serves as a press-fit coupling for the fused silica column end protruding into the inlet liner (Figure 4-2). The constricted section at the top only serves to guide the needle of a syringe into the glass sleeve during sample deposition and reduces the risk of back diffusion of the sample into the GC inlet at the low volume flow rates employed with the capillary column.

The function of the woven glass sleeve is to spread and keep in place the liquid modifier by capillary action, ensuring that a large surface area is made available for vapour equilibration at the set temperature of the GC-inlet. It simultaneously prevents the modifier from entering the column in the liquid form, which would result in non-uniform deposition along the length of the column and on local areas of stationary phase thickness that are unacceptable in terms of column separation efficiency.

The loading insert was deactivated with dimethyldichlorosilane (Appendix 3) in an attempt to reduce adsorption and catalytic activity at elevated temperatures.

4.1.4 Dynamic modification and selectivity evaluation

In order to prevent re-condensation of a pure modifier in the column and to achieve the highest practical vapour concentration, the inlet is held at a temperature just below that of the column oven. The column temperature is chosen to obtain a reasonable retention time of the modifier and thereby limit the time required for the dynamic vapour loading process. Ideally, the correct amount of the modifier is placed on the insert to provide a constant concentration stream of vapour only until the time when the front of the plug enters the detector. In practise, it is easier to use a slightly larger amount of

the modifier in the insert and then to cool down the inlet, together with the column, as soon as the vapour plug reaches the detector, thereby completing the loading process. During the cooling down phase, care is taken to prevent the oven from cooling faster than the slow cooling inlet in order to prevent re-condensation of the modifier vapour still emitting from the inlet. The insert is then removed from the inlet.

After loading it with up to 30 microliters (the maximum volume that the system will hold by capillary action) of a solution containing the modifier in a concentration of ca. 0.2 $mg.\mu l^{-1}$, the insert is lowered into the inverted quartz inlet liner with a tweezer (Figure 4-2, page 4-5), finally resting with its press-fit coupling on the fused silica end-section of the glass capillary column. This allows on-column transfer of the modifier to the column, even when the splitter valve is open - a precaution against possible back-diffusion of the modifier with resulting contamination of the upstream inlet area under low carrier flow rates. The septum nut is screwed back on and the carrier gas supply started while the inlet and column are still cool, preventing air contact with modifier and stationary phase at the later higher evaporation and column temperatures. The inlet and column are then heated up to the required temperature, allowing sufficient time for the solvent to evaporate below its boiling point. The column heats up rapidly but the loading process really only starts when the preset temperature for modifier evaporation in the inlet is reached. The whole process is monitored with the FID at the end of the column.

To establish a dynamic loading procedure for a new combination of modifier and stationary phase thus involves finding (a) a convenient temperature for elution of the modifier in not too long a time, say between 10 and 60 min, (b)

the minimum amount of modifier required to provide a long enough vapour plug length - at a concentration defined by the (inlet) saturation temperature 5°C below that of the column temperature - to cover the length of the column.

4.1.4.1 Glycerol modified Carbowax 20M

After deciding on a column temperature of 205°C - based on the retention time of glycerol - and an inlet temperature of 200°C, tests were done to establish the amount of glycerol required to uniformly coat the column. This was done by injecting 1 µl, 3 µl, 6 µl and 15 µl of a 0.2 $mg.\mu l^{-1}$ solution of glycerol in ethanol onto the loading insert and recording the FID trace. Ten minutes were allowed for the solvent to evaporate and pass through the column at room temperature before switching on the inlet and column heaters (Figure 4-3).

Dynamic modification was then done with 15 µl of the same solution, starting the cooling phase at 28.5 min (Figure 4-4, *a*). In order to calculate the percentage composition of the dynamically modified column, the insert with residual modifier was first removed and then the column was heated to 205°C in order to record the area of the glycerol peak eluting from the column (Figure 4-4, *b*). The residual amount of modifier on the loading insert was then determined by monitoring the glycerol peak arising from its desorption under the same conditions (Figure 4-4, *c*).

The dynamic modification procedure was then repeated before the column was evaluated with test mixture I: methanol, ethanol, n-octane, n-nonane, n-decane and n-undecane in hexane (Figure 4-9); and test mixture II: 1-hexanol, n-undecane, n-dodecane, n-tricdecane, n-tetradecane in methanol (Figure 4-10).

The GC conditions for tests I and II were; Inlet and detector at 200°C, linear flow rate of hydrogen carrier: 50 $cm.s^{-1}$, splitter flow: 40 $cm^3.min^{-1}$, column: 50°C isothermal.

4.1.4.2 Diglycerol modified Carbowax 20M

Dynamic modification was with 15 µl of a 0.29 $mg.\mu l^{-1}$ solution of the diglycerol in methanol. The column and inlet temperatures were 205°C and 200°C respectively. A period of 10 minutes was allowed for the solvent to evaporate before the inlet and column were switched on (Figure 4-5).

4.1.4.3 N-lauroyl-L-valine-t-butylamide modified SE-30

Dynamic modification was with 30 µl of a 0.22 $mg.\mu l^{-1}$ solution of the diamide in chloroform. The column and inlet temperatures were 295°C and 290°C respectively. A period of 10 minutes was allowed for the solvent to evaporate before the inlet and column were switched on (Figure 4-6).

The modified and unmodified columns were evaluated with test mixture III (1-decanol, n-undecane, n-dodecane, n-tridecane, n-tetradecane in hexane) at an isothermal oven temperature of 120°C (Figure 4-11).

The modification was also evaluated with the test mixture IV (Altech p.n. 19268) consisting of the TFA-isopropyl ester derivatives of amino acids; D,L-alanine, D,L-valine, D,L-leucine, D,L-aspartic acid, D,L-methionine. The temperature programme of 70°C to 100°C at $3°C.min^{-1}$ was used (Figure 4-12).

4.1.4.4 Cobalt (II) acetylacetonate modified SE-30

Dynamic modification was with 15 µl and 30 µl of a 0.29 $mg.\mu l^{-1}$ solution of the cobalt (II) acetylacetonate in tetrahydrofuran . The column and inlet temperatures were 295°C and 290°C respectively. A period of 10 min was allowed for the solvent to evaporate before the inlet and column were switched on (Figure 4-7).

4.1.4.5 Permethylated β-cyclodextrin modified PS-086

A column loading temperature of 350°C was selected (Figure 8) as this temperature eluted the cyclodextrin from a normal split injection at 87 minutes, comparable with its elution reported by Schomburg *et al.* [18] on their shorter column with a thinner film. This temperature was a compromise in an attempt to minimize thermal decomposition that has been shown by thermoanalysis to start at 300°C in an inert atmosphere [59]. Tests with various inlet temperatures were performed.

Evaluation was by injection of (1R)-(+)- and (1S)-(-)-cis-pinane, comparing the resolution obtained between the dynamically modified (Figure 4-13) and 10% statically modified (Figure 4-14) columns.

4.2 Results and Discussion

4.2.1 Monitoring the dynamic modification process

The FID traces in Figure 4-3 clearly show the expected overloaded peak shape (see e.g. [73]) of the increasing size of the vapour plug of glycerol. The ever increasing size of the solvent peak is also visible. The fronting peak shape expected from the non-linear partition isotherm is superimposed on the fronting shape expected from the slowly increasing temperature of the injector.

From about 26 min hardly any change in the concentration eluting from the column is observed (flat portion of peak), indicating that a dynamic equilibrium has been established in this region, with the same amount of modifier exiting (and entering) the column per time unit .

The FID trace in the chromatogram *a* of Figure 4-4 represents the dynamic loading process, with cooling commencing at 28.5 min. The chromatograms *b* and *c* were recorded during the unloading of the column and insert respectively. From the ratio of the corresponding peak areas and the amount of modifier loaded onto the insert, it can be calculated that the loading percentage of 4.7%(m/m) glycerol/Carbowax 20M was achieved.

Figure 4-5 is the FID trace obtained for the dynamic modification of Carbowax with diglycerol. It is clear from this trace that there is no diglycerol passing through the column. This is most likely due to polymerization of the concentrated diglycerol solution. Diglycerol can not be coated onto a open tubular column by this modification procedure.

The curves obtained for the coating of the diamide selector on SE-30 looks virtually identical to the one obtained in Figure 4-4, *a* (Figure 4-6). Considering the mass of the diamide loaded onto the insert, the mass of the SE-30 phase on the column and the appropriate three peak areas, a modifier mass percentage of 7.1%(m/m) could be calculated.

The use of cobalt (II) acetylacetonoate for the dynamic modification on SE-30 was not succesful. According to Figure 4-7 less cobalt (II) acetylacetonate passed through the column than expected and the cobalt (II) acetylacetonate shows peak tailing. After the GC run, a metal deposit was found at the front

end of the column. This deposit may be decomposition products of the concentrated cobalt (II) acetylacetonate solution.

The loading curves for the cyclodextrin selector were not as easy to interpret and consistently indicated thermal degradation of this compound at the high column and injector temperatures (Figure 4-8). Not only were more volatile compounds indicated in the curves, but the inlet sleeve became very dark in colour, indicating condensation reactions were taking place under these conditions of high concentration and temperature. No equivalent calculation of the modifier mass percentage could be made due to the undefined peak shapes of the loading and unloading traces.

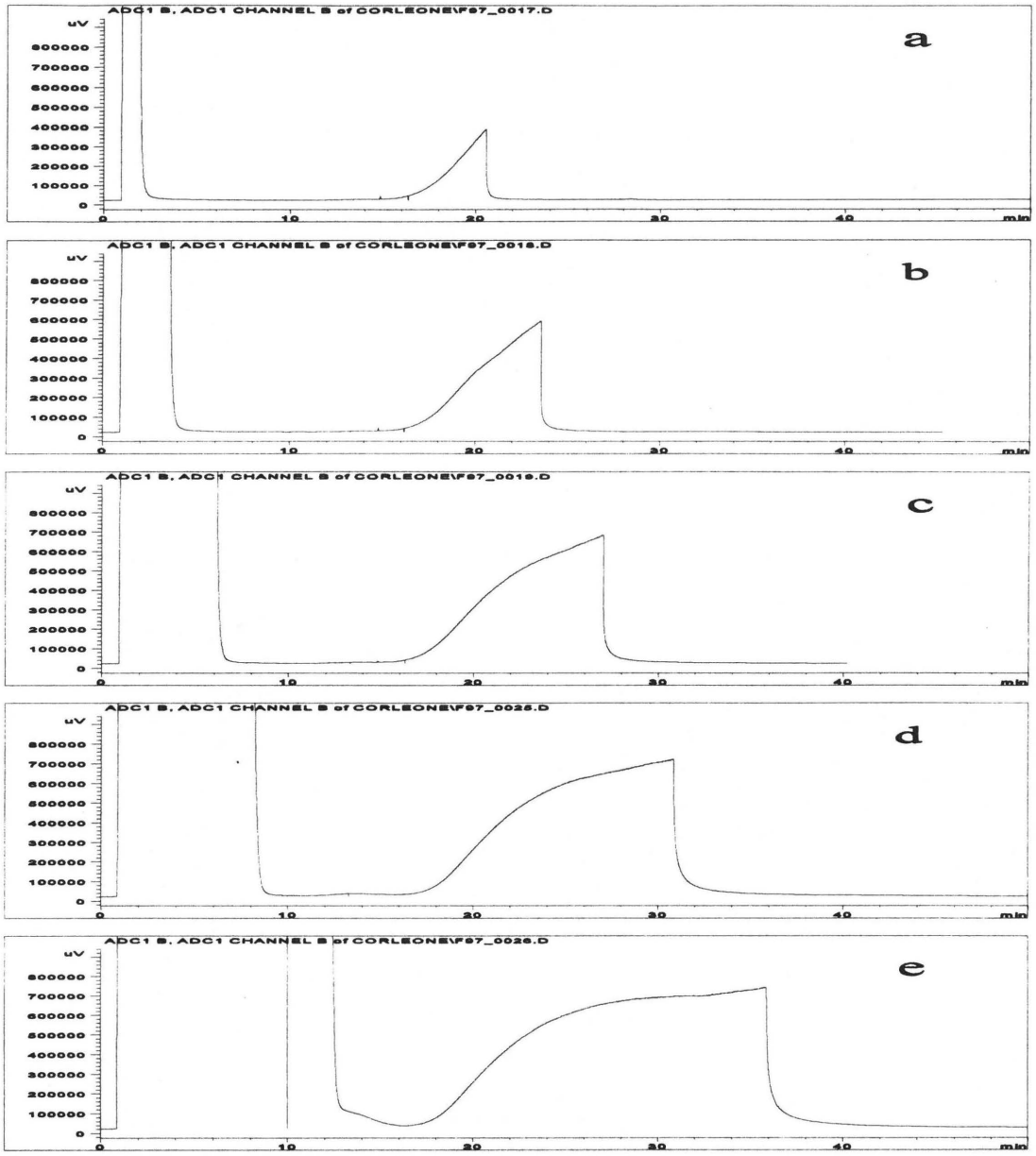


Figure 4-3. GC elution profiles of glycerol vapour plug. (a)1 µl, (b) 3 µl, (c) 6 µl, (d) 9 µl and (e) 15 µl of a (ca.) 0.19 mg.µl^{-1} solution of glycerol in ethanol were used under identical conditions.

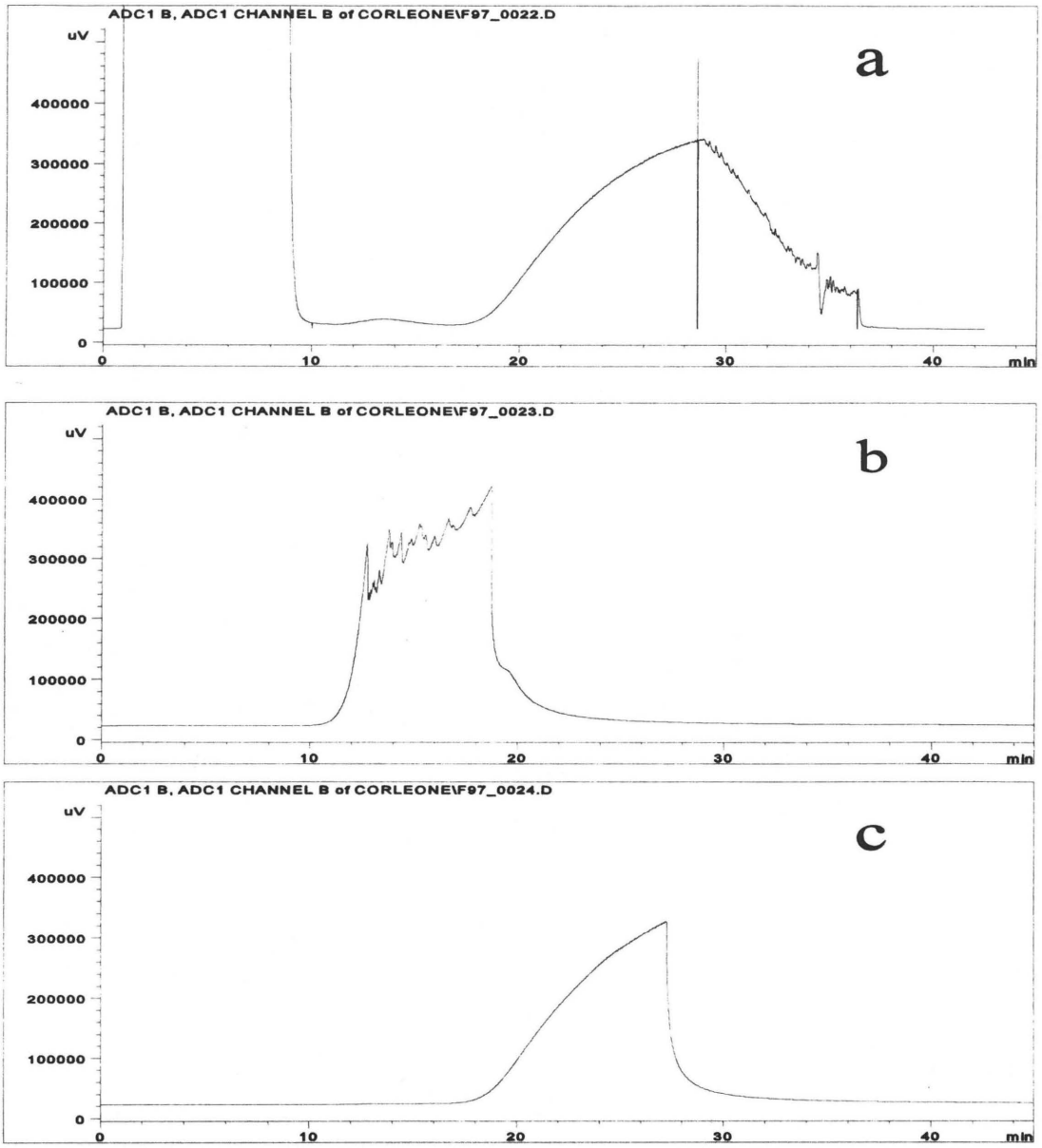


Figure 4-4. (a) Dynamic modification of a Carbowax 20M column by glycerol. (b) Thermal removal of glycerol from the stationary phase. (c) Thermal removal of glycerol from the loading insert.

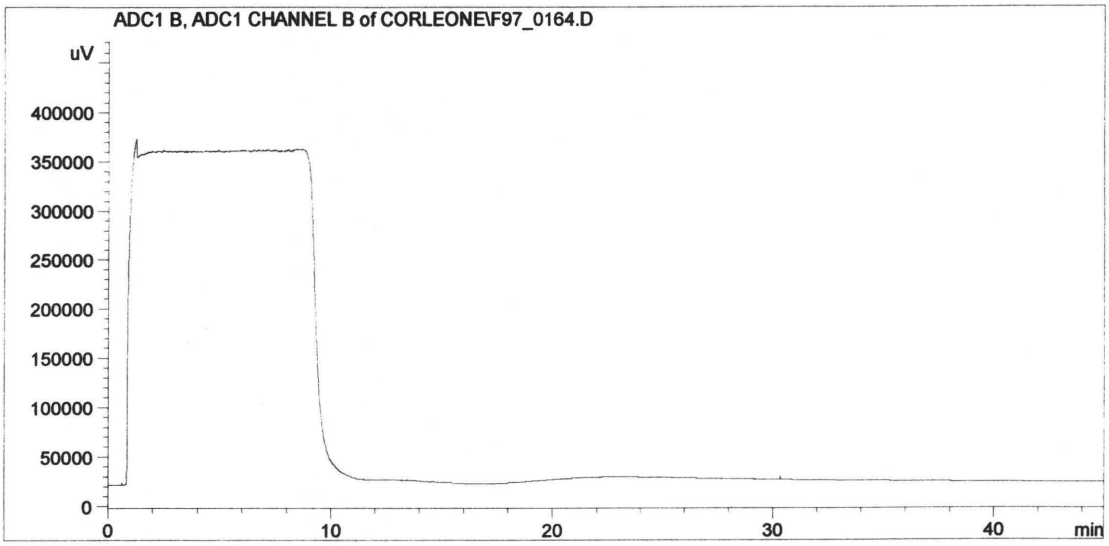


Figure 4-5. Dynamic modification of a Carbowax 20M column by diglycerol.

