Characterization of hydrocarbons by gas chromatography : means of improving accuracy

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CHARACTERIZATION OF HYDROCARBONS BY GAS CHROMATOGRAPHY; MEANS OF IMPROVING ACCURACY

(MET SAMENVATTING IN HET NEDERLANDS)

J. A. RIJKS

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(MET SAMENVATTING IN HET NEDERLANDS)

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Dit proefschrift is goedgekeurd door de
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Aan mijn vrouw,
Aan Jackie, Marion,
    Joske en Paul,
Aan mijn ouders.

CONTENTS

INTRODUCTION

Among the most important techniques in analytical chemistry are the separation methods. The separation is followed by the characterization of the individual compounds.

For volatile compounds gas chromatography is the method of choice considering:

- the separation power which is unparalleled by any other technique,
- the minimum sample quantity which is some orders smaller compared with spectroscopic techniques. (Except mass spectrometry).

Although primarely a separation method the retention data directly give qualitative information. Peak areas are proportional to the quantitative composition of the sample.

The most recent developments in gas chromatography have been made in the direction of more sensitive and specific detectors, faster separations and improvement of the quantitative aspects of gas chromatography. Considerably less attention has been paid to the precise determination of retention data. The number of gas chromatographic analysis can be estimated to be about 10^6 a day. The number of publications in this field is about 2000 a year.

In contrast to the importance of qualitative analytical data, the number of papers dealing with improvement of precise and accurate retention data is disappointingly low.

Apart from the characterization of unknown compounds retention data are also used for the calculation of thermodynamic quantities. In both cases the importance of accurate measurements can not be emphasised enough.

For the characterization of a compound by purely gas chromatographic means it is obvious that the more precise the results, the easier and faster the characterization. The number of substances having the "same" retention diminish with the decreasing range of error of the measurements.

Apart from an increase in accuracy the characterization of a compound may be enhanced by using stationary phases of different polarity (ΔI) or one stationary phase at different temperatures (dI/dT) or both.

Structure retention relationships, on two stationary phases of different "polarity" can also give additional information about the identity of an unknown compound in a chromatogram. Logarithmic plots of retention data on two stationary phases allow us to determine the class of compounds, with a limited number of reference compounds (homology lines). In such plots isomers are upon or slightly scattered around straight lines (isomer lines). A further division in sub-isomer groups is possible.

Also in this case the accuracy of the measurements is essential to bring out sufficient structural detail.

The purpose of this thesis is firstly to make a precision of one tenth of a retention index unit attainable in every laboratory and secondly to make an international agreement possible between the data so obtained. The latter purpose can only be achieved if it is realised that it is both necessary and possible to correct for systematic errors.

This will also result in an increasing accuracy of thermodynamic quantities, calculated from gas chromatographic retention data.

Therefore in the first part of this investigation, the influence of several sources of error on repeatability is studied. The measurement and control of instrument factors affecting the reliability are improved as far as possible and reasonable from the point of view of systematic error.

Computer programs were developed for the off-line calculation of reliable peak parameters, including absolute and relative standard deviation of a series of measurements.

Another limitation for the characterization of an unknown compound in a chromatogram is the separation. The properties of different column types have been compared. (Chapter 3).

For the separation of complex mixtures long capillary columns are to be preferred (high resolution and permeability, sharp peaks and low concentrations, no solid support, easier temperature and flow control). For the separation of hydrocarbons a procedure is worked out for the reproducible coating of stainless-steel openhole columns.

The low boiling hydrocarbons are separated with micropacked columns. These columns prepared in our laboratory, according to a new technique, compare favourably with other types of columns. The efficiency of this column type make these very well suited for high resolution work, in those cases where open tubular columns with an appropriate stationary phase are not available.

A low cost self assembled system is described which enables reproducible measurements in the same laboratory of about 0.05 index units for hydrocarbons.

The main reasons for the choice of hydrocarbons in this investigation are:

- the great number of possible isomers, a special complication in qualitative analysis of these compounds,
- reaction chromatography enables the conversion of many classes of organic substances into hydrocarbons. This means an important extension

of the scope of the hydrocarbon retention data,

- a lot of standard hydrocarbons (the complete API collection) are available in our laboratory.

With the refined equipment the influence of several parameters (e.g. temperature, pressure, time measurement etc) is studied. This resulted in the conclusion that a major factor limiting the accuracy of retention data, is the adsorption of the solute at the gas-liquid and gas-solid interface.

A method is proposed to estimate the contribution of adsorption to retention behaviour. Plotting the relative retention time of the solute against the inverse of the capacity ratio, retention data corrected for this adsorption effect are obtained - "ideal" retention data - by extrapolation.

The difference between the retention index measured on a particular column and the corresponding "ideal" value for benzene (adsorption shift), is proposed as a measure for the column inertness.

For a particular substance under identical conditions, the agreement of the retention index, measured in different laboratories, will be the better the smaller the adsorption shift for benzene. Of course the best agreement will be obtained if this shift is zero.

From this point of view this thesis may be seen as a contribution to bridge the gap between a precision of about 0.1 index unit and an accuracy of the same order.

A list of retention data of 170 odd hydrocarbons (C_4-C_9) is presented on a non polar (squalane) and a polar (acetyltributylcitrate-Citroflex A_4) stationary phase.

The potentialities of accurate measurements as a tool for the characterization of compounds in a complex mixture are demonstrated in the last chapter.

CHAPTER I

CHARACTERIZATION OF G.C. PEAKS BY MEANS OF THEIR RETENTION BEHAVIOUR.

1.1 INTRODUCTION.

The analytical as well as the physicochemical significance of gas chromatographic retention data has been recognised since the beginning of gas chromatography.

Up to now gas chromatography is lagging in its identifying ability compared with its separation power, which is unsurpassed by any other technique. The main reason is the poor reproducibility of retention data which characterize a substance.

The standard method for the characterization of a peak in a chromatogram is most frequently based upon measured retention times or related functions of known substances and the coincidence of these values with those of unknown in the chromatogram.

Unfortunately there are several factors which limit the usefulness of this procedure:

- Determination of retention data on a particular column is a tedious process. Because of instability, chemical change (e.g. oxidation) and the loss of stationary phase, this determination is not always a once for all operation.
- For hydrocarbons the pure substances necessary for calibration may be expensive. In many laboratories they are not available.
- The use of not unambiguously defined stationary phases.

- The precision of the determination of retention times may be insufficient.
- More than one compound may have the "same" retention time.
- Incomplete resolution of peaks can lead to the shifting of the peak maximum as discussed by Huber and Keulemans (1) and reported by Ettre (2).
- Mixed separation mechanisms (solution in the stationary phase and adsorption at the phase boundaries) can lead to systematic deviations.

Summarising these limitations it may be concluded that there is a need for tables with accurate retention data, high resolution columns with a negligible adsorption effect and stable and well defined stationary phases.

To increase the precision of the measurements refinement of instrumentation is the first step (3-6). With a high quality instrument the influence of the variation of the process parameters on the quantity to be measured can be estimated. For slight changes of the set values of these parameters a linear relationship between these parameters and the measured quantities may be assumed. The proportionality constants corresponding with these relationships enable the calculation of the extent of control, which is required to achieve a desired precision.

A decrease in the variation of the critical parameters will result in a higher precision of the measurements.

1.2 STANDARDIZATION OF RETENTION DATA.

In order to enable the general use of published retention data, their standardization is required.

Since the beginning of gas chromatography the proper expression of retention data was one of the most discussed problems. This subject is briefly discussed in the appendix.

The retention index was introduced in 1958 by Kováts (7). It was designed in first instance to minimise the discrepancies in the reporting of retention data. In this system retention data are given on a relative basis. n-Alkanes, bracketing the compound to be characterized, are used as reference standards. The interpolation is logarithmic.

Since that time the Kovats index has been advocated by many authors (2, 8-11), as a means for the standardization of retention data. It appears to be widely accepted as such or after appropriate modification nowadays.

A survey of the literature on the retention index, dealing with the concept, its origin, the pro and contra is given by Walraven (12).

1.3 EXCHANGE OF RETENTION DATA BETWEEN LABORATORIES.

The characterization of a compound by purely gas chromatographic means, requires standards for calibration. In many laboratories, for hydrocarbons, these standards are not available.

Therefore comparison of measured data and literature data is necessary. That is, for future work sets of collected retention data will be essential for characterization purposes. Of course the value of such a collection is dependent on the accuracy of the data.

The interlaboratory irreproducibility is the main problem in the characterization of G.C. peaks. A review of the literature, partly accumulated in compilations of retention data (8, 13-15), reveals a lack of agreement between different sources. Large ranges of retention values can be observed for compounds chromatographed under identical conditions on supposedly similar columns. For instance $I_{cyclohexane}$ on squalane as the stationary phase at 100°C shows a discrepancy of 60 index units. On this basis Kovats was right in not expecting decimal places in his index system.

To gain insight into primary factors which are responsible for this large discrepancy, inter laboratory tests have been organised, with standard mixtures.

In 1964 the first test (8) was made with 9 participating laboratories. A six-component mixture was analysed at 3 different temperatures. Packed columns were used, filled with Celite, coated with 20% squalane. For toluene the maximum difference found, was 24 index units. As a chief point came to the fore that the use of a stationary phase of the same batch and preferably unambiguously defined is essential to enable the study of the influence of other parameters.

In 1966 a subgroup of the " Groupement pour l'Avencement des Méthodes Spectrographic (GAMS)", the " Commission de Chromatographie en phase gazeuze", started a series of inter laboratory tests, with 12 participating laboratories.

Loewenguth (16) reported about the evaluation of the results.

A standard mixture of 7 compounds was analysed on capillary columns (length 50-100 m, i.d. 0.25 mm) with squalane as the stationary phase, at 50°C. Methane was considered to be an unretained compound under these conditions. The choice of the equipment and the method of time measurement was free. The number of measurements in each laboratory was 11. The results of this test are summarised in Table 1.1

As a matter of interest the mean values of all the laboratories are compared with retention data finally obtained in our laboratory, with a high quality instrument. Considering the differences between the participating laboratories, the agreement of the mean results of this ring test and our results is surprisingly good.

Table 1.1 Interlaboratory comparison of retention indices of a test mixture of hydrocarbons.

Name	Lowest value	Highest value	Mean	Our Results
Methylcyclopentane	626.37	629.08	626.7	627.9
Benzene	635.33	644.77	638.2	637.2
Cyclohexane	660.70	664.15	662.4	662.7
2,3-Dimethylpentane	670.71	672.30	671.5	671.7
3-Methylhexane	675.42	676.41	676.1	676.2
Trans-3-heptene	686.02	687.83	687.2	687.5
Cis-3-heptene	688.85	690.89	690.2	690.4
standard deviation	0.05<s.d.<0.04			≤ 0.03

As a result of a statistical evaluation Loewenguth concluded that the main factors contributing to the variance of the retention index determinations are the measurement of retention time and the column temperature control.

In our opinion another factor has to be considered. The discrepancy in the maximum difference of the retention index for benzene (9.5 I.u) and cyclohexane (3.5 I.u) cannot be explained by insufficient temperature control alone. The temperature dependence of the retention index for benzene (dI/dT = 0.23 per ^{0}C) and cyclohexane (dI/dT = 0.22 per ^{o}C) is of the same order. Most likely this difference may be attributed to the phenomenon of mixed separation mechanisms, as discussed in Chapter 2.

Exchange of retention data in this way can give only an idea about the order of the deviations and the main reasons for these deviations. Improvement of the accuracy of retention data can be obtained only up to a certain limit by this procedure and it will require much effort.

Therefore we have chosen for a different approach, which enables independent variation of the parameters influencing the precision and accuracy. A correlation between the variation of the individual experimental parameters and the resulting fluctuation in the retention, enables the calculation of the extent of control of these parameters which is required to achieve a desired precision.

1.4 PREDICTION OF RETENTION DATA.

In addition to a direct characterization by coincidence methods (comparison of the retention quantity of the unknown with standard compounds or tabulated retention data), the calculation of retention data must be mentioned.

A large number of correlations between molecular structure and gas chromatographic retention has been established. It was Kovats (17) who summarised these regularities in six general rules.

Since that time many authors have proposed calculation methods to predict retention data. Roughly these methods can be divided in two groups:

- Methods using only physical constants (e.g. boiling points, vapour pressures and activity coefficients calculated theoretically).
- Calculations based on the additivity principle of structural increments, which have been determined by gas chromatography.

The advantage of the first group is that the retention data can be calculated without any information about the gas chromatographic behaviour of the compounds. The agreement between calculated and measured values, however, is poor. Even for hydrocarbons differences up to 15 index units can be expected.

The second type of calculations is based upon:

- Number, nature and position of functional groups.
- Number, type and position of bonds.
- Polarity and geometry of the stationary phase.

In this case the agreement between calculated and measured values is dependent on the quantity of pre-information used in the calculation. The reliability of the predicted retention value increases with an increasing number of structural elements involved in the calculation.

This means that the number of compounds which have to be measured, to enable the calculation of the contribution of the structural increments, also increases. The consequence is that the number of compounds for which the retention can be predicted is decreasing. Amongst others Schomburg (18-20) and Soják (21-23) have studied extensively the potentialities of these calculation methods, to predict retention data for hydrocarbons.