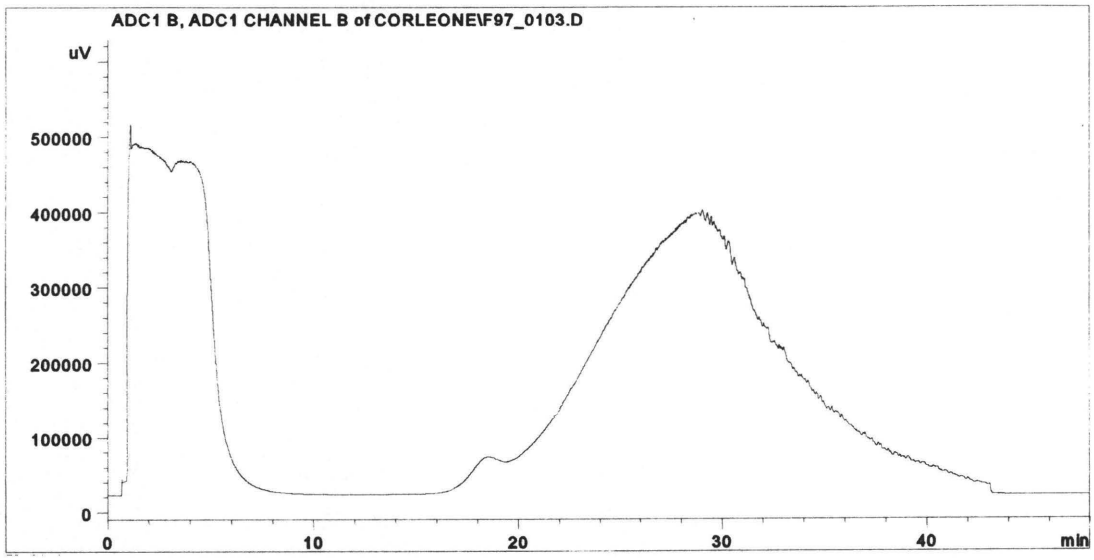


Figure 4-6. Dynamic modification of a SE-30 column by N-lauroyl-L-valine-*t*-butylamide.

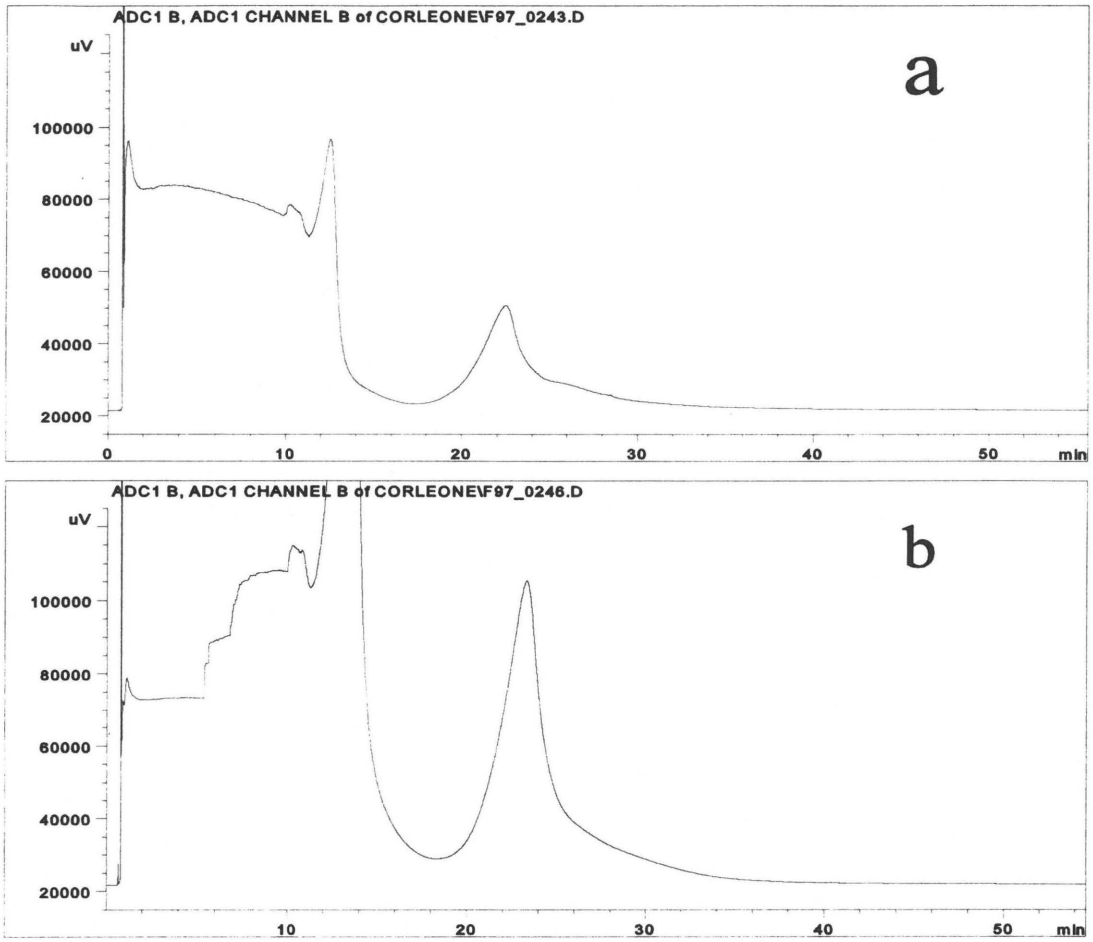


Figure 4-7. Dynamic modification of a SE-30 column by cobalt (II) acetylacetonate. (a) 15 µl ; (b) 30 µl of a (*ca.*) 0.29 mg.µl^{-1} solution of Cobalt (II) acetylacetonate in tetrahydrofuran.

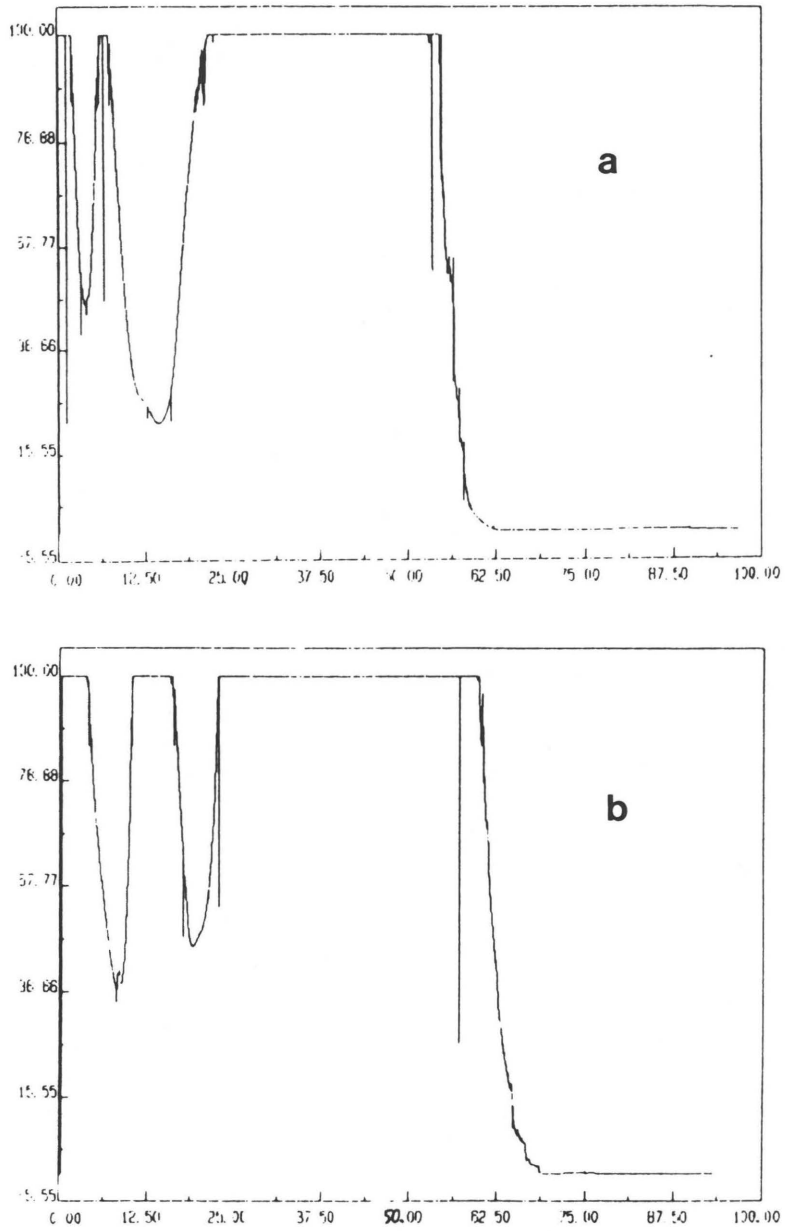


Figure 4-8. Dynamic modification of a PS-086 coumn by heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin: (a) injector set to 320°C; (b) injector set to 350°C.

4.2.2 Evaluation of glycerol modified Carbowax 20M column

From Figure 4-9 it is clear that the column polarity has increased appreciably - the retention times of alkanes have reduced, those of the alcohols have increased. A similar trend is visible in the chromatogram of the less volatile test mixture II (Figure 4-10), from which a Kovats retention index (see Chapter 2) for hexanol of 1335 and 1378 could be calculated for the unmodified and modified columns respectively ($\Delta I_{hexanol} = 43$).

Table 4-1. Capacity factors of the test mixture II on the same Carbowax 20M column without and with glycerol, under identical conditions.

Capacity factor, *k*	Without glycerol	With glycerol
Undecane	1.724	1.400
Decane	3.924	3.170
Tridecane	8.789	7.11
1-Hexanol	11.594	13.264
Tetradecane	19.5	15.79

The column efficiency remains unchanged, proving that no droplet formation occurs.

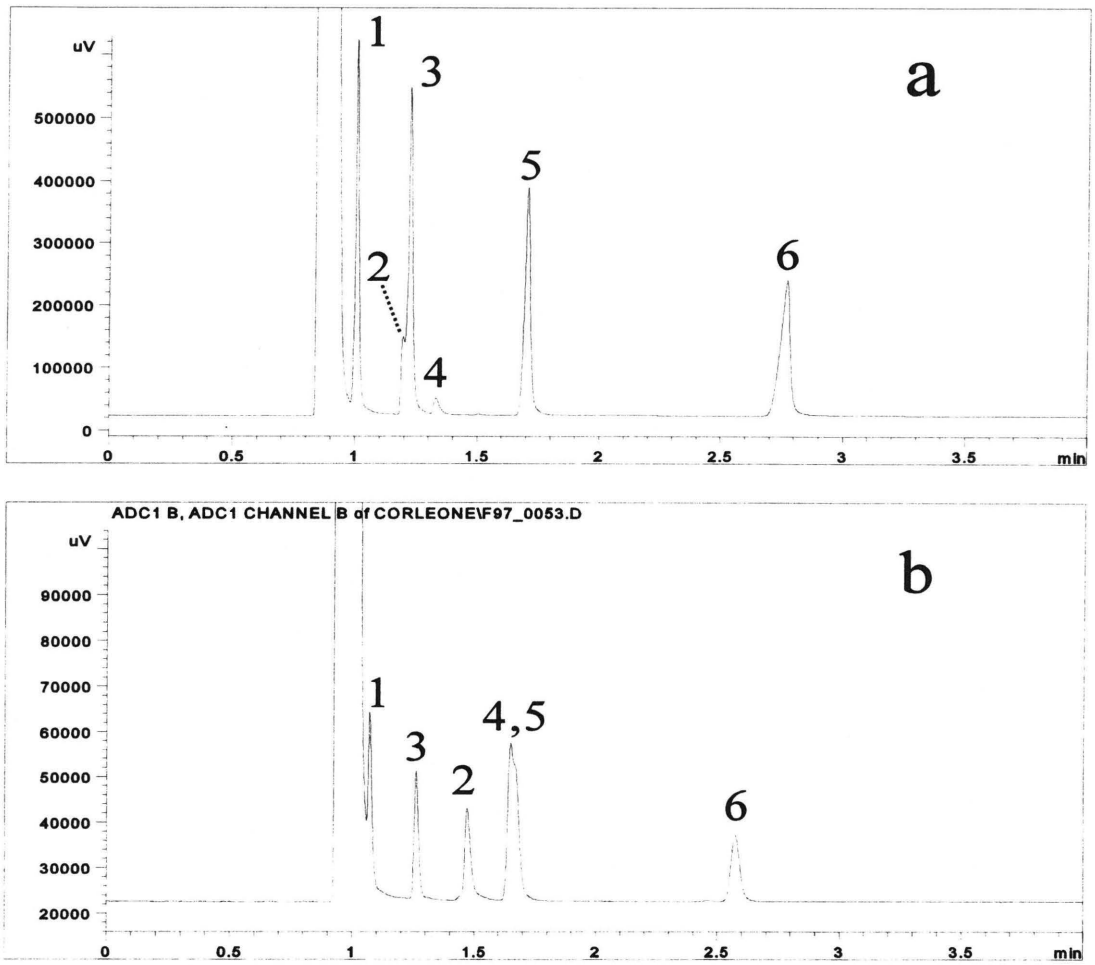


Figure 4-9. Separation of the test mixture I on a Carbowax 20M column (a) without and (b) with glycerol, under identical conditions. Column: 25 m × 0.3 mm i.d. at 50°C. Peaks: 1= octane; 2= methanol; 3= nonane; 4= ethanol; 5= decane; 6= undecane.

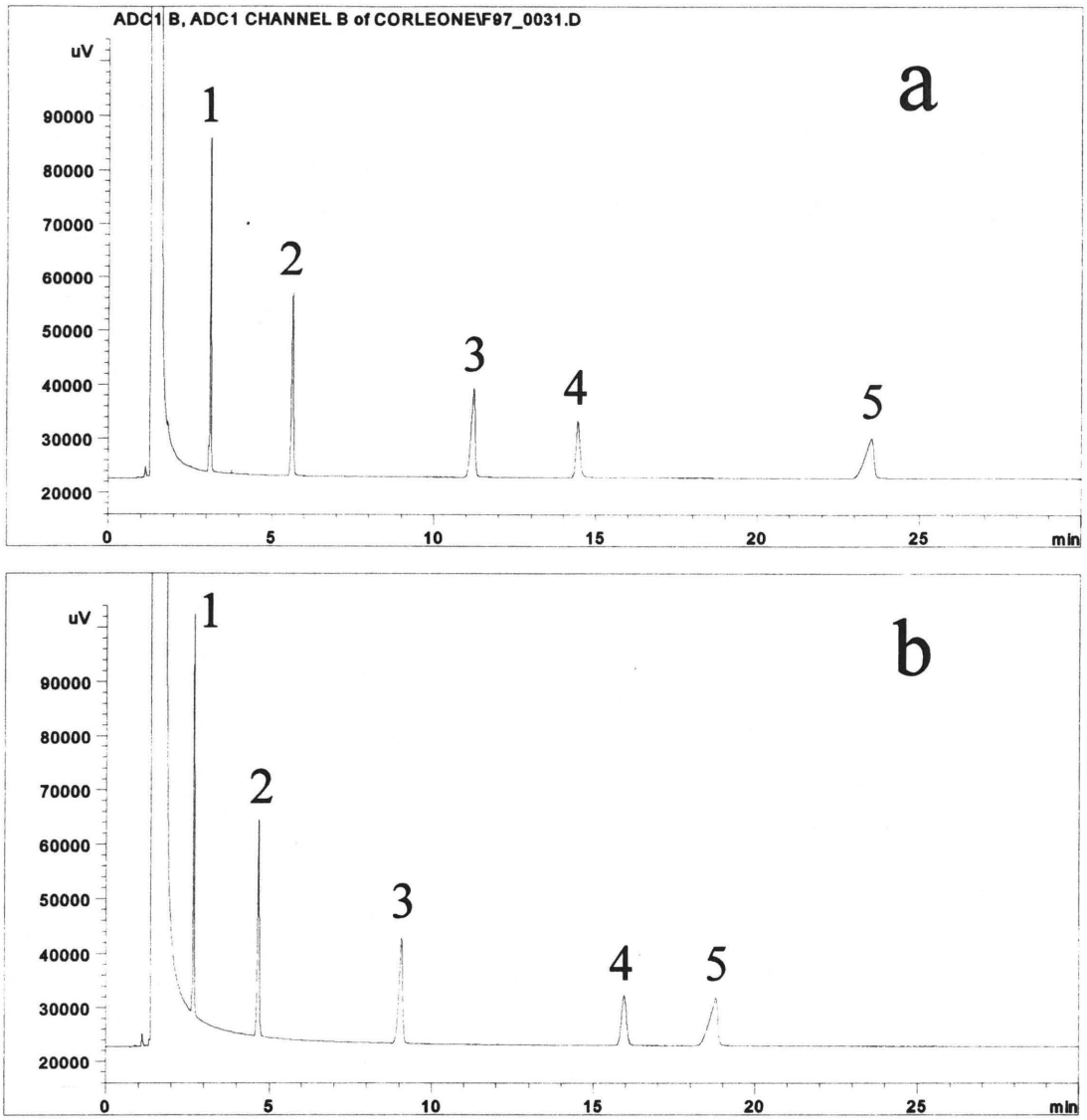


Figure 4-10. Separation of the test mixture II on a Carbowax 20M column (a) without and (b) with glycerol, under identical conditions. Column: 25 m × 0.3 mm i.d. at 50°C. Peaks: 1= undecane; 2= dodecane; 3= tridecane; 4= 1-hexanol; 5= tetradecane.

4.2.3 Evaluation of N-lauroyl-L-valine-t-butylamide modified SE-30 column

The chromatograms shown in Figure 4-11 indicate that again the polarity of the modified column has increased appreciably but in this case the alkanes also elute later. This alkane shift is typical of a less polar column, where the increased amount of stationary phase determines the shift in alkane retention, not the increase in polarity.

The chromatogram in Figure 4-12 clearly shows the power of the novel dynamic modification procedure. Chiral separation of all derivitized amino acids except aspartic acid could be achieved. The latter enantiomeric pair is only just separated on the Chirasil-Val® column as shown in the Alltech catalog. The concentration of the modifier in our case is clearly a lot less than that chemically bonded in the Chirasil-Val® column, as expected from the measured 7.1% (m/m) loading. It is significant that racemisation of the chiral moiety has not occurred at the high loading temperature of 290°C. (See Appendix 4 for a schematic presentation of the Chirasil-Val/N-lauroyl-L-valine-*t*-butylamide and stereoselective interaction)

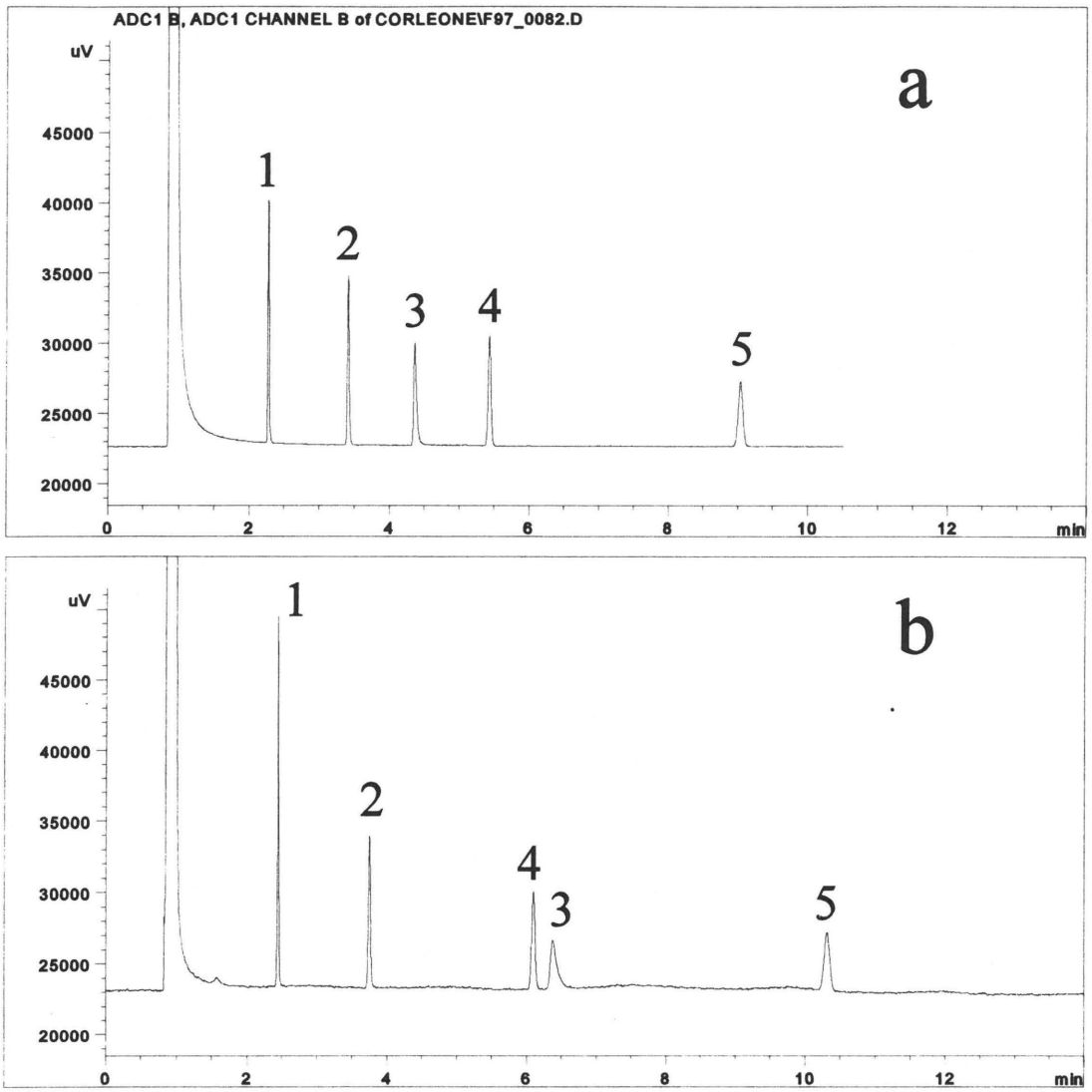


Figure 4-11. Separation of the test mixture III on a SE-30 column (a) without and (b) with N-lauroyl-L-valine-*t*-butylamide, under identical conditions. Column: 25 m × 0.3 mm i. d. at 120°C. Peaks: 1= undecane; 2= dodecane; 3= 1-decanol; 4= tridecane; 5= tetradecane.