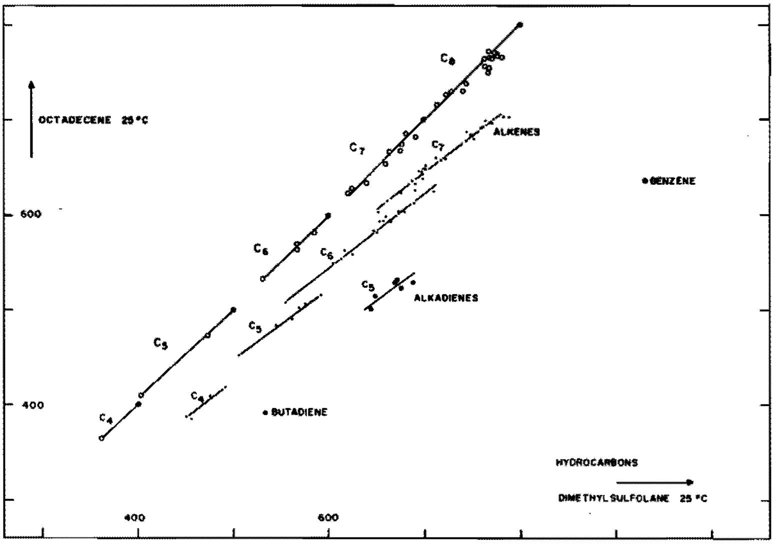

Fig. 1.1 Retention index plot on two phases of different polarity.

The possibilities of the application of structure retention correlations for characterization purposes have been demonstrated by Tourres (24) and Walraven (12). They have plotted retention indices measured at two different temperatures or two different stationary phases. Isomers differing only in the degree of branching, thus containing the same functional groups, are scattered around parallel lines of slope 1. They form imbricated series as demonstrated in Fig. 1.1 and 1.2.

The reliability of predicted retention data, by graphical or calculation methods, is also dependent on the accuracy of the measured retention data.

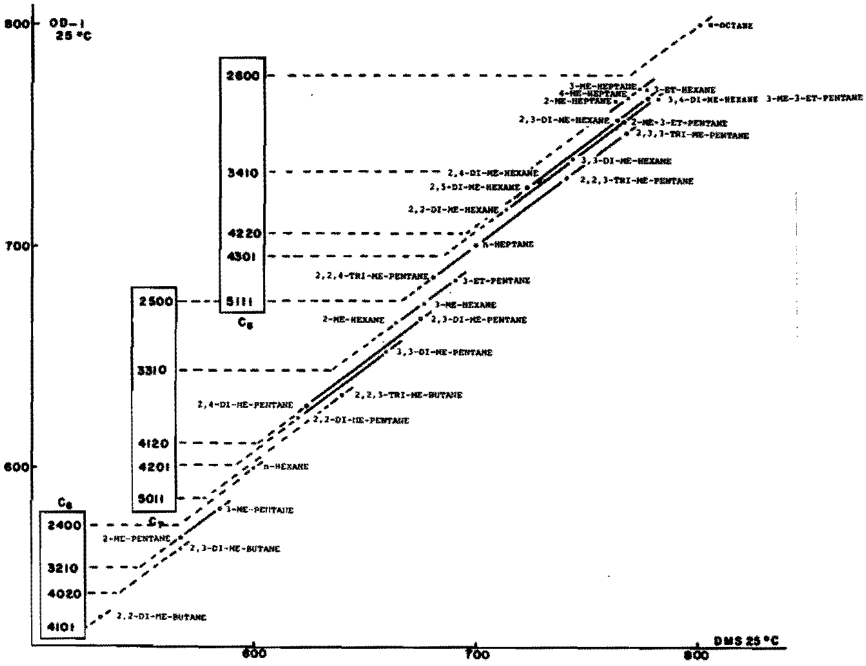

Fig. 1.2 Fine structure of retention index plot.

1.5 COMBINATION OF GAS CHROMATOGRAPHY AND MASS SPECTROMETRY.

Because of its importance in qualitative analytical chemistry the combination GC-MS will be discussed very briefly. The direct coupling GC-MS appears to be an ideal combination. That is, if the conditions of both methods are optimal and the problems involved in the coupling are solved.

The mass spectrometer provides more structural information than any other technique with the small sample size offered by capillary columns, which have an unsurpassed separation power.

The area in which a mass spectrometer is not entirely satisfactory for component identification is distinction between structural and geometrical isomers (e.g. hydrocarbons).

The high cost and complex data handling are severe obstacles to the wider use of this combination. For the moment there are also some practical difficulties limiting their applicability:

- sensitivity for column bleeding (choice of the stationary phase).
- capillary link between gas chromatograph and mass spectrometer (delay of sample, peak broadening, condensation of sample and stationary phase).

It may be expected that in near future these problems will be solved. Considering the development of chemical ionisation sources and single - or multiple - ion detection, the potentialities of this combination are obvious.

For qualitative analysis of hydrocarbons this combination may not be considered as the final solution. The identification in this case is complicated by the great number of possible isomers.

1.6 STATEMENT OF THE PROBLEM.

The use of subsequent analytical techniques for the identification of compounds separated by gas chromatography is limited seriously because of the quantities of sample required. For instance, the only instrument which can be used in conjunction with open-hole tubular columns for identification purposes is the mass spectrometer. For several reasons this combination can not be used in any laboratory and for any identification problem.

It is mainly due to this lack of complementary identification methods that, in spite of their excellent separation power and high sensitivity, open tubular columns equiped with ionisation detectors, have found very limited application in the qualitative analysis of complex mixtures.

Considering this, it may be concluded that it is highly desirable to have means available for the characterization of gas chromatographic peaks by retention data only.

The poor interlaboratory agreement of retention data has given rise to the belief that G.L.C. methods are fundamentally unsuited for characterization purposes.

Nevertheless these techniques provide a powerfull method for the characterization and structural studies of organic compounds. It will be evident that the process of characterization on the basis of G.C. retention parameters will gain in efficiency and reliability if highly discriminating columns are used and the accuracy of the measurements is improved.

The importance of the resolution can be reduced by using more columns of different polarity or the same columns at different temperatures. However, for complex mixtures the recognition of corresponding peaks is rather complicated, even if a quantitative estimation of the peak areas is involved in the determination.

Isolation of a peak eluted from the first column and rerunning on a second column of different polarity can overcome this difficulties. In most cases the application of this techniques is limited by the quantity of sample, especially for open tubular columns. Therefore a system (described in Chapter 4) is developed for open tubular columns with which all the components separated on the first (apolar) column can be directed to a second (polar) column individually.

Since the characterization of a compound by gaschromatographic means is an elimination method, obviously, the number of compounds to be differentiated from the unknown compound decrease if the accuracy with which a particular retention value can be measured is increased.

To improve the accuracy of the measurements the influence of the fluctuations of experimental and physical process parameters have to be investigated and decreased to such a level that the required accuracy is achieved.

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CHAPTER II

SOURCES OF INACCURACY IN THE MEASUREMENT OF RETENTION DATA.

2.1 INTRODUCTION.

Improvement of the interlaboratory agreement of retention data will greatly enhance the usefulness of published retention data, for the characterization of unknown peaks in a chromatogram. However, there is always some element of doubt in any assignment which is based merely upon retention data.

Improvement of the accuracy of retention data can always be justified if tabulation of retention data is intended. For the characterization of a compound in a complex mixture improvement of accuracy will be only significant when the separation power keeps step with this improvement. The interval in retention time, within which a compound can be expected (window), reflects the uncertainty in both, reference values and measured values.

According to Klein and Tyler (1), who assume that the retention times of the peaks in a chromatogram will be randomly distributed, the probability (p_n) of finding n solutes in a time interval δt is given by:

$$p_n = \frac{e^{-\rho} . \rho^n}{n!} \qquad (2.1)$$

where ρ is the peak density, which may be generally be expressed as:

$$\rho = \frac{\text{total number of peaks}}{\text{total number of divisions}} = \frac{N}{\Delta t/\delta t} \qquad (2.2)$$

where N is the number of possible eluates in a time interval Δt and δt is the smallest difference in retention time, which can be detected. At a 95% confidence interval $\delta t = 4\sigma_t$, σ_t being the standard deviation of a series of measurements.

The probability of simultaneous elution (p_{se}) of two or more solutes in an interval δt follows from equation 2.1:

$$p_{se} = 1 - e^{-\rho}\,(1+\rho) \tag{2.3}$$

Calculation of ρ for different values of p_{se} allows the prediction of the maximum permissible number of compounds (N_p) in a given time interval Δt, if the accuracy of the measurements is known, as shown in Table 2.1.

Table 2.1 Influence of the accuracy on the maximum permissible number of compounds (N_p) in a time interval Δt.

p_{se}	ρ	N_p	
		$\delta t = 0.01\ \Delta t$	$\delta t = 0.001\ \Delta t$
0.1	0.53	53	530
0.05	0.36	36	360
0.01	0.15	15	150
0.001	0.05	5	50

In order to reduce the probability of confusion between two or more compounds to the one-in-a-thousand level, for $\delta t = 0.01\ \Delta t$, no more than 5 compounds are acceptable in a time interval Δt.

Obviously the degree of certainty of the characterization ($1-p_{se}$) can be increased by a reduction of ρ. This is equivalent to a reduction of δt or N. The former involves improvement of accuracy. The number of possible eluates (N) can be reduced by making use of alternative information (e.g. pre- information about the origin of sample), multidimensional systems (columns of different polarity or different temperatures) and class-separations in preceding steps.

Improvement of accuracy on to a certain level, which requires more sophisticated instrumentation, is only justified when resolution is sufficiently high. The relation between accuracy and resolution will be discussed below.

The calculation of the maximum permissible number of compounds (N_p) in a time interval Δt, as presented in Table 2.1, was based on the assumption that two or more compounds in a time-interval δt cannot be distinguished. The resolving power needed, expressed as the required plate number, is determined by the last two compounds in the interval Δt, having the largest peak width (Fig. 2.1).

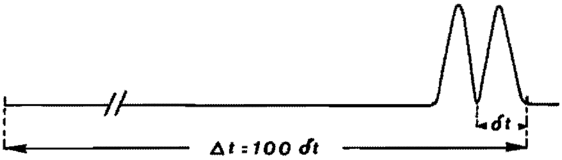

Fig. 2.1 Situation of the maximum required plate number.

Assuming that these 2 compounds have identical σ_p values (standard deviation of a Gaussian peak), the required plate number can be calculated by the equation:

$$n = \left(\frac{t_{R,1} \cdot R_{min.}}{\delta t} \right)^2 \qquad (2.4)$$

where $t_{R,1}$ is the retention time of the last compound in the interval Δt. $R_{min.}$ is the minimum resolution required to separate these compounds to such an extent, that the peak shift caused by incomplete separation, can be neglected. The minimum resolution required depends also on the ratio of the peak areas, as will be discussed in section 2.6.3.

The maximum plate number, required to separate the maximum permissible number of compounds in a time interval Δt (N_p) as given in Table 2.1, is presented in Table 2.2.

Table 2.2 Maximum number of theoretical plates required ($n_{req.}$) for the separation of the maximum permissible number of compounds (N_p), in a time interval Δt

	$n_{req} \times 10^{-6}$	
Δt	δt = 0.01 Δt	δt = 0.001 Δt
Δt_{C6-C5}	2.89	289
Δt_{C7-C6}	1.22	122
Δt_{C8-C7}	0.74	74

Although N_p is decreasing with decreasing p_{se}, the required number of plates will be the same for all p_{se} values, because in all cases the last two compartments of length δt can be occupied.

For the calculation an interval (Δt) is chosen between two n-alkanes. The retention times used for this calculation were measured on an open hole tubular column (length: 100 m. int.diam.: 0.25 mm. stationary phase: squalane, temperature: 50°C, $k_{n\text{-hexane}} = 0.65$ at 50°C) The ratio of the peak areas is assumed to be 1, $R_{min} = 4$.

As can be seen from Table 2.2, at a given precision, the maximum required plate-number is decreasing with increasing carbon number. The number of possible isomers, however, is increasing rapidly with increasing carbon number. The probability that the last two compartments of length δt, in the interval Δt, both will be occupied is also dependent on the number of possible compounds in this interval. If the last two compartments are not occupied both, the separation power needed will be determined by the last two compounds, which occupy two neighbouring compartments in the interval considered.

Therefore the prediction of the required plate number, which is in agreement with a given precision, is hardly possible. The great importance of the use of high resolution columns for the characterization of a compound in a complex mixture, however, is clearly demonstrated in this way.

Of course for thermodynamic studies and listing of retention data of pure compounds, improvement of accuracy is less strongly dependent on the resolution.

The performance of the column, which sets limits to the separation attainable, is compared for different types of columns in Chapter 3.

To improve the precision and accuracy of the measurements the elimination of the relevant sources of error in the measurement of retention data, as far as possible and reasonable, is necessary.

Therefore in this thesis, as a contribution to improve the agreement of retention data between laboratories, not only random errors but also systematic errors will be discussed.

The distinction between random and systematic errors cannot be made very sharply in all cases. However, in this thesis a subdivision in these two types of errors is preferred for the sake of clearness.

2.2 LITERATURE SURVEY.

Considering the importance of accurate retention data for qualitative analysis, it is surprising that the first publications, reporting precise retention data (2.3) appeared only in 1967. Since that time an increasing interest in this subject can be observed.