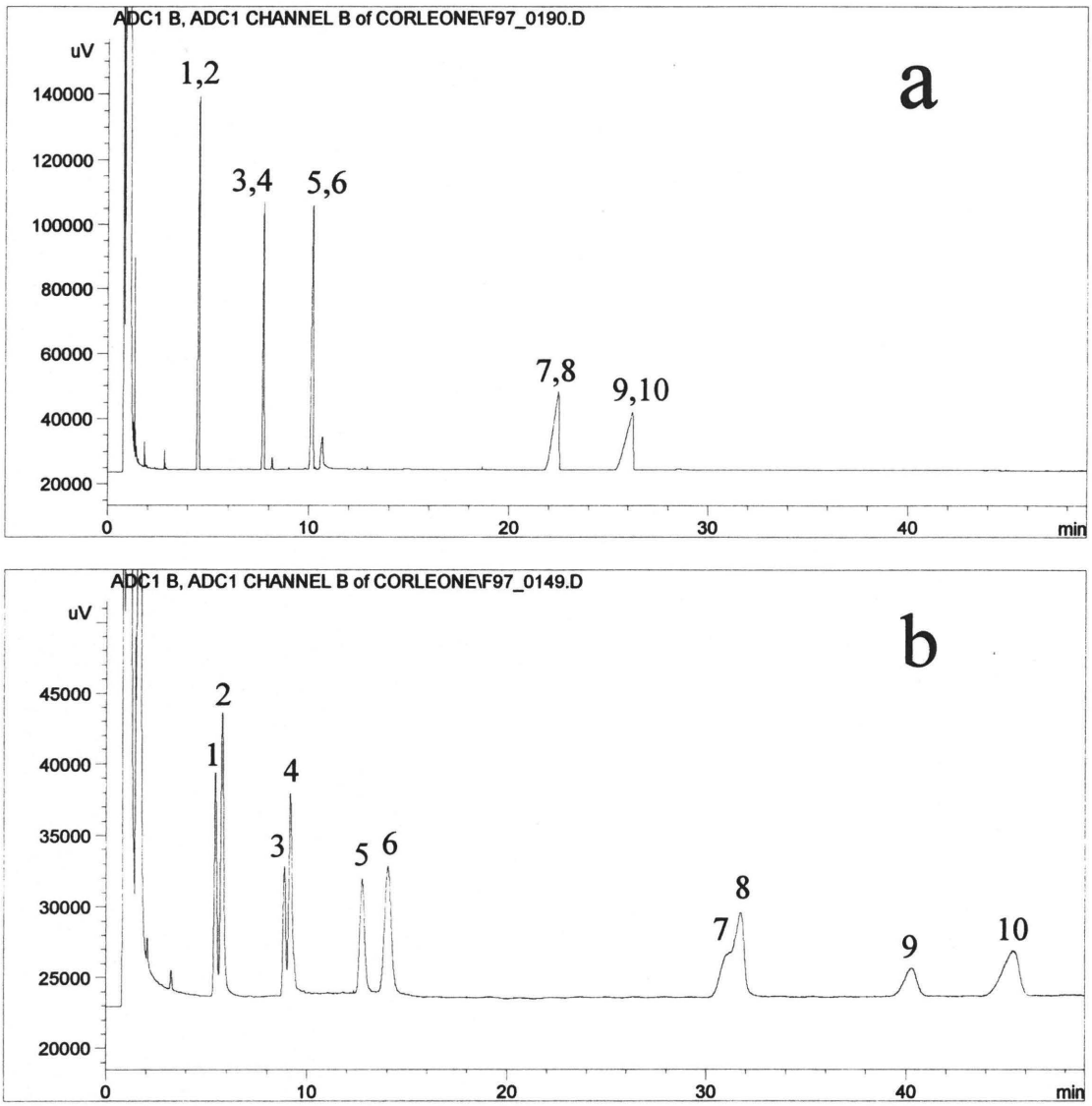


Figure 4-12. Separation of the test mixture IV (Alltech p.n. 19268) on a SE-30 column (a) without and (b) with N-lauroyl-L-valine-*t*-butylamide, under identical conditions. Column: 25 m × 0.3 mm i. d., temperature programme: 70°C to 100°C at 3°C.min^{-1}. Peak: 1,2= D,L-alanine; 3,4= D,L-valine; 5,6= D,L-leucine; 7,8= D,L-aspartic acid; 9,10= D,L-methionine.

4.2.4 Evaluation of permethylated β-cyclodextrin modified PS-086 column

Figure 4-13 shows only the incomplete separation obtained from the dynamically modified column of the cis-pinane enantiomeric pair at 40°C. The 10%(m/m) conventionally loaded column gave baseline separation of this pair, even at a temperature of 88°C, at about the same retention time (Figure 4-14). This is clear indication that insufficient dynamic loading was obtained due to thermal degradation of the modifier at the loading temperature of 350°C.

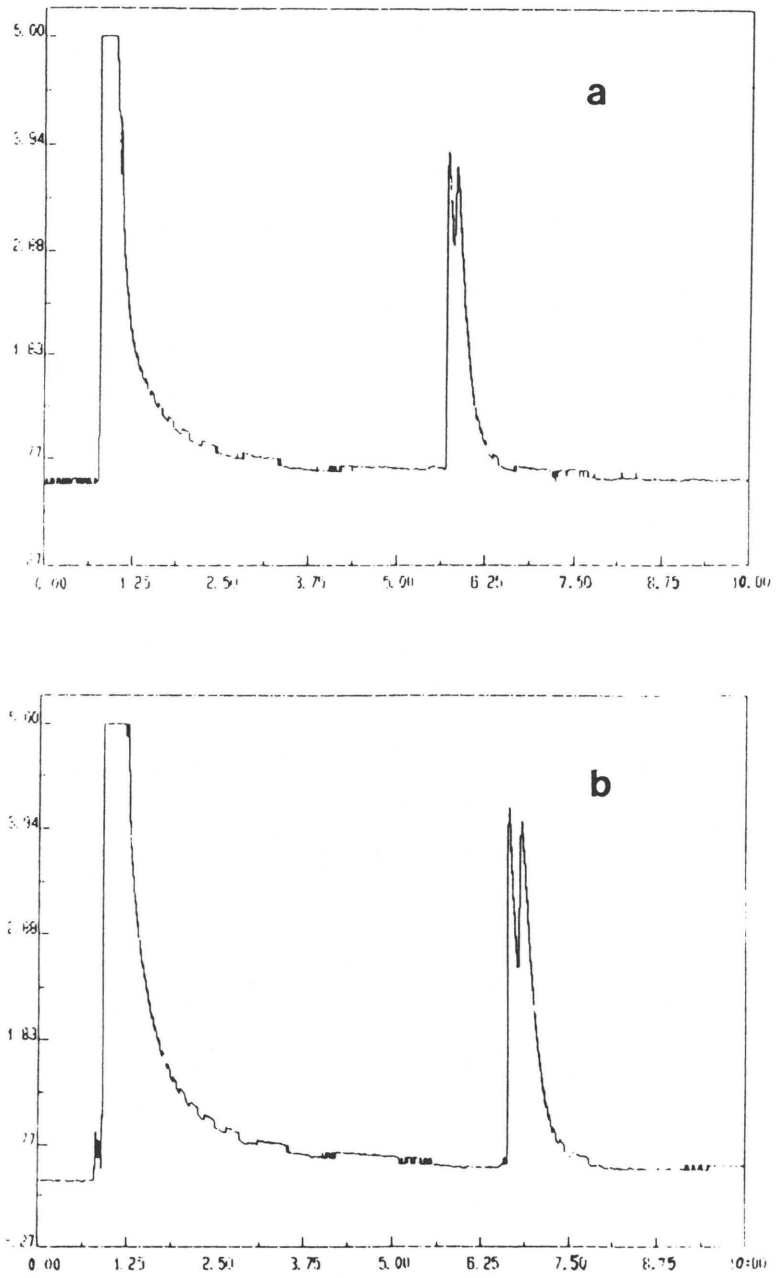


Figure 4-13. Separation of (1R)-(+)- and (1S)-(-)-cis-pinane, under identical conditions, column: 25 m × 0.3 mm i. d. at 40°C. The PS-086 column modified with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin at injector temperature of (a) 320°C (b) 350°C.

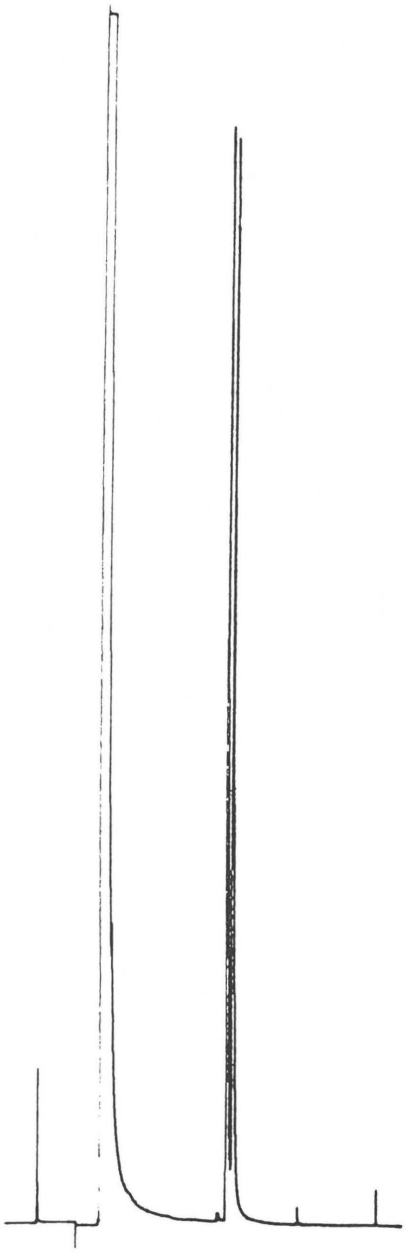


Figure 4-14. Separation of (1R)-(+)- and (1S)-(-)-cis-pinane on the PS-086 coated with 10%(m/m) heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin by the static coating. Column: 25 m × 0.3 mm i. d. at 40°C, temperature programme: 70°C to 120 at 3°C.min^{-1}.

CHAPTER 5

DYNAMIC MODIFICATION OF GLASS CARBOWAX 20 M COLUMNS WITH WATER VAPOUR

This chapter demonstrates the use of a volatile modifier that is continuously fed to the carrier gas stream. This form of dynamic modification relies on the prerequisite that compounds do not give a detector response. Continuous introduction of water as modifier is possible as it gives no response in the flame ionization detector. The water is dissolved in the Carbowax 20M to form a binary stationary phase.
Water vapour was added to the carrier gas, at levels approaching saturation, to study its effect on the retention properties of a polyethylene glycol coated capillary GC column. The retention is increased by forming strong hydrogen bonds between solutes and the binary phase. The choice of this modifier enables a comparison with the dynamic modification of the Carbowax column by glycerol, as presented in the previous chapter.

5.1 Experimental

5.1.1 Gas chromatographic equipment

A Carlo Erba Fractovap Series 4200 equipped with a FID, split/splitless injector and a Carbowax 20M column, 25m x 0.3 mm i. d. x 0.25 µm film thickness, was used for all the experiments. The column was prepared from borosilicate glass in our own laboratory according to the method by Grob [36] as described in Chapter 3. Hydrogen was used as carrier gas, at 50 $cm.s^{-1}$ linear flow rate, for all experiments. The splitter flow was permanently set to a value of 50 $cm^3.min^{-1}$. The only non-standard device used is the water vapour generator, described below.

5.1.2 Continuous introduction of water vapour

The system designed for the controlled addition of water vapour to the carrier gas can be explained with reference to Figure 5-1. The basic principle is to provide a controlled flow rate of carrier gas saturated with water vapour at the same isothermal temperature as the column oven. This stream is then mixed with the conventional, dry carrier gas stream coming from the column head pressure regulator. The water vapour concentration is controlled by setting the secondary hydrogen flow through the flow controller, FC, to any value lower than the splitter flow. As long as this condition is met, no re-condensation of water can take place in the column and no back diffusion of the water into the primary hydrogen supply line will occur. The closer the secondary flow rate is to the splitter (+column) flow rate, the higher the water vapour concentration.

The secondary flow reaches the injector from a 100ml Schott bottle inside the GC oven (Figure 5-2) via a heated transfer line (DANI SPT 37.50) kept at 120°C. (See Figure 5-3). A needle(Figure 5-4), connected to the transfer line, pierces the septum. This is removed briefly during injection of the sample.

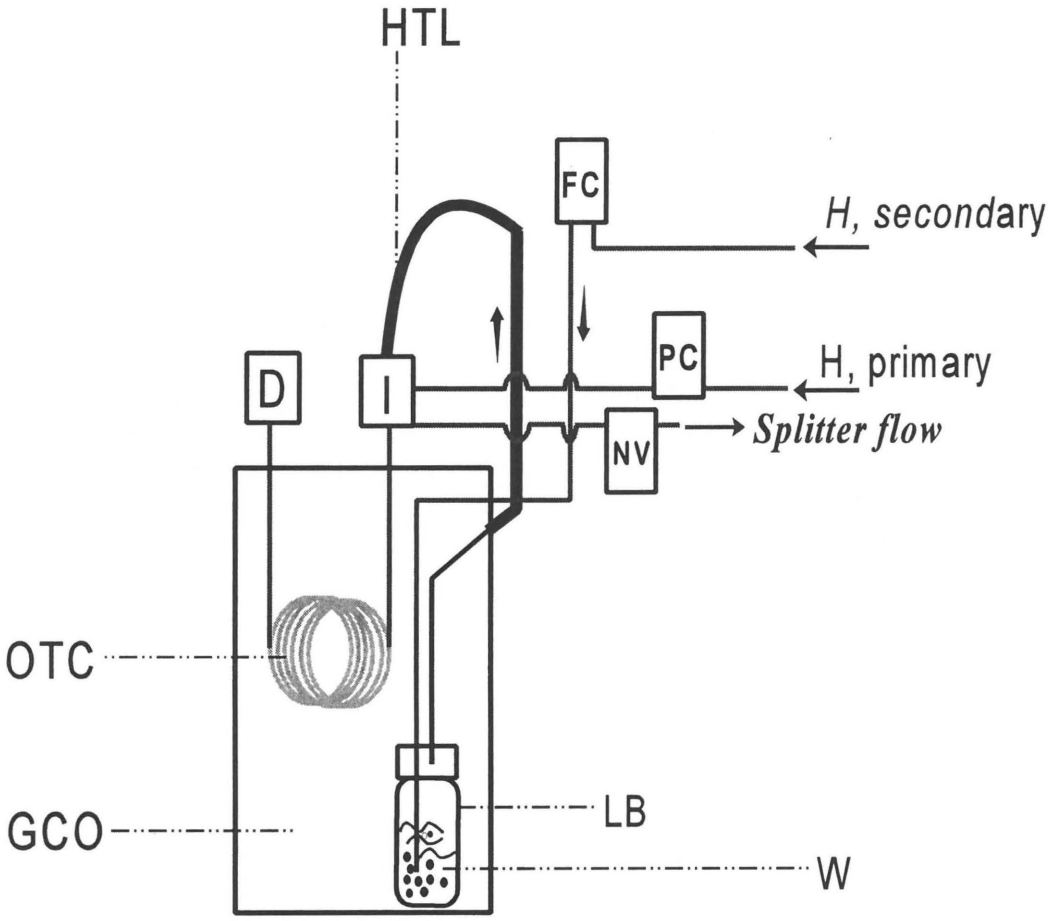


Figure 5-1. The system for the dynamic modification with continous introduction of water vapour.
HTL= Heated transfer line; FC= Flow controller; PC= Pressure controller; NV= Needle valve; H= Hydrogen; LB= Laboratory bottle; W= Water; GCO= GC oven; OTC= Open tubular column; D= Detector; I= Injector.

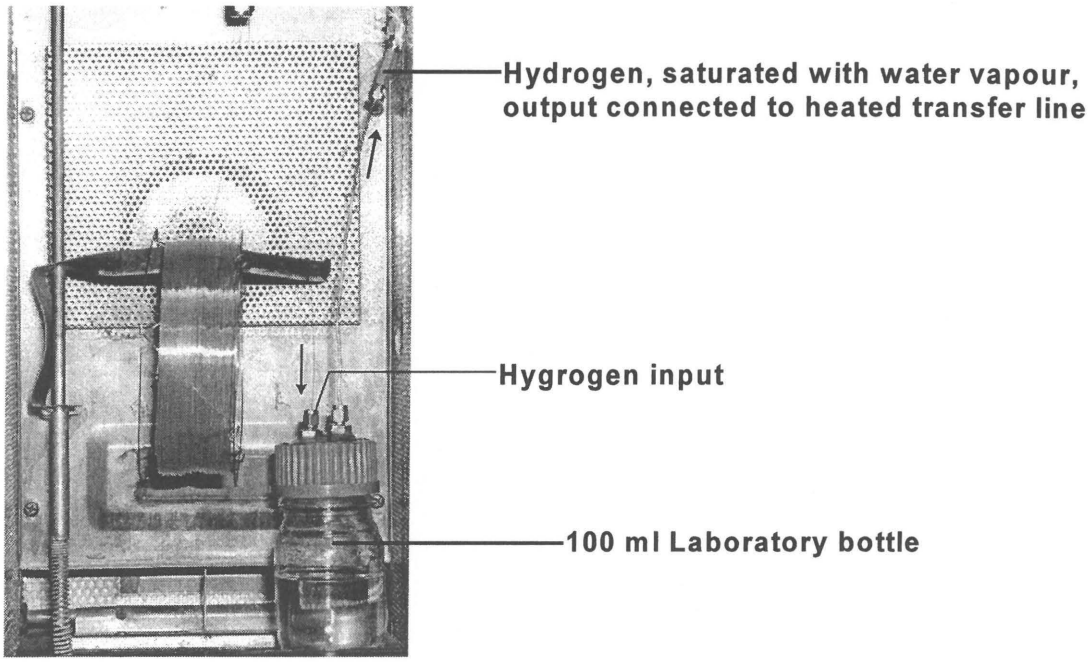


Figure 5-2. The facilities for the modification with continuous introduction of water vapour from the inside of the instrument

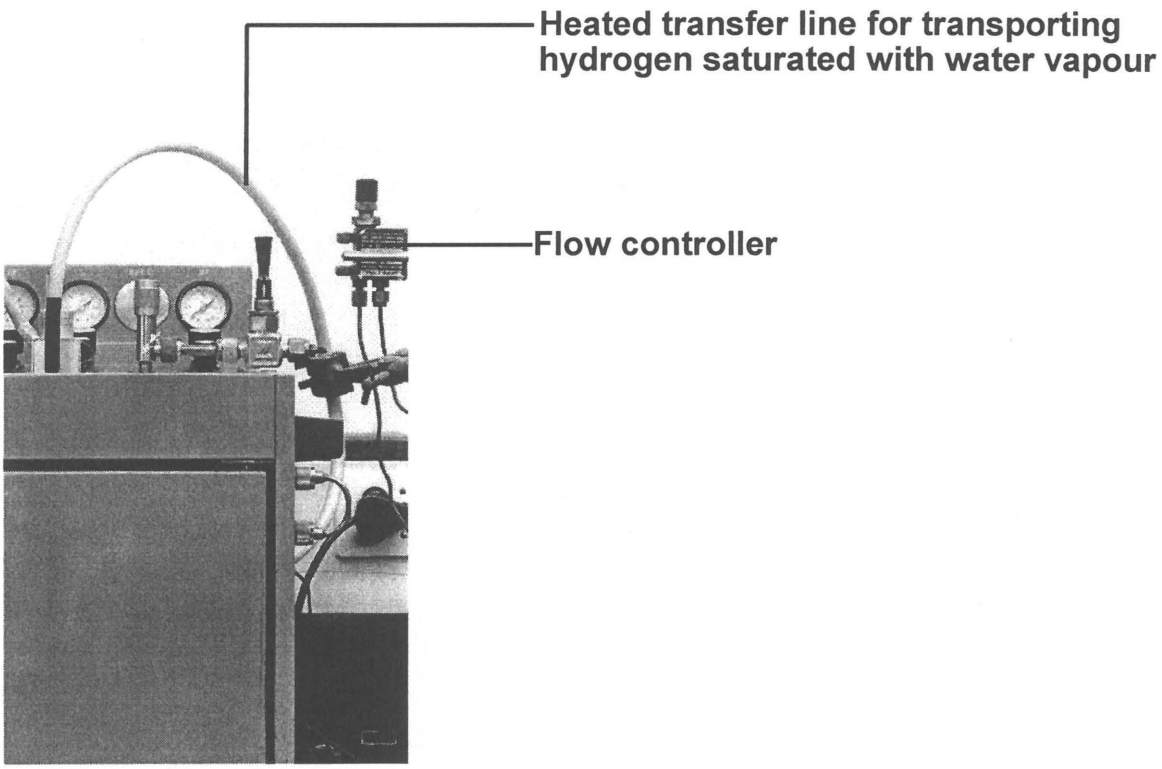


Figure 5-3. The facilities for the modification with continuous introduction of water vapour from the outside of the instrument

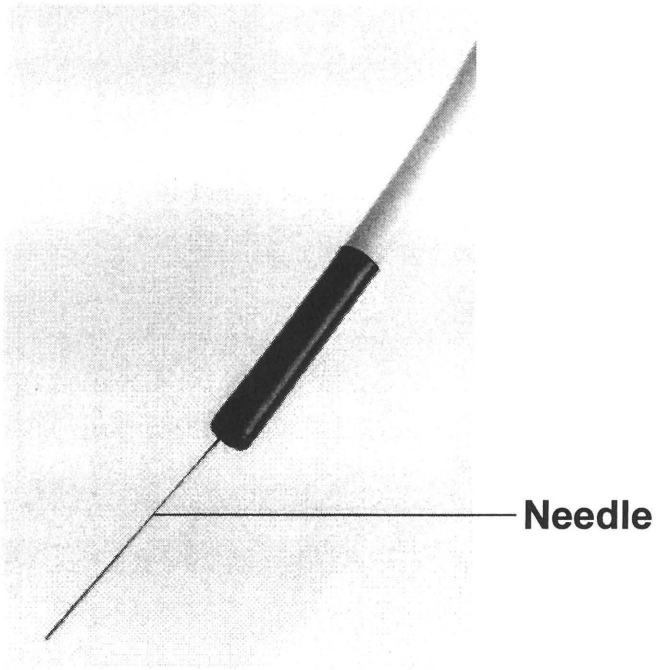


Figure 5-4. The needle of the heated transfer line

SAFETY WARNING: *The use of hydrogen inside the GC oven is an explosive hazard, especially when using modified systems as described here; a hydrogen leak warning system is essential and special care must be taken to prevent leaks.*

5.1.3 Monitoring selectivity changes

Our first interest was to observe the changes in the retention times of alcohols relative to those of alkanes and aromatics. Brief tests were, however, also done with acetic acid and some ketones and ethers. Initial non-reproducible results prompted us to check whether the shifts in retention time were also a function of the contact time of the vapour with the stationary phase.

5.1.3.1 Changes of selectivity over time

This test was done with a secondary hydrogen flow rate of 42.5 $cm^3.min^{-1}$, providing a carrier gas almost saturated with water. Test Mixture IX containing methanol, ethanol, propanol, benzene, toluene, xylene, n-octane, n-nonane, n-decane and n-undecane, dissolved in hexane was injected at fixed time intervals.

It was first suspected the water temperature was not in equilibrium with the oven temperature and that slowly changing vapour concentration was responsible for the shifting of the retention time. Care was therefore taken to allow the water temperature to stabilize overnight at an oven temperature of 50°C.

To compare the polarity change with that obtained with glycerol, in Chapter 4, Test Mixture II consisting of 1-hexanol, n-undecane, n-dodecane, n-tridecane and n-tetradecane was injected with dry carrier gas and with the above concentration of water vapour, allowing time for the retention behaviour to stabilize.

Test mixture VI (*tert*-butyl methyl ether (MTBE) and *tert*-amyl methyl ether (TAME)), test mixture VII (Acetone, methyl ethyl ketone, diethylether) and test mixture VIII (acetic acid) were also injected with dry carrier gas and with the

above concentration of water vapour, allowing time for the retention behaviour to stabilize.

5.1.3.2 Changes in selectivity with different vapour concentrations

A test mixture of methanol, ethanol, n-octane, n-nonane, n-decane and n-undecane in hexane (test mixture I) was injected at different volume flow rates of secondary, moist hydrogen, corresponding to different final vapour concentrations in the carrier gas. Sufficient time was given between settings for the elution profiles to stabilize.

5.1.4. Application to the analysis of alcohol in petrol

As an example of a possible application, petrol samples were analysed. A sample of commercial 91 octane petrol was analysed under dry and moist conditions, as was a sample of the same petrol spiked with 5% (m/m), each of methanol and ethanol. The concentration of alcohols was chosen to be in the range typically sold in blends in the times of local oversupply of alcohols.

5.1.5. Monitoring the stability of the Carbowax column

The stability of the Carbowax column was monitored using the standard Grob test at regular intervals, after careful drying the column at 50°C. This is a stringent test for monitoring column efficiency, surface activity and possible loss of stationary phase.

5.1.6 Test mixtures :

Column performance was evaluated by means of nine test mixtures :

Test Mixtures I to III was described in Chapter 4.

Test mixture V -- benzene, toluene and xylene in hexane.

Test mixture VI -- *tert*-butyl methyl ether (MTBE) and *tert*-amyl methyl ether (TAME) in dichloromethane.

Test Mixture VII -- acetone methyl ethyl ketone and diethylether in hexane.

Test Mixture VIII --acetic acid in hexane.

Test Mixture IX -- octane, methanol, nonane, ethanol, benzene, decane, propanol, toluene, undecane and xylene in hexane.

5.2 Results and Discussion

5.2.1 Selectivity changes

Dynamic modification with water vapour produces a more polar column giving separations that are increasingly based on polarity differences of analytes. The non-polar alkanes are *expected* to elute first, followed by the aromatic groups with the oxygenated compounds eluting last. Figure 5-5 illustrates that when water vapour is added to the carrier gas, the alcohols do in fact elute as a separate, late eluting group. Test Mixture II was analyzed using dry and humid carrier gases. In Figure 5-5, Chromatogram *b* all the alkanes shifted to earlier retention times while 1-hexanol eluted later, at about 18 minutes, after the tetradecane peak. This selectivity is far more pronounced than observed with the glycerol modified Carbowax column. It should be useful for alcohol analysis in petrol samples they usually elute with the alkanes. This is further demonstrated in Figure 5-6 where the retention time of methanol changed from less than that of nonane to greater than that of undecane.

The aromatic compounds are more polar than the alkanes and were expected to shift to later retention times. However, as demonstrated by Figure 5-7 and Figure 5-8 they all shifted to earlier retention times. This indicates that hydrogen bonding is the dominant interaction introduced by

the dynamic modification with water vapour. Dipole-induced dipole interactions are not increased.

Ketones and ethers do not undergo strong hydrogen bonding . Test Mixtures VI, VII and VIII were analyzed using dry and humid carrier gases to investigate the effect of dynamic modification with water on retention of ketones, ethers and acids. As illutrated by Figure 5-9 and 5-10 *tert*-butyl methyl ether (MTBE), *tert*-amyl methyl ether (TAME), acetone, methyl ethyl ketone and diethylether, showed only a very minor increase in k values. Acetic acid, however was much more retained by the modified stationary phase(Figure 5-11). With a dead time of 0.9 minute the acetic acid eluted on the unchanged ('dry') Carbowax column in 24 minutes, increasing to 90 minutes with water vapour close to saturation. This represents an increase in capacity factor, *k*, of about a factor of 4. The equivalent increase in interaction strength with methanol is about a factor of 5. This clearly indicates a strong additional interaction with OH containing compounds, the result of hydrogen bonding with quasi-stationary water in the column.

5.2.1.1. Changes in selectivity over time

When some experimental results were repeated under identical conditions variance in the retention time of ethanol was observed. (see Figure 5-12). The observed polarity of the modified column appeared to increase with time. The retention of the alcohols were still increasing after three runs while the xylenes were continously less retained.

Figures 5-13 , 5-14 and 5-15 show the chromatograms of test mixture IX obtained after (15 minutes,) 1 hour, 2 hours, 3 hours and 4 hours (and 5 hours) of introduction of moist carrier gas at different flow rates.
In Figure 5-13 the chromatogram *d* shows that methanol and ethanol are partially separated after three hours of the saturation at 42 ml.min^{-1}. No

significant change was observed after about 3 hours of carrier flow. It is clear that more than an hour is required to completely stabilize the system.

5.2.1.2 Changes in selectivity with different water vapour concentration

The concentration of water vapour in the carrier gas influences the polarity of the Carbowax 20M stationary phase. The concentrations were changed by adjusting the relative flow rates of the water saturated carrier flow to the dry hydrogen flow.

The chromatograms in Figure 5-16 show the influence of the water vapour concentration on the stationary phase polarity of the Carbowax column. An increase in polarity with increased flow of water vapour is demonstrated by the cross-over of the alkanes and the alcohol as the secondary carrier flow rate is changed from 20 to 27 $cm^3.min^{-1}$. Changing values from 42 to 48 $cm^3.min^{-1}$ (the latter giving almost 100% water saturation at the column temperature of 50^0C) no longer had a dramatic effect on the elution profiles of the test mixture I. Hence it was preferred to work at 42 $cm^3.min^{-1}$. This ensures that no water vapour enters the inlet system by back diffusion - a potential hazard to the Carbowax column when it is baked out under 'dry' conditions and prepared for the Grob test at high temperatures. Traces of water in the carrier gas are known to shorten the lifetime of Carbowax columns at high temperature.

The hump in Figure 5-16(d-f) corresponds to the returning water profile after brief interruption when the wet carrier line is removed for injection of the sample through the septum. The hump varies in size and could be the result of contaminants desorbed by the water near the FID. For other possible explanations the reader is referred to the work of Grob and Habich [22]. A minor modification to the system, eliminating the requirement for the brief interruption of the moist carrier during injection, should thus be beneficial.

5.2.2 The maximum shifts obtained

Chromatograms in Figure 5-17 are presented to emphasize the extent of the change in polarity between dry (a) and moist (b) carrier gas after 1 hour stabilizing time. Both alkanes and aromatics are eluted far quicker under moist conditions whereas there is strong additional retention for the alcohols. Based on this figure, the change in Kovats retention index, Δ I, when going from dry to wet carrier gas, was calculated for the following compounds (ΔI is given in brackets): Methanol (353), ethanol (314), benzene (55), toluene (39), m-xylene (27). Important to see is that methanol (I = 1232) will elute after n-dodecane and far beyond the xylenes under the wet carrier gas conditions. This can be of practical importance to petrochemical analysis as no capillary column is available with this selectivity.

5.2.3 Application to the analysis of alcohol in Petrol

The chromatograms in Figure 5-18 show petrol spiked with methanol and ethanol under dry (a) and moist (b) carrier gas conditions after 1 hour stabilization. The peak allocation could be done reliably by recording some interim chromatograms during the first hour of stabilization (Figure 5-19 and 5-20). The gradual alteration of the elution profiles with time is in itself a powerful tool for peak identification. Peak allocation across two chromatograms obtained from different polarity columns cannot normally be done reliably. Note that a number of windows exist in the chromatogram into which the alcohol peaks can be positioned by appropriate choice of the vapour concentration of the carrier gas. This would allow reliable quantitation.

5.2.4 Stability of the carbowax column

The chromatograms in Figure 5-21 show analysis of the Grob test mixture, before (a) and after (b) the first series of experiments performed under moist conditions. Apart from a slight deterioration of the butanediol peak shape, the rest of the peaks elute without any additional peak tailing - an essential prerequisite for the dynamic modification with water to be used in practise. There is however a slight reduction in the phase film thickness as indicated by the peaks eluting about 2 minutes earlier. It also shows a slight residual increase of polarity after the modification, which can be seen on the separation of methyl dodecanoate and 2,6-dimethylaniline. Grob tests done after subsequent experiments with moist carrier were however identical to the one in Figure 5-21(b), indicating a once-only initial loss of phase(see Figure 5-22).

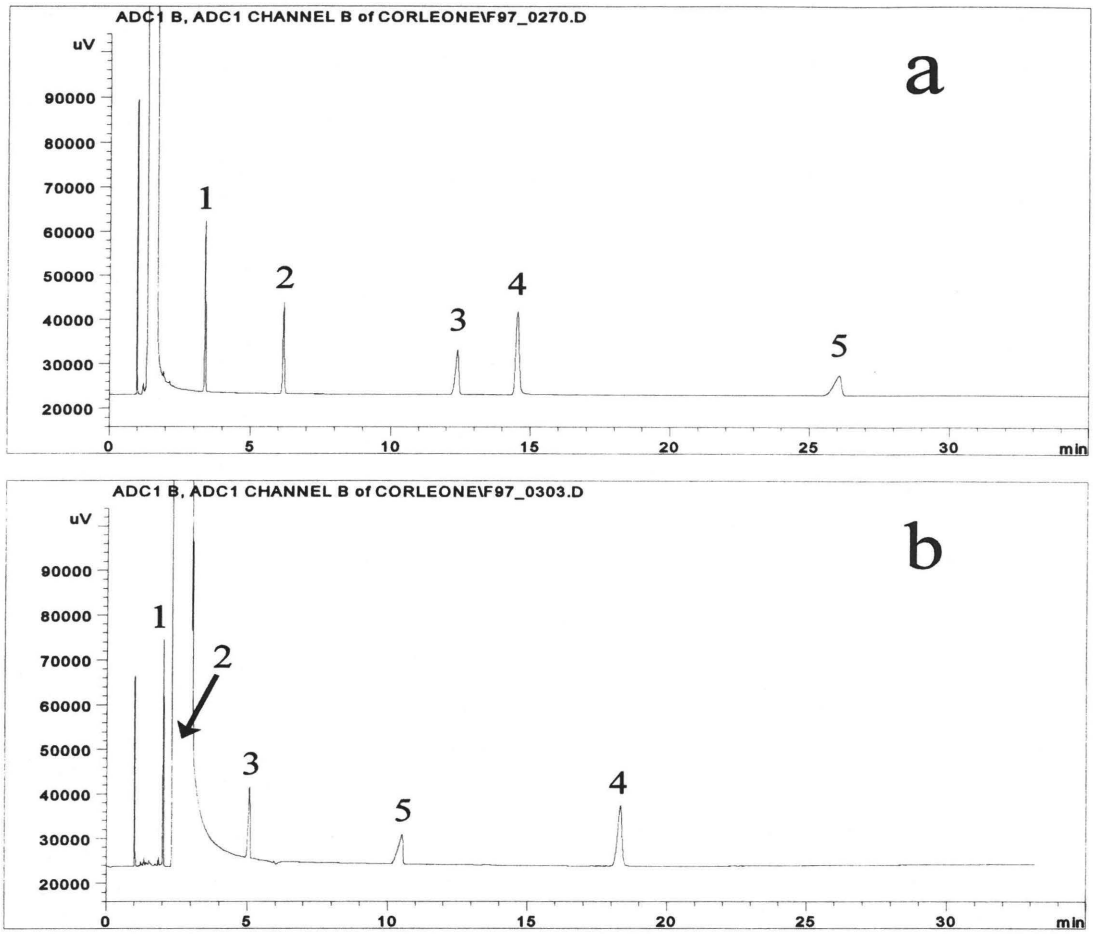


Figure 5-5. Separation of the test mixture II on a Carbowax 20M column (a) without and (b) with water vapour, under identical condtions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= undecane; 2= dodecane; 3= tridecane; 4= 1-hexanol; 5= tetradecane.

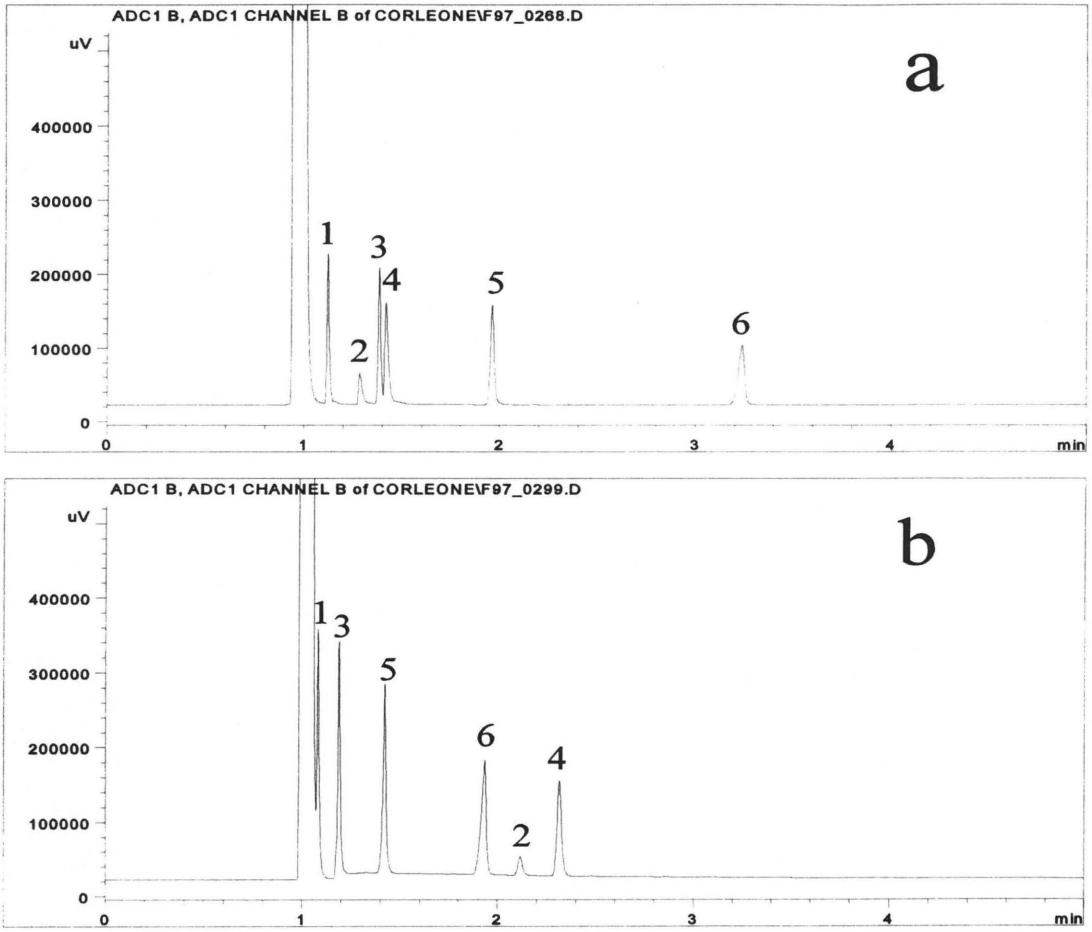


Figure 5-6. Separation of the test mixture I on a Carbowax 20M column (a) without and (b) with water vapour, under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= octane; 2= methanol; 3= nonane; 4= ethanol; 5= decane; 6=undecane.