Several authors (4-8) studied the influence of fluctuations of experimental parameters on the retention time and related functions. They calculated statistically the error in the final retention value from the error propagation coefficient of the experimental parameters, using the existing theoretical relationships.

With packed columns and a specially designed gaschromatograph Wicarova, Novak and Janâk (6) obtained a precision corresponding to a coefficient of variation of 0.2% for the specific retention volume.

Goedert and Guiochon (4) reported an excellent agreement between the overall precision calculated from the error propagation coefficients of the individual parameters and the experimental values of absolute retention time. They concluded that the main limiting factors are the measurement and control of temperature, in- and outlet-pressure and the method of retention time determination. At a precision level of 0.01%, which is extremely difficult to obtain, even with the most sophisticated instrumentation, retention time measurements can only be carried out with a computer.

Oberholtzer and Rogers (5) constructed a gaschromatograph which is capable of measuring relative retention data with a precision better than 0.02%.

The importance of precise retention data for structure-retention correlations is demonstrated by Tourres (4) and Keulemans et al. (9-13).

2.3 DEFINITIONS AND TERMS.

To avoid confusion in terminology, random and systematic errors, precision and accuracy, repeatability and reproducibility, in this thesis will be defined as follows:

Random error is the effect of a sequence of errors beyond control.

Systematic error is the significant discrepancy between the average of measurements and the true value of a quantity.

Precision is an indication for the agreement between successive measurements, with one instrument under similar conditions, independent of any systematic error involved.

Accuracy refers to the closeness of a measured value and the true value of a quantity.

Repeatability is the random variation of successive measurements of one sample, analysed with one instrument, by one operator, under similar conditions.

Reproducibility is the random variation of a quantity obtained by different operators, on different instruments, in one or more laboratories, under similar conditions.

2.4 RANDOM ERRORS.

2.4.1 Introduction.

The error sources which cause the random variation of retention times can be divided into two categories. The first category concerns the experimental parameters (e.g. temperature and carrier gas-flow) and the time measurement itself. The second category consists of errors resulting from the partition process and concerns deviations from the linear distribution (overloading, adsorption and composition of sample), resulting in concentration dependent retention times.

The relation between these factors and retention time, for an ideal carrier-gas, is given by the equation (14):

$$t_R = \frac{4.L^2.\eta}{\kappa} (1+k) \frac{P_i^3 - P_0^3}{(P_i^2 - P_0^2)^2} \qquad (2.5)$$

t_R = retention time.
L = column length.
η = viscosity of carrier-gas.
κ = permeability of the column.
P_i = inlet pressure of the column.
P_o = outlet pressure of the column.
k = capacity ratio.

Because of stringent requirements on the constancy of temperature and pressure of the chromatographic system, if precision measurements are intended, the influence of the viscosity of the carrier-gas and the permeability of the column is negligible. The capacity ratio (k) depends on both the temperature and the average column pressure. However, measurements of Desty and others show (15-17), that the pressure dependence of k is very small. A variation of 10^{-3} atm. corresponds with a variation of k in the order of 0.003%, with carbon dioxide as the carrier-gas. With nitrogen or hydrogen the effect is much smaller.

Therefore, in the following sections the influence of temperature and time measurement will be discussed, as are overloading effects and composition of sample.

2.4.2 Variations of Flow-rate due to Pressure Fluctuations.

Carrier-gas flow-rates for open-hole tubular columns are usually of the order 0.5-5 $ml.min.^{-1}$. Regulation of these small flows with flow regulaters is practically impossible. Therefore with this column type regulation of flow is restricted to in- and outlet pressure regulation and control. Experimentally there are two methods to control the flow-rate through an open tubular column:

- Both inlet and outlet pressure are controlled.
- Only the pressure drop over the column is controlled.

A variation in the outlet pressure is partly compensated in the inlet pressure with the last method. For practical reasons this is the method of choice, for measurements of relative retention data.

The error propagation coefficients for the absolute retention times are given in this case by the equation (14):

$$\frac{dt_R}{t_R} = \frac{p^2P_o}{(2P_o+p)\ (3P_o^2+3P_op+p^2)}\ \frac{dP_o}{P_o} - \frac{(P_o+p)\ (6P_o^2 + 3P_op + p^2)}{(2P_o + p)\ (3P_o^2 + 3P_op + p^2)}\ \frac{dp}{p} \qquad (2.6)$$

where $p = P_i - P_o$.

Calculation of the error propagation coefficients for P_o and p shows, that the influence of fluctuations in p is one order of magnitude higher than the influence of the variation of P_o.

For a constant outlet pressure (P_o = 1 atm.) and a fluctuation of 0.002 atm. in the inlet pressure, the relative variation of the absolute retention time is given as a function of the pressure-drop (p) in Fig. 2.2.

For open-hole tubular columns longer than 50 m, at normal operating conditions ($p > 1$ atm., P_o = atmospheric), a precision better than 0.2% in absolute retention time can be obtained when the fluctuation of in and outlet pressure is less than 0.002 atm.

Assuming that the variation of atmospheric pressure will not exceed 0.002 atm., during one analysis, the precision of relative retention times will be even better.

The influence of long term drift of in- and outlet pressures is neglected here because only relative retention data are considered in this investigation.

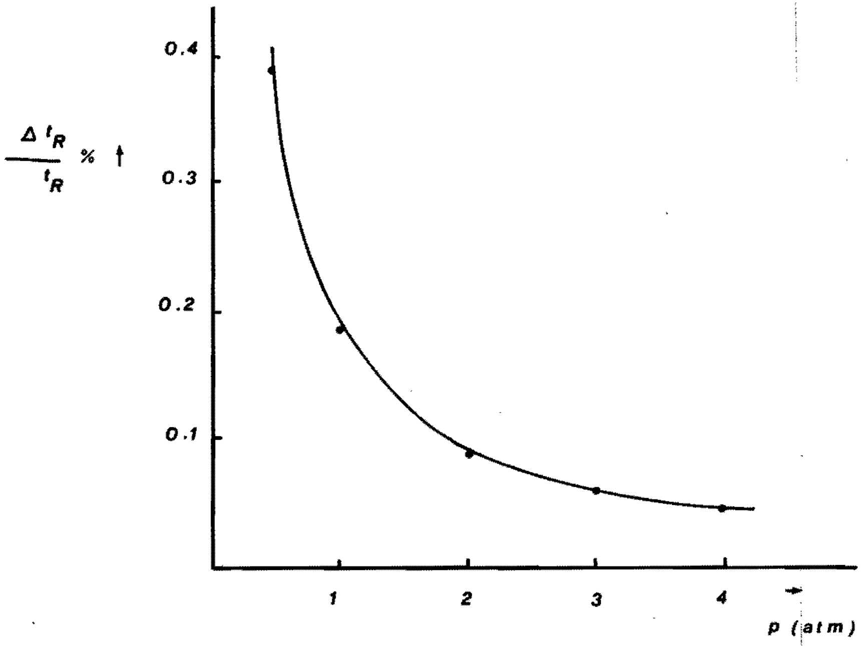

Fig. 2.2 The relative variation of the absolute retention time as a function of the pressure drop (p), for a fluctuation of 0.002 atm. in the inlet pressure, at constant outlet pressure.

The influence of upstream pressure (supply pressure) fluctuations (ΔP_s) on the performance of pressure controllers, normally used in our laboratory, is studied with the equipment given in outline in Fig.2.3.

The operating conditions (flow-rate, in- and outlet pressure) are in agreement with the conditions normally encountered in a G.C. system provided with long open tubular columns (100 m) and an inlet system with splitter.

For two representative measurements the results are given in Fig. 2.4. The upstream pressure variations after pressure controller 1 may be estimated to be less than 3%.

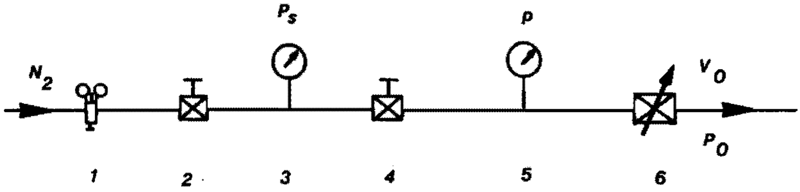

Fig. 2.3 Measuring schematic for the comparison of pressure controllers.

1. Conventional 2 stage pressure controller (LOOS CO,Amsterdam).
2. Pressure controller (BECKER,Delft, Type MB 19936).
3. Differential manometer (WALLACE AND TIERNAN, FA 145).
4. Pressure controller to be tested.
5. Differential manometer (WALLACE AND TIERNAN, FA 145).
6. Needle valve substituting the open-hole tubular column with inlet splitter.

With a Becker pressure controller at position 2 and 4 a pressure drop variation (Δp) of less than 0.1% has been obtained, for a variation of 1 atm. of the supply pressure, P_s.

Long term stability and thermal stability of the pressure controllers are not considered because only relative retention data are the subject of interest in this study.

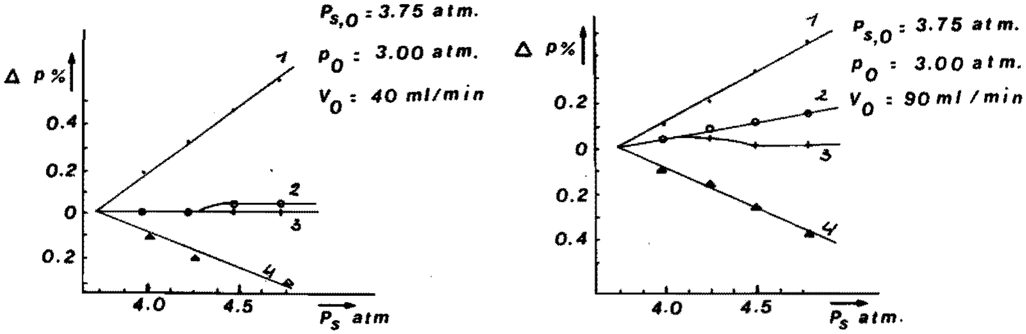

Fig. 2.4 Performance of pressure controllers as a function of upstream pressure fluctuations (P_s). 1 = NEGRETTI AND ZAMBRA (R/182), 2 = NORGREN (11-018), 3 = BECKER, Delft (MB -19936), 4 = BROOKS (8600).

2.4.3 Temperature Fluctuations.

The acceptable fluctuation of the column temperature is related to the temperature coefficient (dI/dT) of the compound of interest. For hydrocarbons, a temperature constancy of 0.03°C allows a precision of 0.01 index units. In other cases, e.g. steroids, this constancy corresponds with a precision of 0.1 index units.

The temperature constancy of the column ovens of a number of commercial gaschromatographs was investigated. Temperature measurements were made at 12 different positions, homogeneously distributed in the oven, with carefully calibrated thermocouples. The results were very disappointing. Dependent on the temperature level, inside one oven temperature differences between 1 and 25°C could be observed (Fig. 2.5).

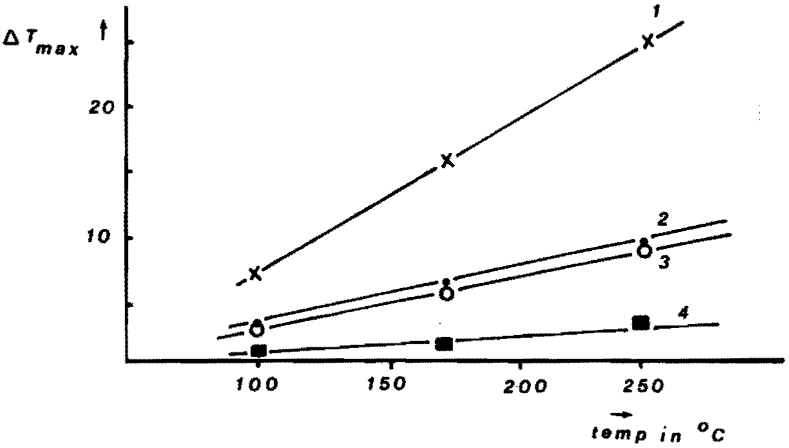

Fig. 2.5 Temperature gradients in thermostats of commercial gaschromatographs. 1 = BECKER, Delft (409-prototype), 2 = BECKER, Delft, G.C. oven (1452 D), 3 = HEWLETT-PACKARD (5750), 4 = PERKIN ELMER (800).

Temperature fluctuations measured at a fixed position, during 8 hours, were between 0.1 and 0.5°C at

100°C. Short term fluctuations were about half these values.

Therefore for accurate measurements a liquid thermostate (TEV 70, TAMSON, Zoetermeer) has been selected, which allows a temperature control at any point inside the thermostate, within 0.01°C. The temperature difference inside the liquid bath was measured with a quartz thermometer (type 2801 A, HEWLETT-PACKARD) provided with two sensors, so that the resolution for differential measurements was about 0.001°C. The reference sensor was fixed in the middle of the bath. The second sensor was placed successively at different positions, symmetrically around the centre; the distance of this sensor from the side wall, the top and the bottom level was more than 5 cm. The maximum difference observed, during 8 hours, between all the points was 0.01°C at 50° and 70°C.

The temperature variations at one point, during 120 hours, corresponds to a standard deviation of 0.005°C.