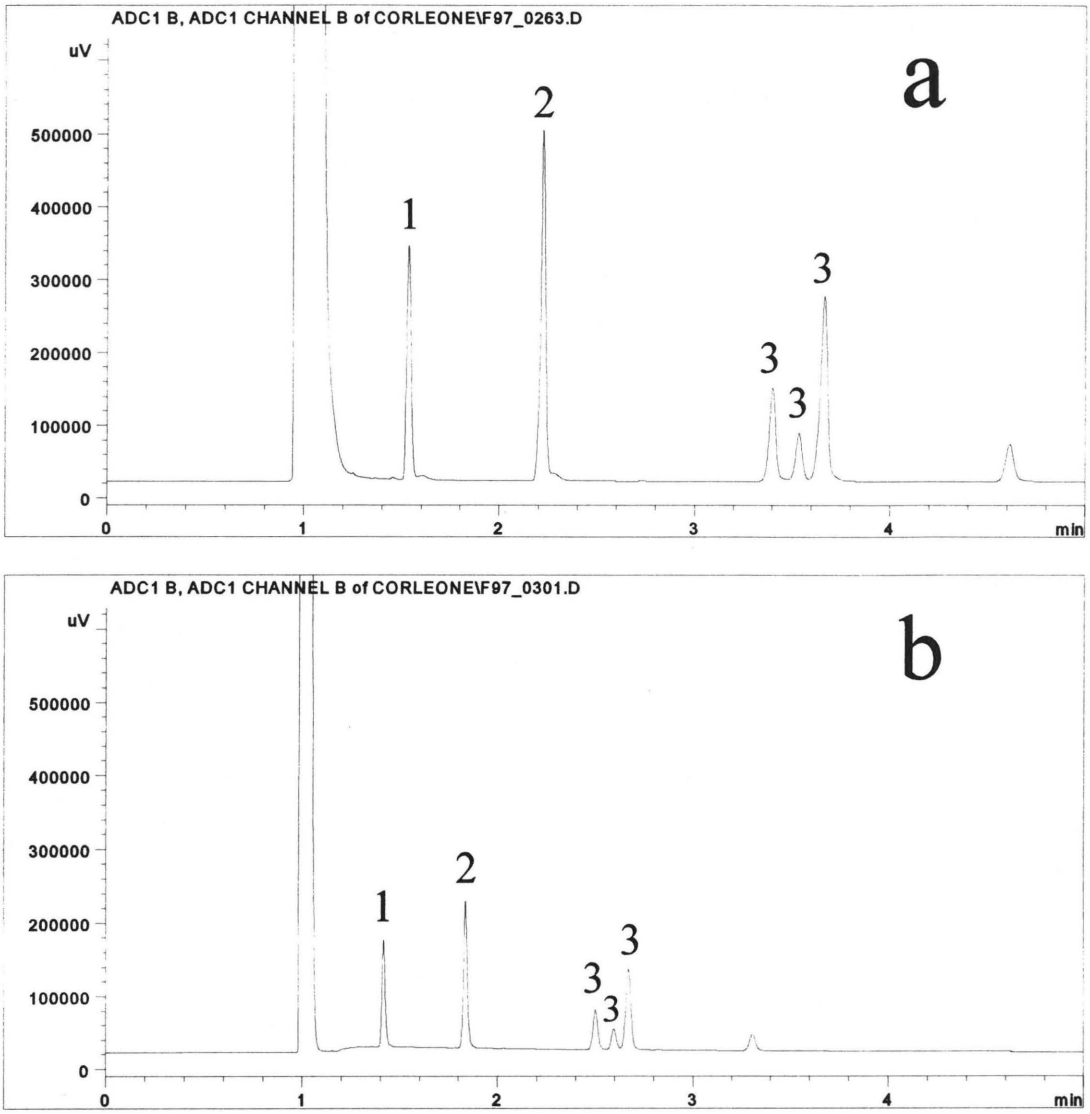


Figure 5-7. Separation of the test mixture V on a Carbowax 20M column (a) without and (b) with water vapour, under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= benzene; 2= toluene; 3= xylene.

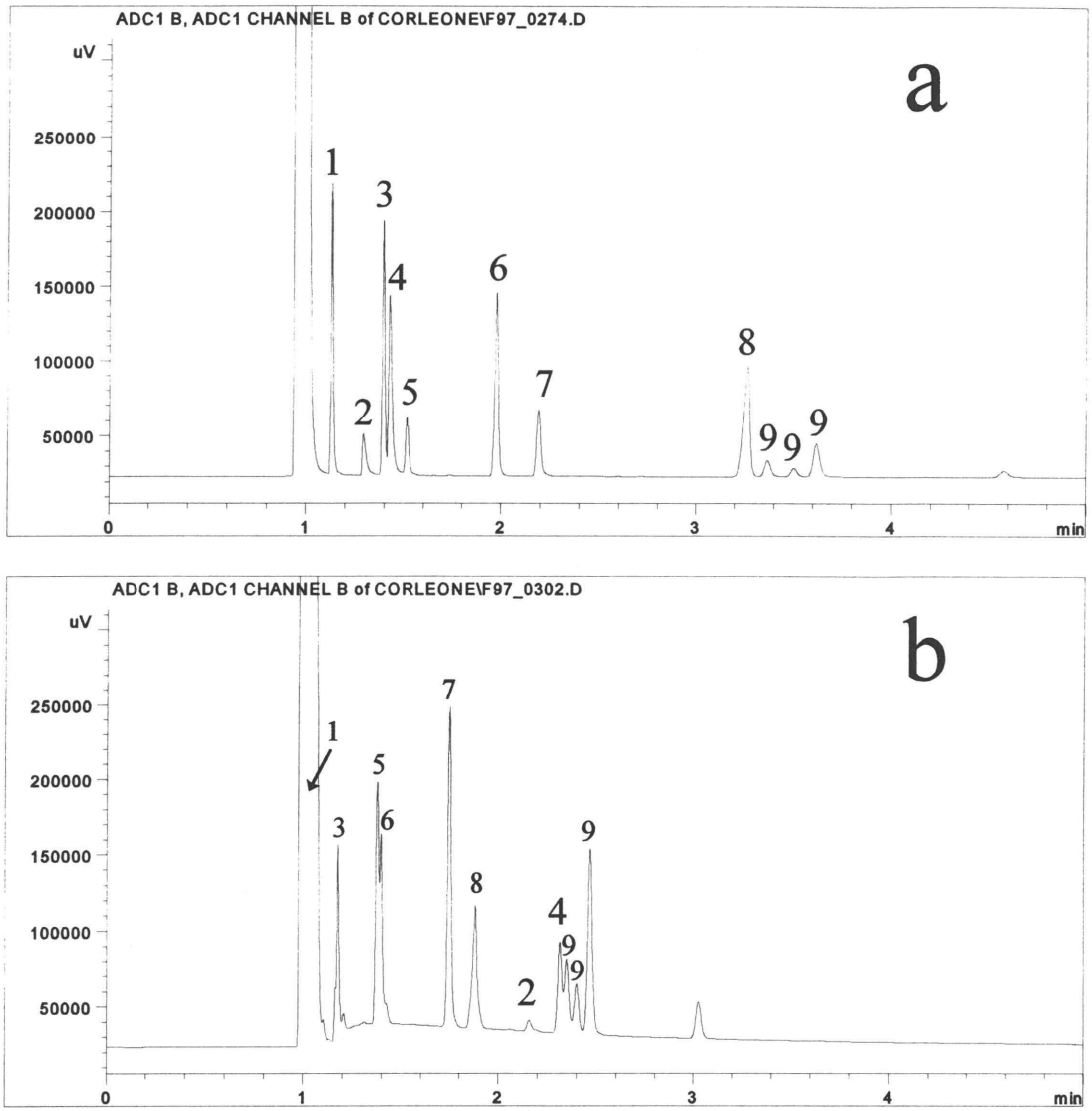


Figure 5-8. Separation of combonation of the test mixtures I and V on a Carbowax 20M column (a) without and (b) with water vapour, under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= octane; 2= methanol; 3= nonane; 4= ethanol; 5= benzene; 6= decane; 7= toluene; 8=undecane; 9= xlyene.

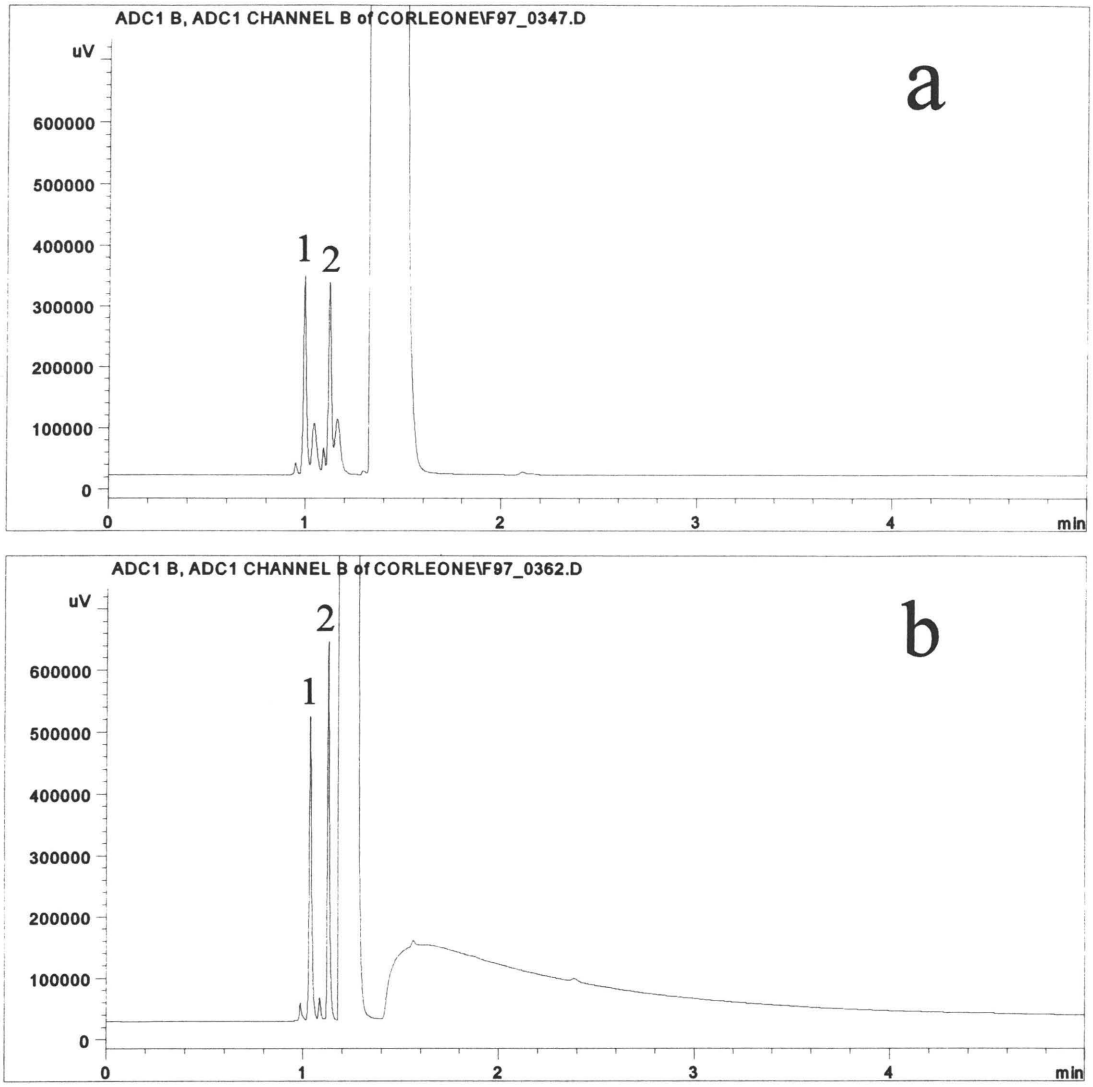


Figure 5-9. Separation of the test mixture VI on a Carbowax 20M column (a) without and (b) with water vapour, under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= *tert*-butyl methyl ether (MTBE); 2= *tert*-amyl methyl ether (TAME).

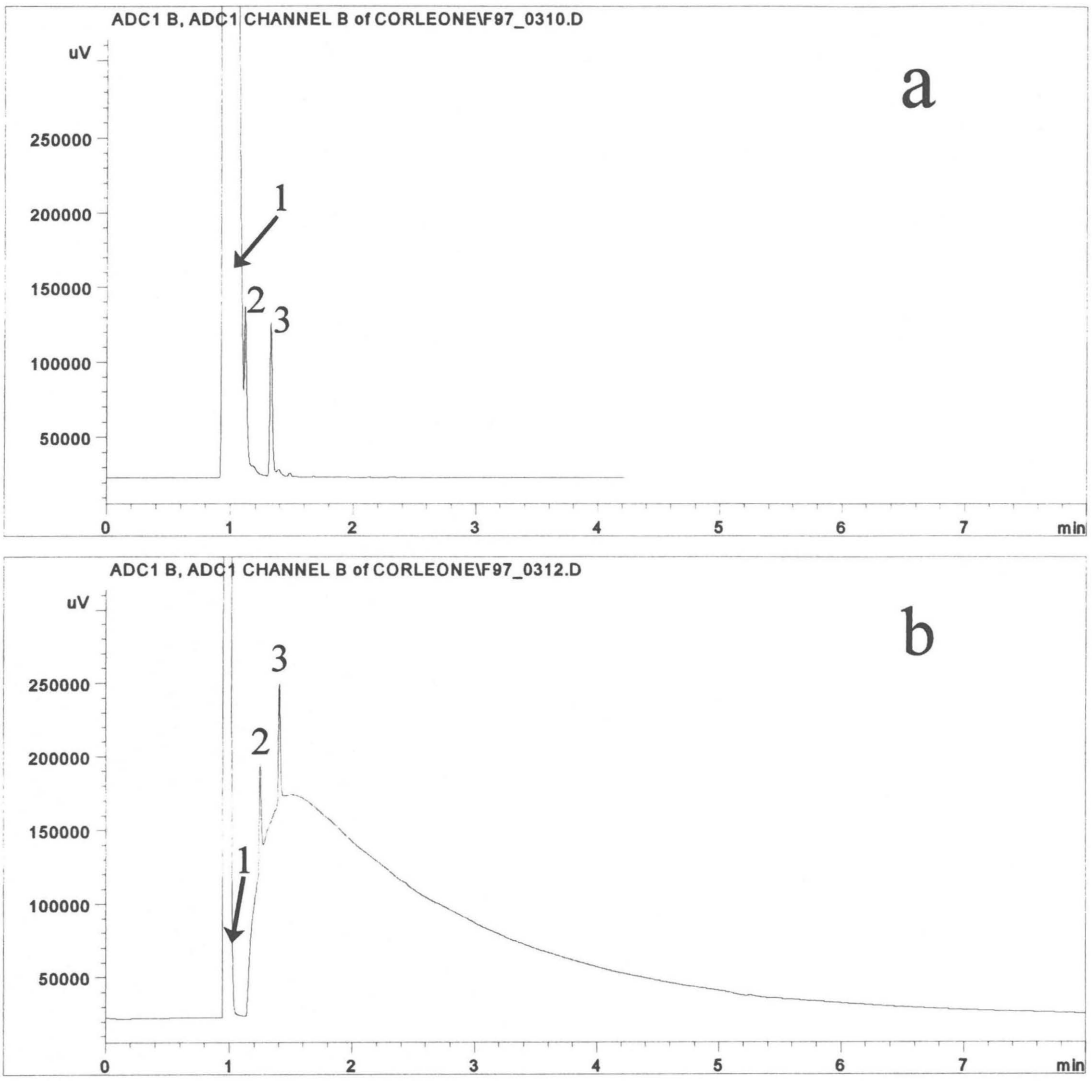


Figure 5-10. Separation of the test mixture VII on a Carbowax 20M column (a) without and (b) with water vapour, under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= diethylether; 2= acetone; 3= methyl ethyl ketone.

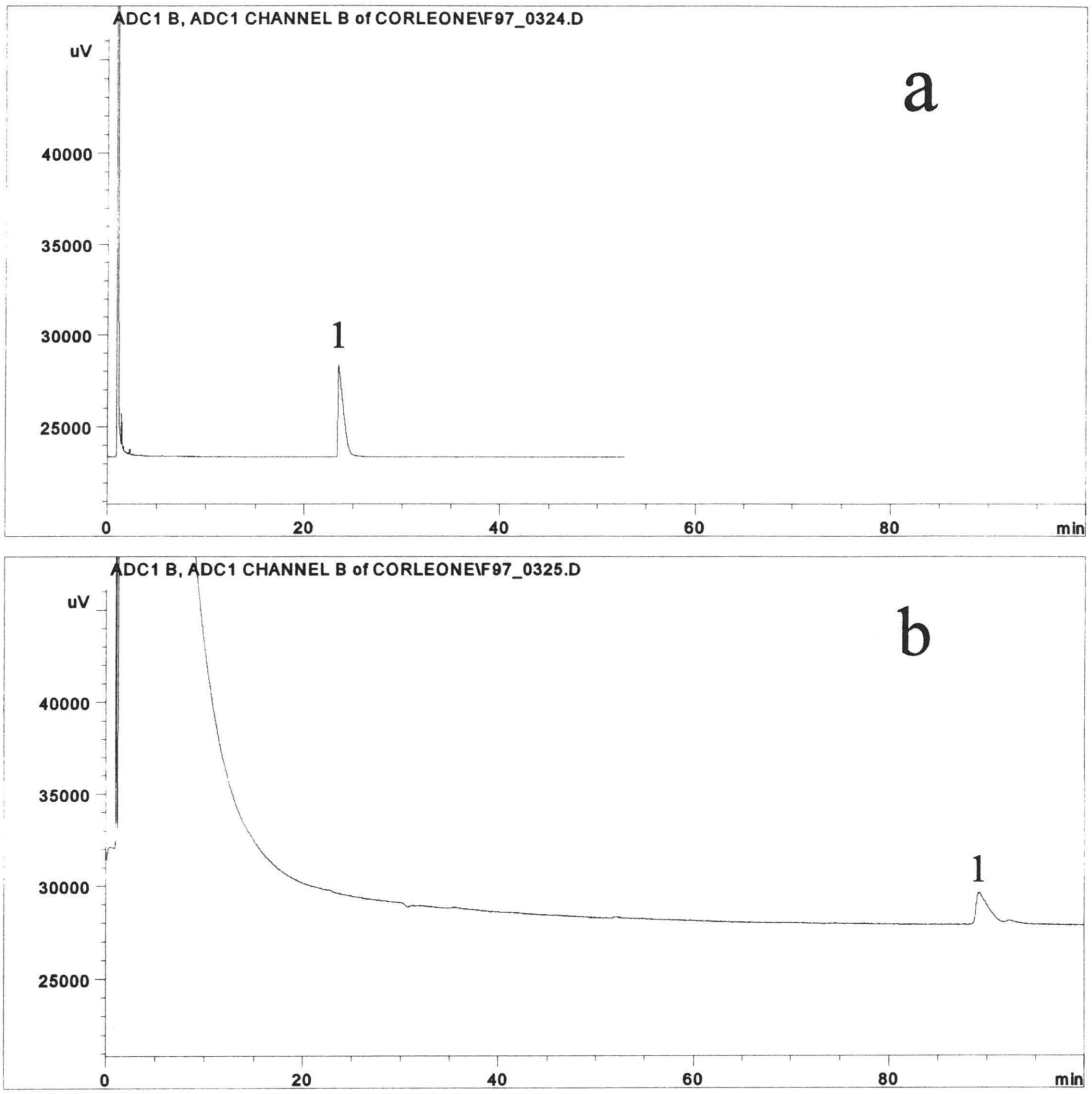


Figure 5-11. Separation of the test mixture VIII on a Carbowax 20M column (a) without and (b) with water vapour, under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peak 1= acetic acid.

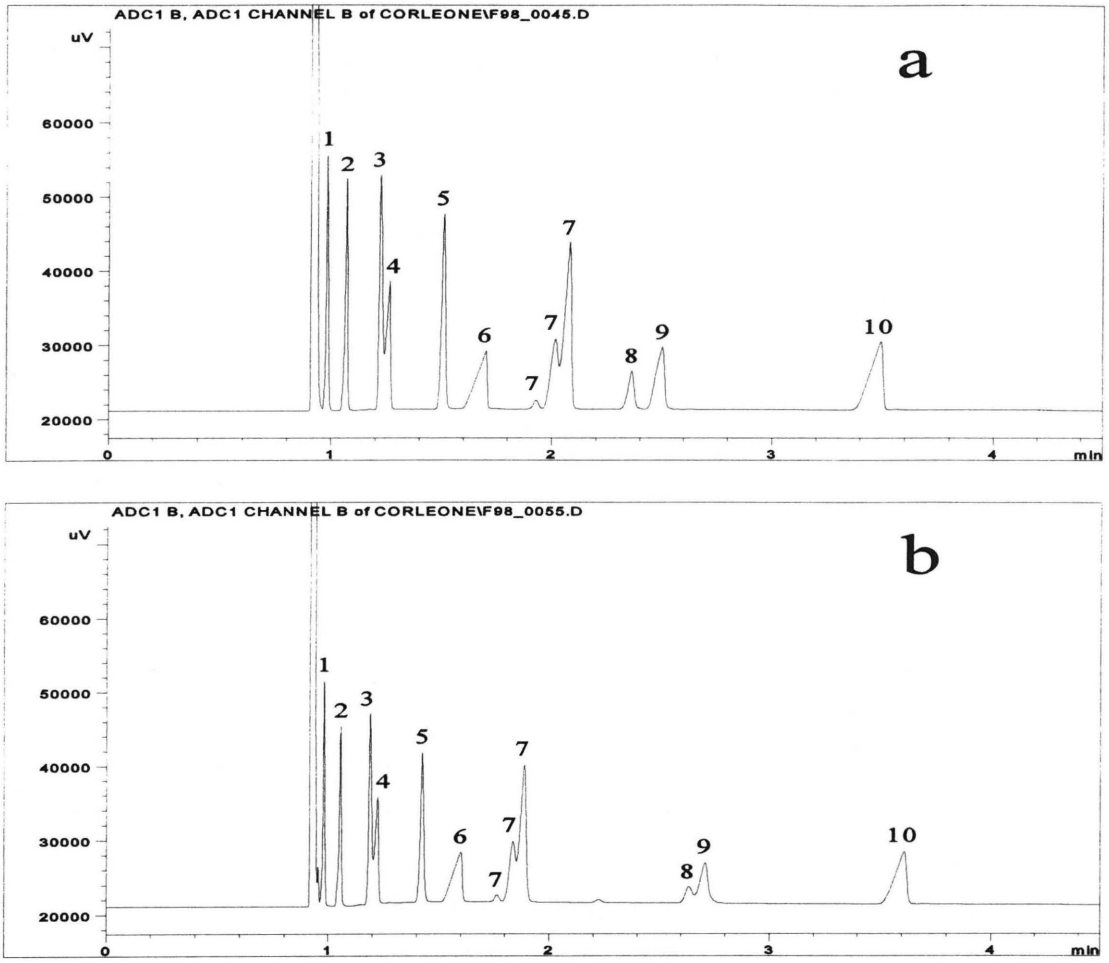


Figure 5-12. Separation of the test mixture IX (a) 10 minutes after continuous introducation of water vapour at 42.5 ml.min^{-1} on a Carbowax 20M column; (b) the identical conditions after 3 GC runs. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= octane; 2= nonane; 3= benzene; 4= decane; 5= toluene; 6= undecane; 7= xylene; 8= methanol; 9= ethanol; 10= propanol.

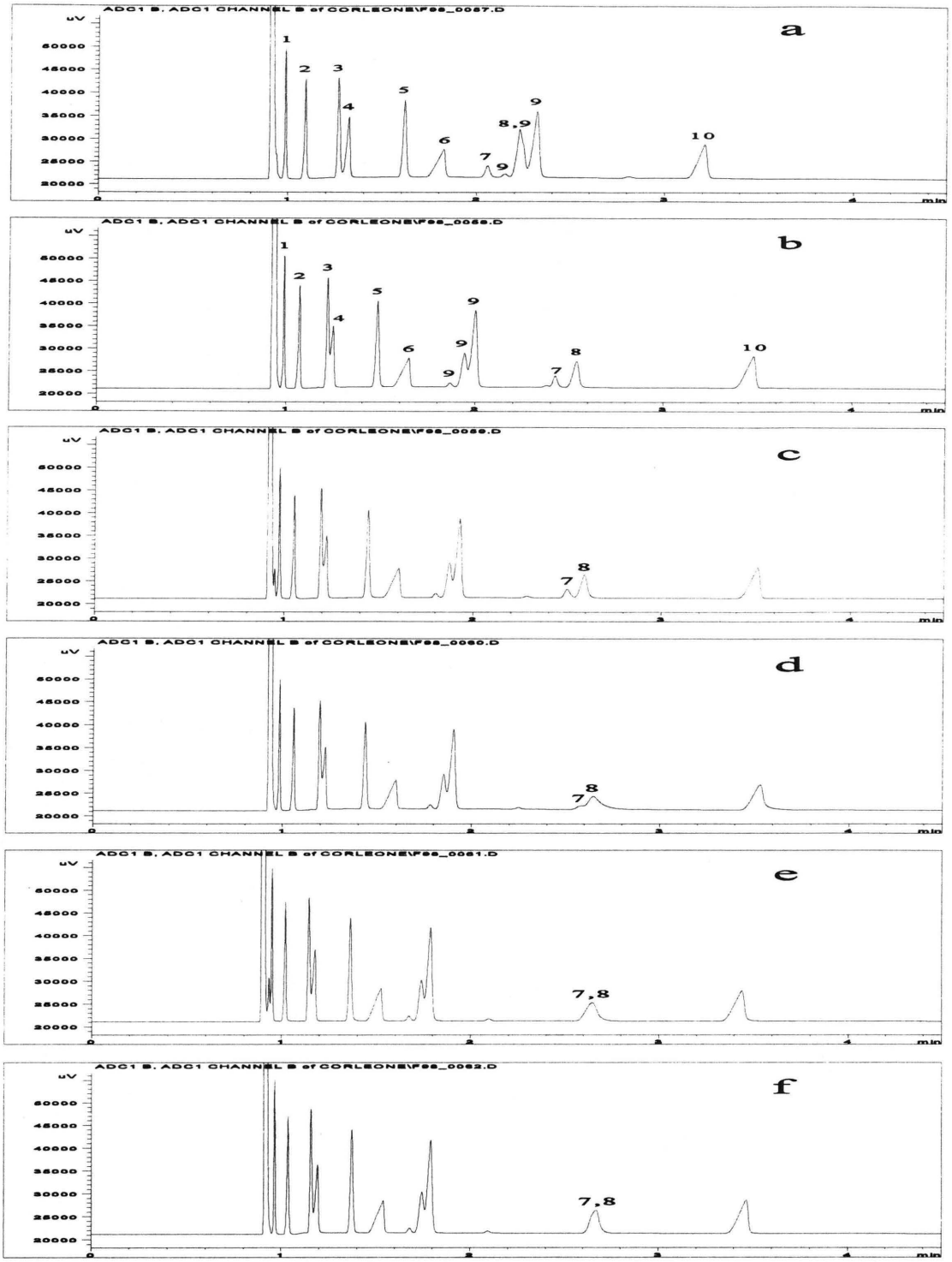


Figure 5-13. Separation of the test mixture IX (a) 15 minutes; (b) 1 hour; (c) 2 hours; (d) 3 hours; (e) 4 hours; (f) 5 hours after continuous introduction of water vapour at 42. 5 ml.min^{-1} to the hydrogen carrier gas on a Carbowax 20M column under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= ocatne; 2= nonane; 3= benzene; 4= decane; 5= toluene; 6= undecane; 7= methanol; 8= ethanol; 9= xylene; 10= xylene.

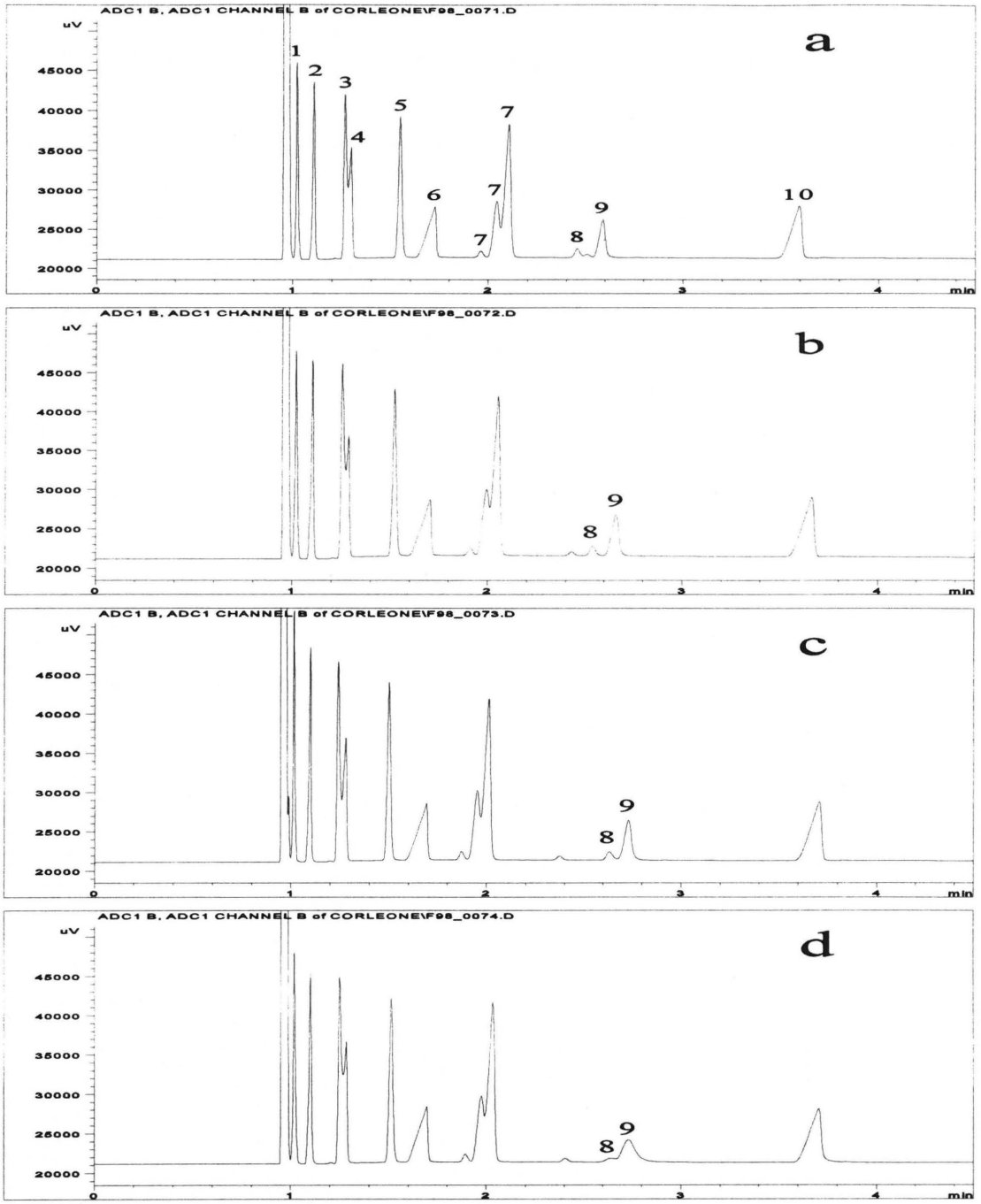


Figure 5-14. Separation of the test mixture IX (a) 1 hour; (b) 2 hours; (c) 3 hours; (d) 4 hours after continuous introduction of water vapour at 28.5 ml.min^{-1} to the hydrogen carrier gas on a Carbowax 20M column under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= ocatne; 2= nonane; 3= benzene; 4= decane; 5= toluene; 6= undecane; 7= xylene; 8= methanol; 9= ethanol; 10= propanol.

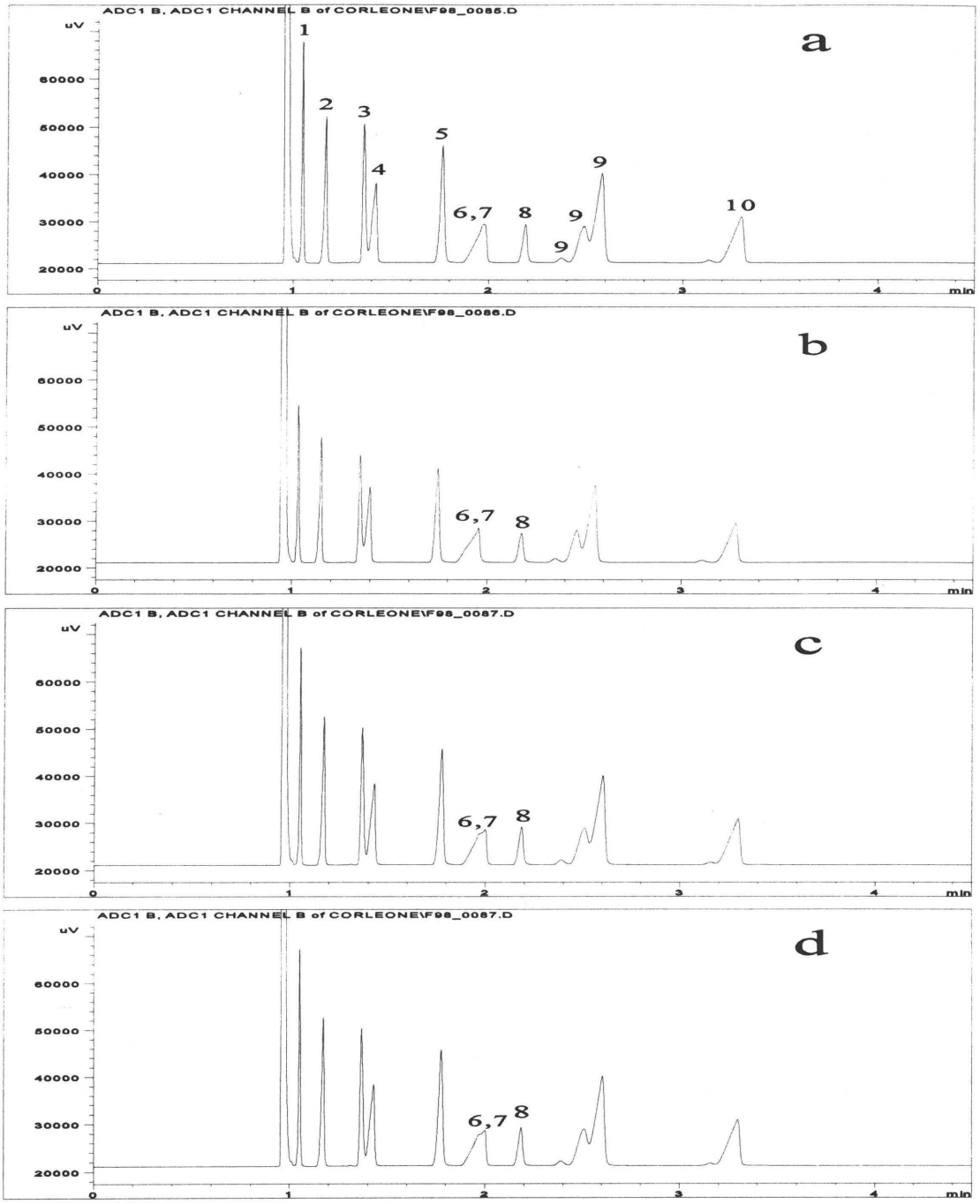


Figure 5-15. Separation of the test mixture IX (a) 1 hour; (b) 2 hours; (c) 3 hours; (d) 4 hours after continuous introduction of water vapour at 22 ml.min^{-1} to the hydrogen carrier gas on a Carbowax 20M column under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= ocatne; 2= nonane; 3= benzene; 4= decane; 5= toluene; 6= methanol; 7= undecane; 8= ethanol; 9= xylene; 10= propanol.

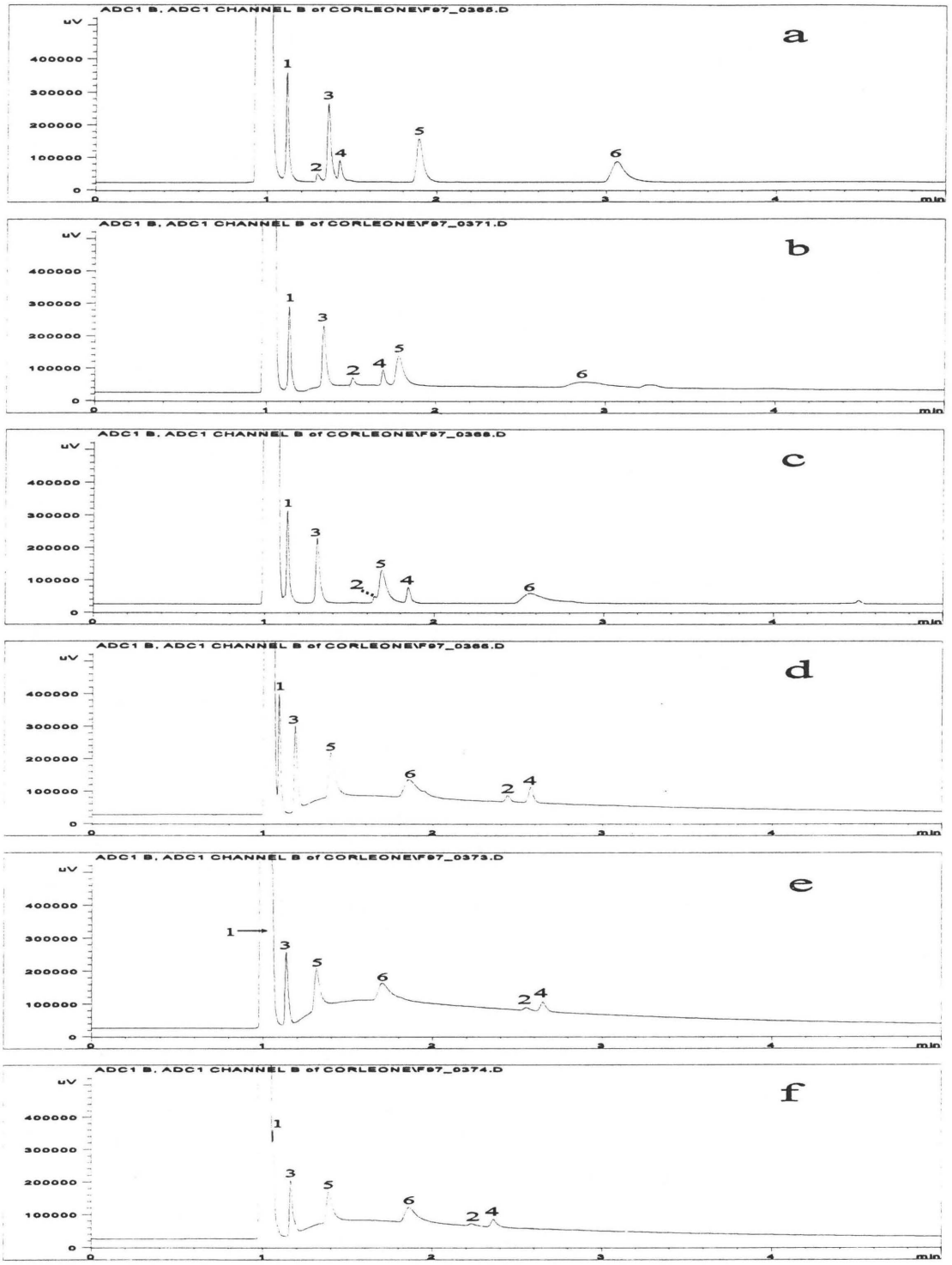


Figure 5-16. Separation of the test mixture I on a Carbowax 20M column without (a) and with water vapour at (b) 20.7 ml.min^{-1}; (c) 27.3 ml.min^{-1}; (d) 38.3 ml.min^{-1}; (e) 42.5 ml.min^{-1}; (f) 48 ml.min^{-1} , under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= octane; 2= methanol; 3= nonane; 4= ethanol; 5= decane; 6=undecane.