2.4.4 Measurement of Retention Time.

To have the full profit of a better measurement and control of temperature and pressure, the random error of the retention time measurement itself should match to the precision of these factors.

This random error is dependent on the method of time measurement, the estimation of the moment of injection and the uncertainty of the position of the peak top.

For compounds with short retention times the influence of errors in time measurement will be relatively high. Because of peak broadening, for compounds with long retention times the estimation of the peak top can be problematic (flat peaks).

Therefore the influence of the method of time measurement and column length, on the precision of retention data, will be briefly discussed.

With a gaschromatograph assembled in our laboratory (described in section 2.5) and a 100 m. open tubular column (stationary phase: squalane, int.diam.:0.25mm., T= 70^{o}C) the following methods of time measurement have been compared:

- electronic integrator,
- stopwatch,
- distance measurements on the chromatogram,
- digitizer unit and subsequent off-line processing of the paper-tape.

The least reliable results were obtained with the electronic integrator (INFOTRONICS - model C.R.S. 11 H.B. /41). With this instrument time measurements are in whole seconds. The registered place of the peak top is dependent on the width of the peak because of quenching and slope detection, both resulting in a shift of the peak top.

For the other methods the repeatability of retention times and retention indices are compared in Table 2.3 and 2.4.

Table 2.3 Repeatability of absolute retention times for different methods of time measurement (paper velocity: 1.15 mm/s).

Component number	Mean retention time in sec.	Standard deviation in %		
		distance	stopwatch	digitizer
1	792.2	0.27	0.04	0.02
2	864.1	0.24	0.04	0.01
3	911.5	0.23	0.05	0.01
4	1086.6	0.19	0.05	0.02
5	1139.6	0.17	0.06	0.02
6	1283.5	0.15	0.07	0.01
7	1349.6	0.14	0.05	0.01
8	1462.5	0.12	0.06	0.02
Number of measurements		11	11	6

Table 2.4 Repeatability of the retention index for different methods of time measurement.

Component number	distance		stopwatch		digitizer	
	I	s.d.	I	s.d.	I	s.d.
1	538.41	0.09	538.40	0.049	538.41	0.005
2	568.95	0.07	568.85	0.041	568.41	0.007
3	585.11	0.07	585.12	0.039	585.10	0.003
4	630.90	0.04	630.93	0.025	630.94	0.006
5	641.91	0.04	641.91	0.031	641.90	0.005
6	667.12	0.03	667.11	0.038	667.02	0.005
7	676.95	0.04	676.91	0.034	676.91	0.005
8	692.07	0.04	692.05	0.025	692.05	0.006
n	11		11		6	

A block diagram and a short description of the digitiser system, developed in our laboratory (18), is given in section 2.5.

The relative standard deviation of retention time and retention index measurements is decreasing in the order, distance > stopwatch > digitizer-computer. For retention index measurements with a precision better than 0.1 index unit, the use of a computer to calculate the retention time from the peak shape is necessary. Measuring retention times with a stopwatch a precision of about 0.1 index unit can be expected.

The influence of column length on precision of relative data is demonstrated in Fig. 2.6. For the interpretation of this figure it must be considered that for a constant relative retention time, an increase of column length results in an increase in absolute retention time. For instance, an adjusted retention time of 500 seconds, corresponds with a relative retention time of 1.3 for the 100 m column and with 5.2 for a 20 m column. For compounds with low capacity ratio's (short retention times), the random error is relatively high, as confirmed by the left hand side of the plots. The difference in precision for compounds with long retention times, on columns of different lengths, can be explained considering the retention time of the standard (n-hexane). The error in retention time of the standard is relatively large for a short column.

It can be concluded that for a 100 m open-hole tubular column with a digitizer-computer system relative retention data can be obtained with a precision better than 0.02%, for hydrocarbons eluting between C_5 and C_9.

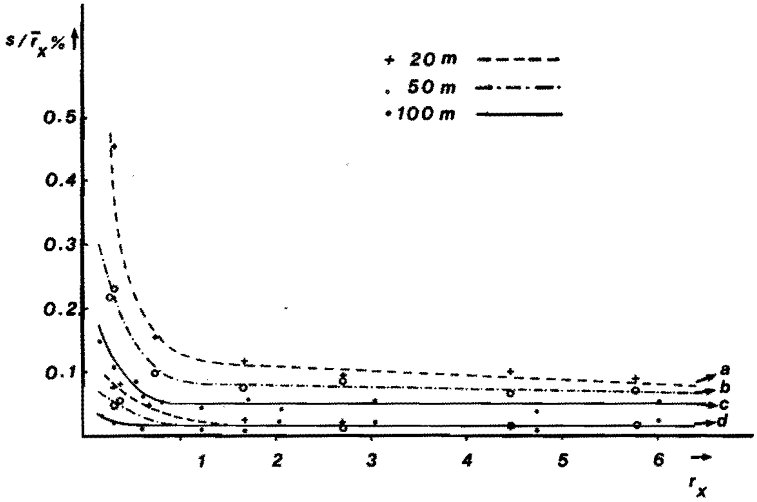

Fig. 2.6 Precision of relative retention time as a function of column length. a-c: measured by stopwatch. d: measured with a digitizer computer system.

2.4.5 Influence of the Quantity of Sample.

Because of the stringent requirements on the symmetry of the peaks, as will be discussed in Chapter 3, the influence of sample size may be neglected in this investigation. With synthetic mixtures it was confirmed, that an increase in sample size with a factor 10, had no influence on the accuracy of retention indices. The quantity of sample per compound was always between 10^{-8} and 10^{-10}g.

2.4.6 Effect of Sample Composition.

Interference effects between compounds slightly differing in partition coefficient may influence the precision of the measurements. Because of solution of

Table 2.5 Retention indices of hydrocarbons in samples of different composition on squalane,at 70°C. M = methyl, B = butane, P = pentane, H = hexane, C = cyclo, D = di, T = tri.

Component	mixt. 1	mixt. 2	mixt. 3	mixt. 4
2,2 DMB	538.40	538.40		
1,1,2 TMCP		550.33	550.33	550.34
3 MP	585.12	585.14	585.10	585.12
2 MP			570.08	570.01
MCP	630.93	630.96		
CH	667.15	667.17		
3 MH	676.91	676.94		
2,2,3 TMP		740.11	740.20	
Number of measurements	12	6	6	6
Mean standard deviation	0.04	0.03	0.04	0.04

one or more compounds of the sample, the properties of the stationary phase would be slightly changed, so that the partition coefficient, at least in principle might be dependent on the composition of the sample.

Experimentally this possible effect was investigated with 4 different synthetic mixtures, consisting each of about 30 compounds, which were completely or almost completely separated. Retention indices measured with a 100 m squalane column at $70^{\circ} \pm 0.01^{\circ}C$, of compounds present in two or more of these mixtures are given in Table 2.5.