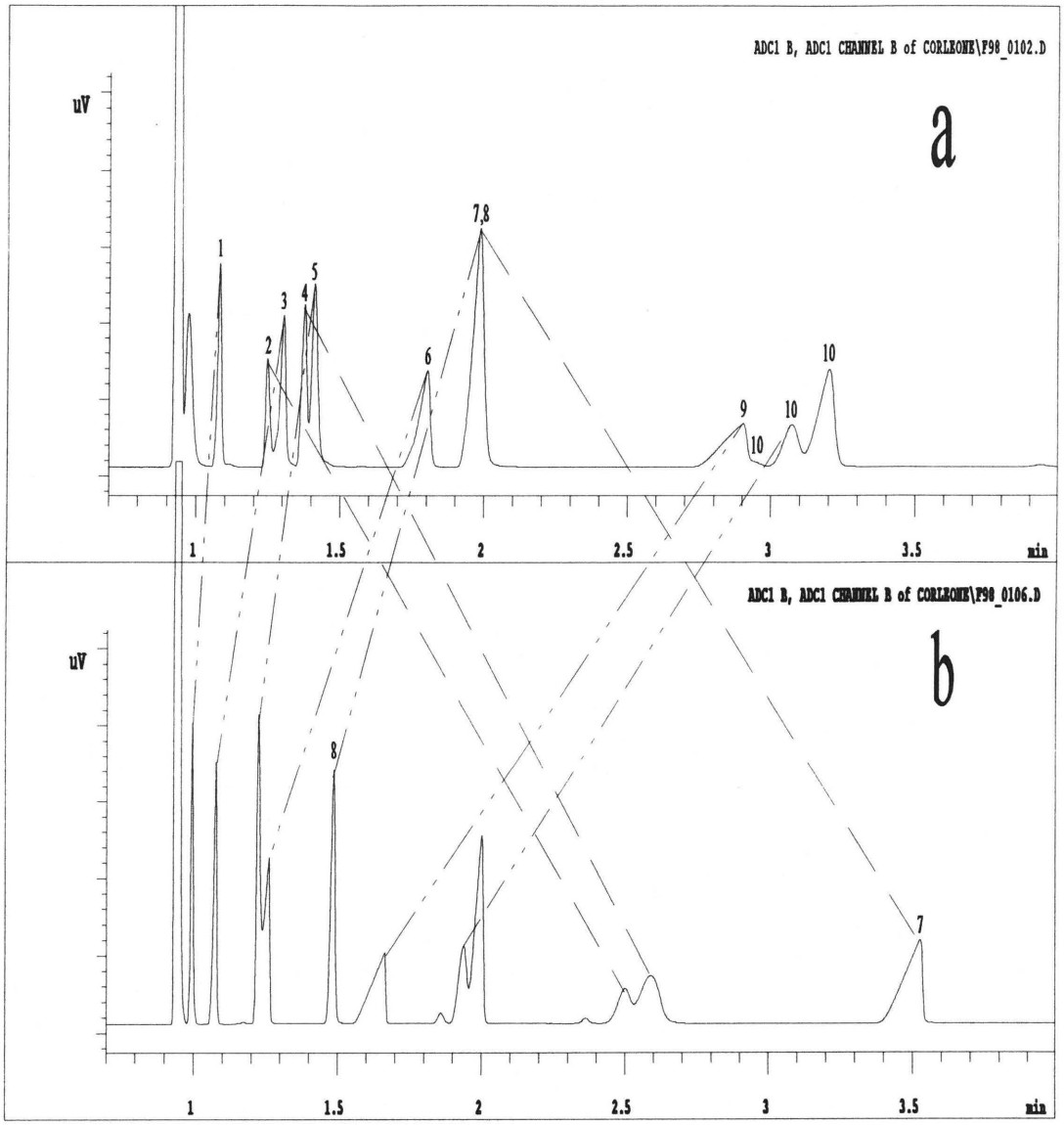


Figure 5-17. Separation of the test mixture IX (a) without water vapour introduction and (b) 1 hour after continuous introduction of water vapour at 42 ml.min^{-1} to the hydrogen carrier gas on a Carbowax 20M column under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= ocatne; 2= methanol; 3= nonane; 4= ethanol; 5= benzene; 6= decane; 7= propanol; 8= toluene; 9= undecane; 10= xylene.

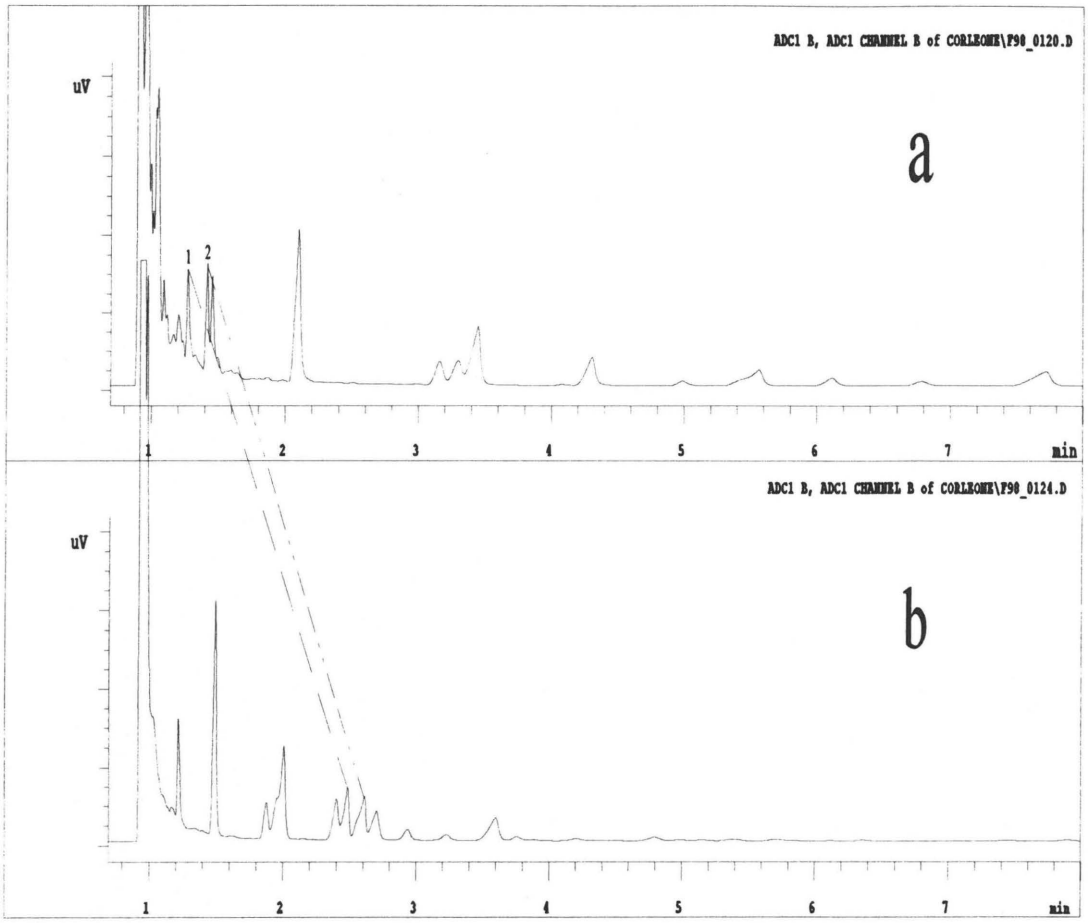


Figure 5-18. Separation of a mixture of commercial 91 ocatne petrol and 5%(m/m) of each of methanol and ethanol; (a) without water vapour introduction and (b) 1 hour after continuous introduction of water vapour at 42 ml.min^{-1} to the hydrogen carrier gas on a Carbowax 20M column under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C. Peaks: 1= methanol; 2= ethanol.

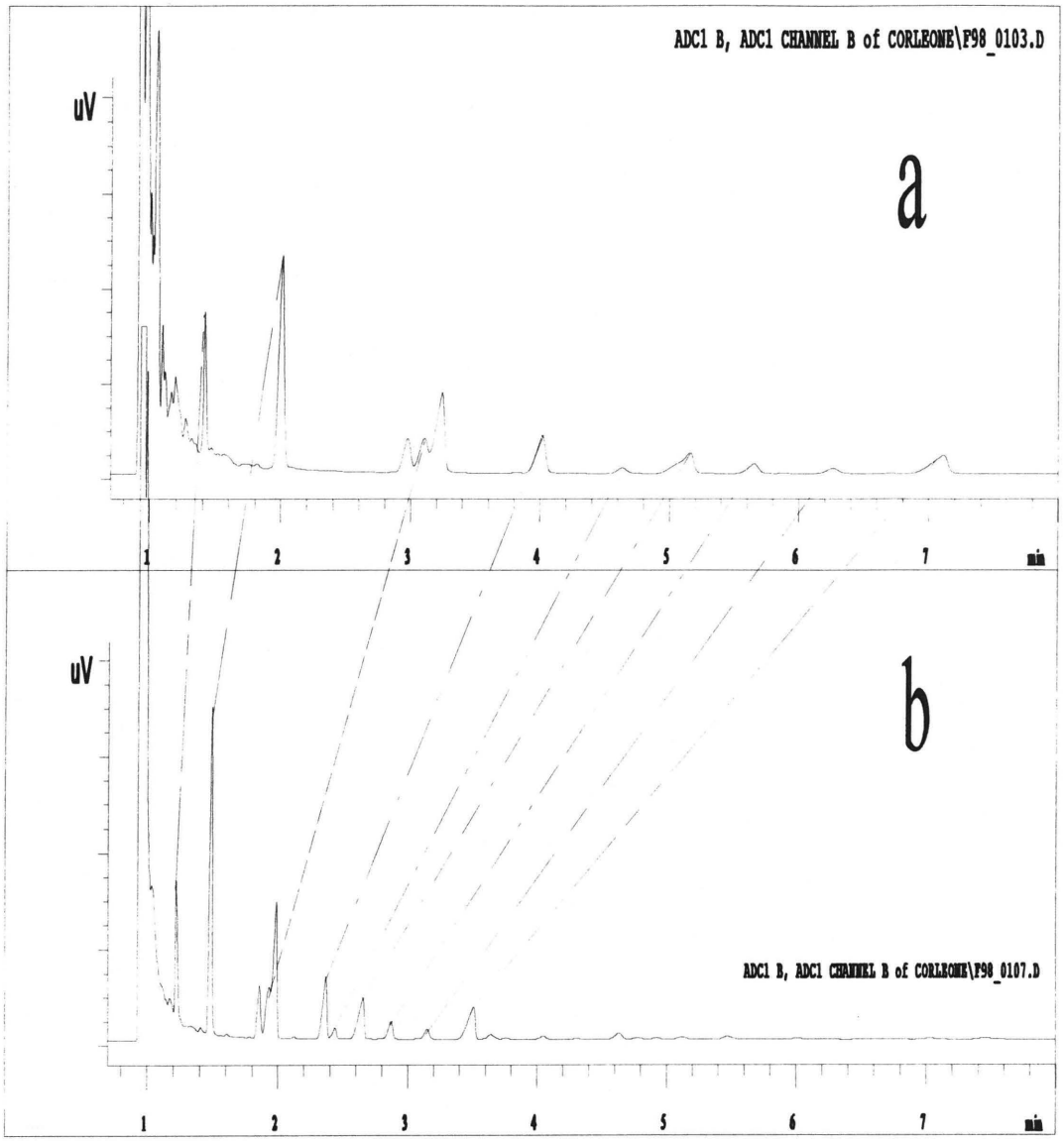


Figure 5-19. Separation of commercial 91 ocatne petrol (a) without water vapour introduction and (b) 1 hour after continuous introduction of water vapour at 42 ml.min^{-1} to the hydrogen carrier gas on a Carbowax 20M column under identical conditions. Column: 25 m × 0.3 mm i. d. at 50°C.

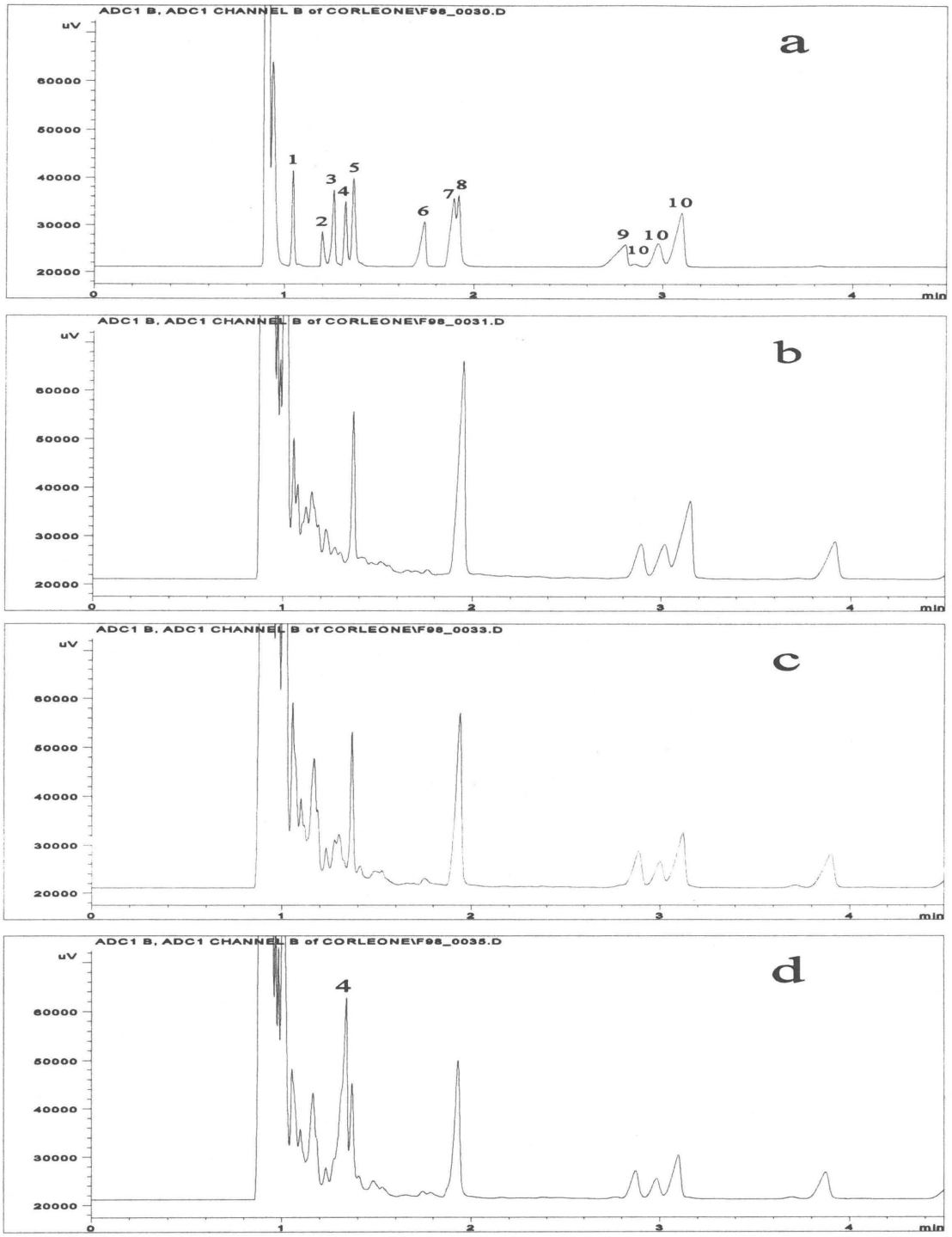


Figure 5-20. Separation of (a) the test mixture IX, (b) commercial 91 octane petrol,(c) commercial 93 octane petrol and (d) commercial petrol with 10% alcohol on a Carbowax 20M column. Column: 25 m × 0.3 mm i. d. At 50°C. Peaks: 1= octane; 2= methanol; 3= nonane; 4= ethanol; 5= benzene; 6= decane; 7= propanol; 8=toluene; 9= undecane; 10= xylene.

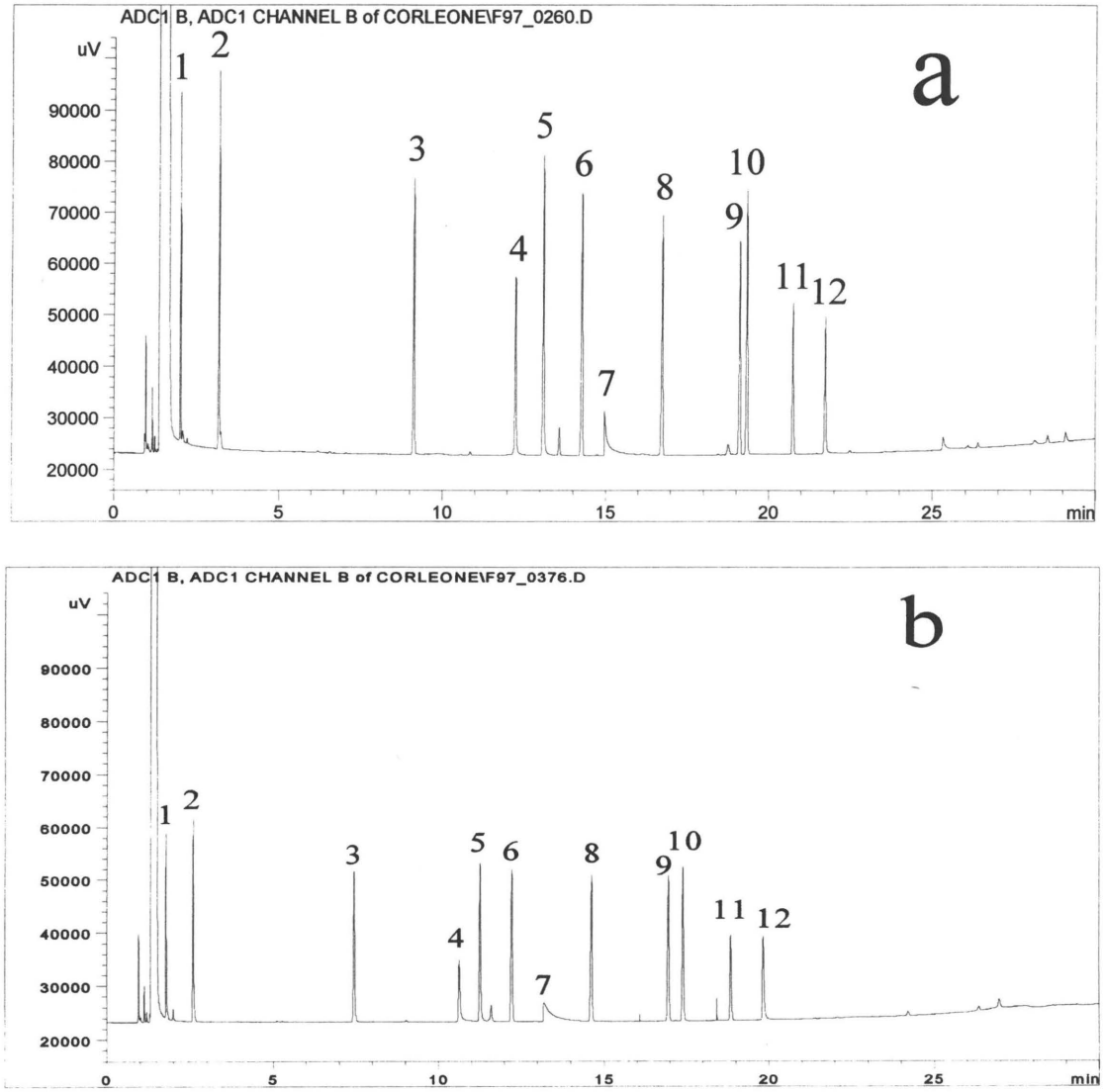


Figure 5-21. Separation of the Grob test mixture on a Carbowax 20M column (a) before and (b) after the modification with water vapour, under identical conditions. Columns: 25 m x 0.3 mm i. d., temperature programme: 50°C to 200°C at 5°C.min^{-1}. Peaks: 1= decane; 2= undecane; 3= nonanal; 4= 2,3-butanediol; 5= 1-octanol; 6= methyl decanoate; 7 = dicyclohexylamine; 8= methyl undecanoate; 9 = methyl dodecanoate; 10= 2,6-dimethylaniline; 11= 2,6-dimethylphenol; 12= 2-ethylhexanoic acid.

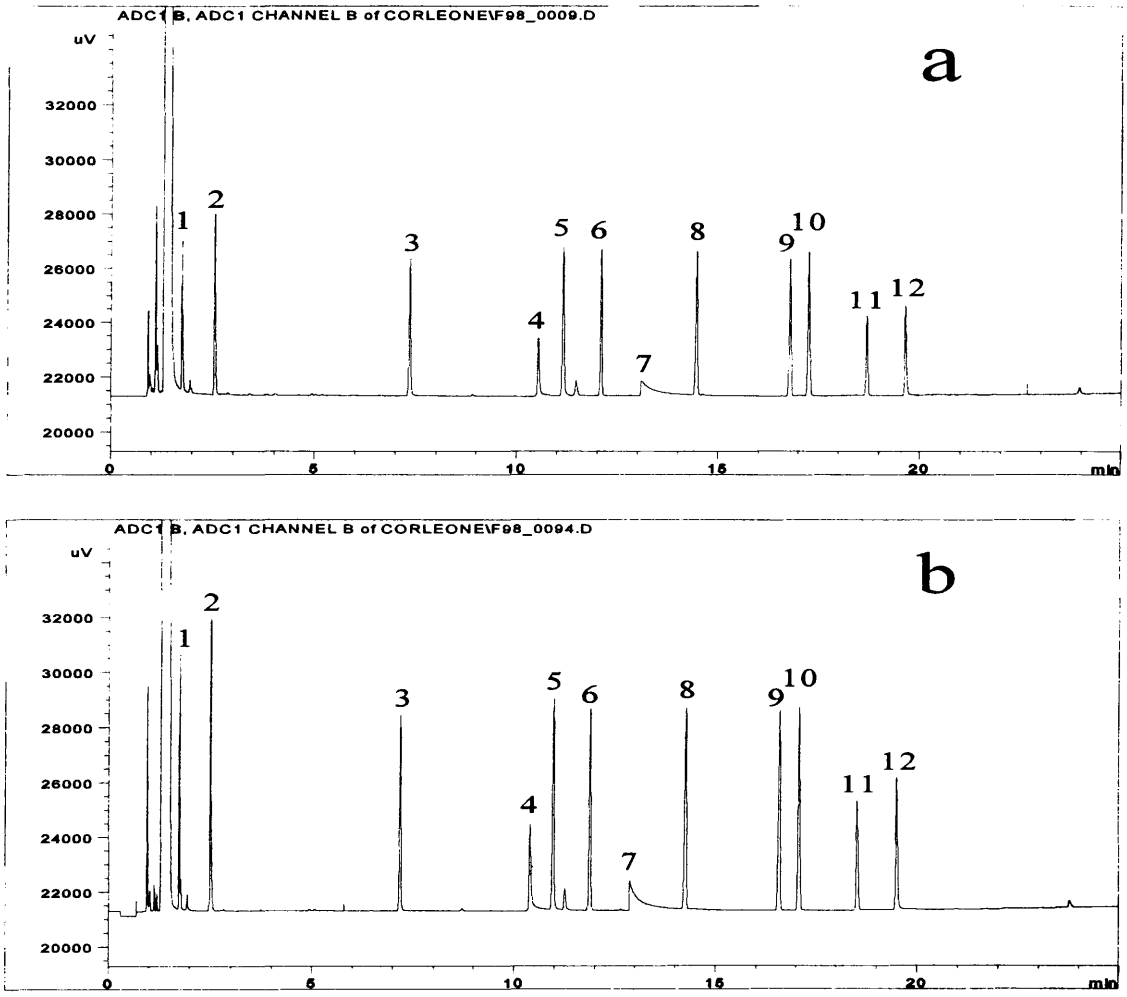


Figure 5-22. Separation of the Grob test mixture on a Carbowax 20M column (a) before and (b) after the modification with water vapour, under identical conditions. Columns: 25 m x 0.3 mm i. d., temperature programme: 50°C to 200°C at 5°C.min^{-1}. Peaks: 1= decane; 2= undecane; 3= nonanal; 4= 2,3-butanediol; 5= 1-octanol; 6= methyl decanoate; 7 = dicyclohexylamine; 8= methyl undecanoate; 9 = methyl dodecanoate; 10= 2,6-dimethylaniline; 11= 2,6-dimethylphenol; 12= 2-ethylhexanoic acid.

Chapter 6

CONCLUSION

Two general methods are presented for the temporary modification of stationary phase selectivity in capillary gas chromatography. In the first a large vapour plug of a modifier of low volatility is injected and immobilised in the stationary phase. As little to no bleeding occurs at the subsequent much lower analysis temperature, no interference with the FID is experienced. In the second case the carrier gas is continuously saturated with a volatile modifier that produces no signal in the FID to dynamically modify the liquid film on the capillary column.

Both methods are reversible and can be implemented in any GC laboratory with minimum alteration to existing equipment. A special loading insert is required in the first instance and a carrier gas saturation chamber in the second.

The process presents a convenient way to study intermolecular interactions and potential selectors for novel GC stationary phases. Dynamic modification may be a general strategy to bypass surface tension related problems in the conventional coating of columns with extremely polar phases.

6.1 Introduction of modifiers of low volatility

A novel method has been developed that dynamically modifies the stationary phase of a capillary column via the vapour phase of selectors that themselves give a strong detector signal. The only proviso is that the volatility of the

selector should be low, preventing excessive bleeding at the temperature required for a particular analysis with the modified column.

A loading of 4.7% (m/m) glycerol in Carbowax could readily be achieved by dynamic gas phase modification. The polarity increase, as measured by the additional retention for the alcohols, is substantial. The original coupling of the Carbowax phase to the glass surface seems to prevent the formation of droplets in favour of the homogenous 'swelling' of the layer with the polar modifier.

Enantiomeric separation could be demonstrated after dynamic modification of a conventional polydimethylsiloxane capillary column with the chiral selector, N-lauroyl-L-valine-t-butylamide. This demonstrates the value of extending vapour phase modification to capillary columns where even small selectivity values, can lead to successful separation by virtue of the high number of theoretical plates available.

The gas phase introduction of the diglycerol, cobalt (II) acetylacetonate and permethylated β-cyclodextrin was not successful due to thermal degradation during the high temperature, high concentration injection step. The lack of success with these modifiers emphasises the role that condensation reactions can play in gas chromatography under conditions of concentration overloading. It seems that these compounds can only be eluted quantitatively in the extreme dilution normally found in GC analysis. It should be kept in mind that other potentially interesting modifiers of low volatility could also present similar problems.

Apart from its application to perform unique analyses on inexpensive, non-specialised capillary columns, the dynamic modification may be useful for preparative packed column separations. The interaction strength between a number of modifiers and sample probe compounds can, furthermore, be studied with a single robust column. This provides a convenient way to test potential selectors for unique gas chromatographic separations and to study the fundamentals of intermolecular interactions without static coating of mixtures or the chemical alteration of existing phases.

6.2 Continuous introduction of water vapour

Dynamic modification of a Carbowax 20M capillary column has for the first time been studied with water vapour at concentration levels close to saturation. Drastic changes to the stationary phase characteristics could be shown with the addition of hydrogen-bonding properties not found in conventional columns. This method can be used with a multitude of volatile modifiers, provided these do not interfere with the mode of detection. Although itself not strongly retained by PEG phases, water has a remarkably strong effect on the retention of OH-containing compounds in comparison with the glycerol introduced as a low volatility modifier.

A shift of 353 Kovats retention units is achieved for methanol, which elutes after dodecane under moist carrier conditions. Hexanol can be eluted far beyond n-tetradecane - a feat difficult to achieve with conventional high polarity phases as these tend to pull into droplets, even during the manufacturing step. The strong retention of alcohols and organic acids may be of interest for the direct analysis of aqueous samples, for example, beverages containing organic acids and alcohols. Elution of alcohols and organic acids

should be after the organic flavour compounds and there should be no additional shifting of retention times due to the water in the sample. The hydrogen-bonding selectivity may be of use in, amongst others, the analysis of alcohol additives in commercial petrol. Controlling the polarity in a continuous way rather than a stepwise manner is of great help with method development. Comparison with the high polarity TCEP column (Alltech) for petrochemical analysis shows that water modified PEG phase gives higher Kovats indices for alcohols and far lower ones for aromatics. Together, this produces a high selectivity for alcohols in the presence of aromatics. Unlike the TCEP phase which provides dipole interaction with polar and polarizable analytes, the PEG/water phase retains mainly OH containing compounds by strong hydrogen bonding interactions. As a result, methanol elutes before benzene on TCEP , whereas it elutes far later than xylene on PEG/water.

An interesting aspect of the modification is that stable retention is only achieved after a couple of hours, indicating slow kinetics in the phase "swelling" process. This process allows the tentative identification of acids and alcohols in unknown samples, as their increasing retention over time clearly distinguishes them from other peaks. The slow kinetics also correlate with the observation by Grob and Habich [12], that not only the amount of aqueous sample injected but also the time interval between subsequent analyses influences the retention of analytes on Carbowax. With the relatively short "conventional" GC retention time of small amounts of water, this would normally not be expected.

This continuous modification could also provide a fast and simple alternative to the qualitative identification of solutes by the comparison of the retention

volumes of known samples. The standard and unkonwn samples could be compared on the same column with and without a modifier in the carrier gas. It is also possible that a small set of standard liquid phases and modifiers could set up to cover the full polarity range covered by the 200~300 commercial liquid phases available [4].

Publications that originated from this work

DYNAMIC MODIFICATION OF THE STATIONARY PHASE IN CAPILLARY GAS CHROMATOGRAPHY: I. INTRODUCTION OF LOW-VOLATILITY SELECTORS

Guay-Chuan Chen and Egmont R Rohwer

Submitted to J. of Chromatography

DYNAMIC MODIFICATION OF THE STATIONARY PHASE IN CAPILLARY GAS CHROMATOGRAPHY: II. CONTINUOUS INTRODUCTION OF WATER VAPOUR

Guay-Chuan Chen and Egmont R Rohwer

Accepted by J. of Chromatography

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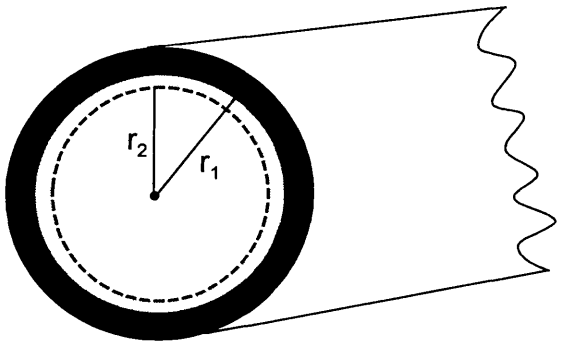
Appendix 1

Preparation of heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin

In an atmosphere of nitrogen, 1 g sodium hydride [55-60 % in paraffin (Fluka)] was washed with petroleum ether 5 times to remove paraffin. It was then transferred into a 0.25-L, three-necked, round-bottom flask equipped with a thermometer, nitrogen inlet, dropping funnel, and reflux condenser fitted with a drying tube. Under nitrogen it was stirred with 10 ml of dry dimethyl sulfoxide (DMSO) at 50°C until the solution became turquoise and the evolution of hydrogen ceased (*ca.* 1 hour). After cooling to room temperature, 1 g of β-cyclodextrin, dissolved in 50 mL DMSO, was added. A gel formed which eventually cleared to a homogeneous, viscous solution. After 1 hour the alkoxide formation was completed. The mixture was cooled to 15°C and 3.5 ml of methyl iodide was added dropwise, at such a rate so that the temperature was kept below 25°C. After *ca.* 20 minutes, the reaction was complete, and a clear, low-viscous solution was formed. A fourfold excess (by volume) of distilled water was added to the solution, and it was extracted 4 times with 35 ml of chloroform. The combined chloroform layers were washed 3 times with 25 ml of distilled water to remove DMSO and then dried over anhydrous sodium sulfate. The solution was concentrated, and the solid residue was recrystallized from chloroform/petroleum ether. Yield: 80%, m.p.: 160°C.

Appendix 2

Calculating the mass of a modifier required for a 10%(m/m) modification of a stationary phase inside a 25 m × 0.3 mm i. d. × 0.25 µm column



Film Thickness = d_f

r_1= Inside radius

$r_1 = 0.3$ mm

$d_f = 0.25\ \mu m = 2.5\times10^{-4}$ mm

Area(A) of film thickness = $\pi(r_1^2 - r_2^2) = \pi[r_1^2 - (r_1-d_f)^2] = \pi (r_1^2-r_1^2 +2r_1 d_f - d_f^2)$

$\because$ d_f^2 is very small

$\therefore A = 2\pi r_1 d_f$

$A = 2\pi r_1 d_f = 2 \times \pi \times 0.3\ mm \times 2.5\times10^{-4}\ mm = 4.71\times10^{-4}\ mm^2$

If column length(L) = 1 m = 1000 mm,

then, volume of film(V) = $A \times L = 4.71\times10^{-4}\ mm^2 \times 1000\ mm = 0.471\ mm^3$

Suppose $1\ mm^3 = 1$ mg, V = 0.471 mg

There is 0.471 mg of a stationary phase per meter

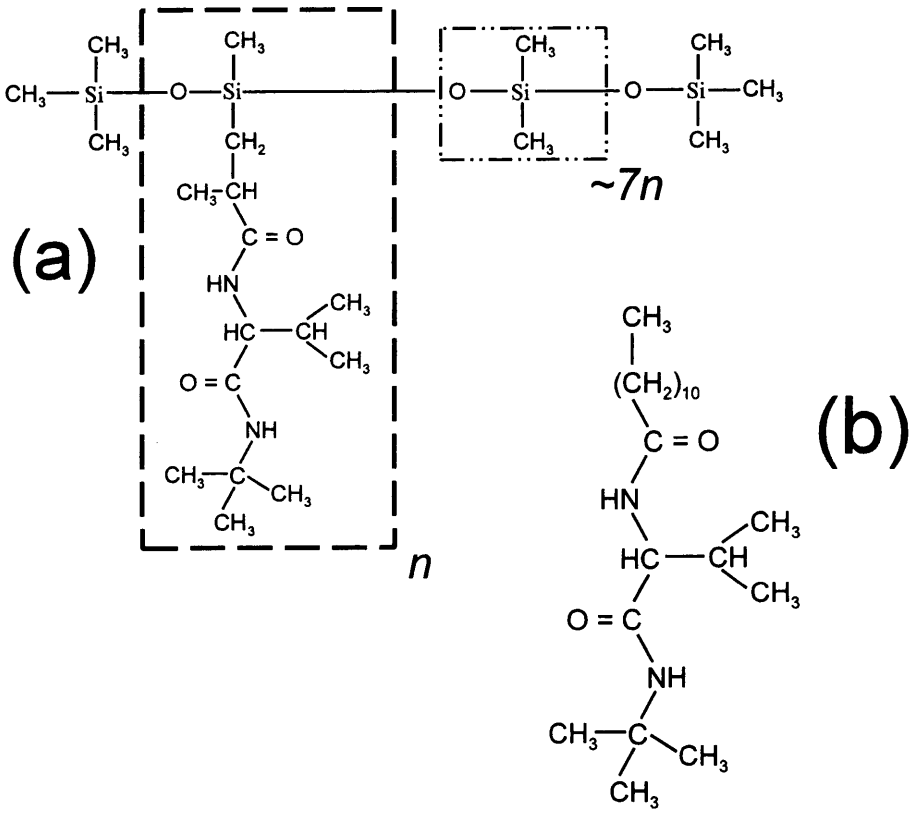
An open tubular column that is 25 meters long will contain 11.775 mg of a stationary phase. Therefore, 1.1775 mg is required for a 10%(m/m) modification of the phase.

Appendix 3

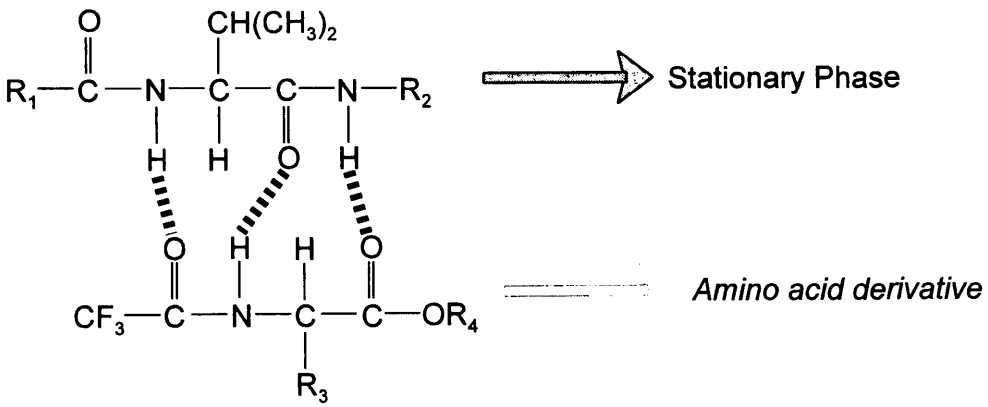
Deactivation of GC glass inlet liner and loading insert with a DMDCS solution (5% dimethyldichlorosilane in toluene)

1. Clean inlet liner overnight with chromic acid.
2. Remove the acid with distilled water.
3. Remove the water with acetone, dry the liner in a oven at 120°C and cool it down to room temperature.
4. Leave the liner in the DMDCS solution overnight.
5. Rinse the liner out with distilled water, dry it in a oven at 200°C for 5 minutes.
6. Rinse the liner out with acetone, dry it in a oven at 200°C for 5 minutes.

Appendix 4



Structures of Chirasil-Val (a) and N-lauroyl-L-valine-*t*-butylamide (b)



The three-point-bonded associate
between *the optical solute* and the chiral stationary phase
(D. W. Armstrong and S. M. Han, in: Critical Reviews in Analytical Chemistry, W. E. Zielinski, Jr. (ed.) **1988**, 19, p. 178)

Appendix 5

Test Mixtures I~IX

Test mixture I -- octane, methanol, nonane, ethanol, decane and undecane in hexane.

Test mixture II -- undecane, dodecane, tridecane, 1-hexanol and tetradecane in methanol.

Test mixture III -- undecane, dodecane, 1-decanol, tridecane and tetradecane in hexane.

Test mixture IV -- the TFA-isopropyl ester (D & L) amino acid mixture (Alltech part no. 19268) made up of alanine, valine, leucine, aspartic acid and methionine in ethyl acetate.

Test mixture V -- benzene, toluene and xylene in hexane.

Test mixture VI -- *tert*-butyl methyl ether (MTBE) and *tert*-amyl methyl ether (TAME) in dichloromethane.

Test Mixture VII -- acetone methyl ethyl ketone and diethylether in hexane.

Test Mixture VIII --acetic acid in hexane.

Test Mixture IX -- octane, methanol, nonane, ethanol, benzene, decane, propanol, toluene, undecane and xylene in hexane.