The results justify the conclusion that this effect can be neglected.

2.5 EXPERIMENTAL CONDITIONS AND SYSTEM DESIGN.

All the experiments are done with a self assembled system, because of special requirements of pressure and temperature control and sample introduction.

The pressure drop over the column is controlled in three stages. The first stage is a conventional pressure controller (e.g. LOOS-CO., Amsterdam) normally used on gas cylinders. It delivers a gas-flow under a pressure of 4-5 atm., with possible fluctuations of less than 3%. For the second and third stage two pressure controllers (BECKER, Delft, type MB-19936) are placed in series.

This permits a pressure constancy, during one analysis, of about 0.002 atm. The pressure drop over the column is measured with a differential manometer (WALLACE and TIERNAN model F.A. 145). This pressure regulation system allows measurement of retention indices, with a precision of about 0.1% (see Section 2.3.2).

No further improvement in precision could be obtained with a TEXAS INSTRUMENTS precision pressure controller (type

150-01 and 150-02), provided with the pressure gauge (model 145). The precision of this system is 10^{-5} atm.

For the temperature control a liquid thermostate(TEV 70, TAMSON, Zoetermeer), is used. The temperature constancy at any point inside the bath is better than 0.01 degree, between 50 and 70°C (see Section 2.4.3).

Since direct injection systems for open hole tubular columns are still in the development stage, a sample introduction system with splitter is employed (HAMILTON inlet system, with splitter). The temperature of the injection was always 20°C above the boiling point of the highest boiling compound, as proposed by Cramers (2).

To permit full utilisation of the column performance and to realise a small input peak, any dead space or mixing volume in the sampling and detection system has to be avoided.

Therefore split ratios between 1/300 and 1/500 have been used in all experiments. The residence time in the mixing tube of the sample introduction system was less than 0.2 seconds.

Varying the split ratio between 1/300 and 1/500, no influence could be observed, on the accuracy of the retention indices of hydrocarbons of different structural type and boiling point.

The measurements are done on stainless steel columns, 0.02" o.d., 0.01" i.d., bright finish (HANDY and HARTMAN Co, Norristown, Penna U.S.A.). The column temperature was held at 50 and 70°C ± 0.01°C. The column liquids were squalane (MERCK, Darmstadt) and Citroflex A4 (APPLIED SCIENCE LAB). The number of theoretical plates for the squalane columns (length 100m) was about 5.10^5, for the Citroflex A4 columns (50 and 75 m) between 2.10^5 and 3.10^5, for n-hexane. The carrier gas was nitrogen of high purity. The detector was a commercially available flame ionisation detector (BECKER, Delft), coupled to a low noise (10^{-15}A) amplifier, developed in our laboratory. The sensitivity of the detection system was 10^{-11}A/10mv (full scale). Sample sizes varied between 10^{-8} and 10^{-10}g per component. The time measurements were done with a stopwatch. Only during the last period of this study a digitizer unit with subsequent off-line processing of the paper tape was available. The outline of this data acquisition system is given in Fig. 2.7.

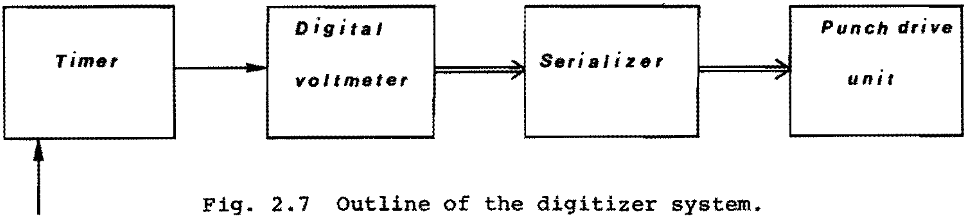

Fig. 2.7 Outline of the digitizer system.

After depressing the start button the timer unit starts sending pulses to the digital voltmeter at a preset rate (0.2, 0.5, 1, 2 and 5 sec.). Each pulse initiates the digital voltmeter to take a reading of the analog signal. When the reading is taken, the digital voltmeter sends a conversion complete signal to the serialiser. After serialising, the measurement is punched on paper tape. The retention time of the peak is calculated on the base of five point fit or centre of gravity. The precision of time measurements with this system is about five times better compared with the stopwatch. The drawback of the system up to now is that with a sampling rate of 2 times per second, the maximum analysis time is about 60 minutes.

2.6 SYSTEMATIC ERRORS.

2.6.1 Introduction.

Sofar, only the influence of random errors on the precision of relative retention data has been discussed.

The interlaboratory agreement of retention data, however, mainly depends on systematic errors, which cannot be easily detected.

The main factors affecting the accuracy of retention data which will be discussed in the next sections, are the following:

- definition and stability of the stationary phase,
- incomplete separation,
- non ideality of carrier gas,
- interfacial adsorption,
- gas hold-up time,
- operator.

2.6.2 Definition and Stability of the Stationary Phase.

The general usefulness of published retention data is drastically limited by the wide and indiscriminate use of stationary phases.

Compilations of literature data present lists of retention data on about 250 stationary phases, at different temperatures (19-21).

Improvement of this situation can only be expected if the number of stationary phases is strongly reduced by international agreement.

This implicates a careful selection of the best stationary phases. To enable this selection the stationary phases must be characterized, so that their properties can be compared. A literature survey of this subject, discussed by several authors, is given by Walraven (12).

The complexity of the standardization of stationary phases was clearly demonstrated at the Lausanne Symposium in 1969 (22). In an informal discussion the general opinion of the participants was that for the time being this procedure is premature. Because of a lack of generally accepted norms for the choice, the most important requirements for the selection and specification of the stationary phase were summarised in 10 statements.

Therefore, in the writers opinion, the stationary phase should be an unambigeously defined pure substance. Mixed stationary phases, e.g. P.E.G., apiezon, etc, should be avoided.

Column characteristics should not change during use, because of loss, or chemical changes of stationary phase. Oxidation due to traces of oxygen in the carrier gas is one of the most common reasons for changes in the nature of stationary phase. Therefore the use of high purity carrier gases and removal of traces of oxygen is strongly recommended.

In addition to these requirements in the ideal case, the stationary phase selected should fulfil the following conditions:

- sample components should have a reasonable solubility in the liquid phase and exhibit different partition coefficients,
- the vapour pressure of the liquid phase must be negligible,
- the liquid phase should be thermally stable,
- the stationary phase should be chemically inert towards the sample components under separating conditons.

Considering these requirements squalane is chosen as the a-polar phase and acetyltributylcitrate as the polar phase, in this investigation. An additional argument for the choice of squalane was that a lot of retention data for this phase are available in the literature for comparison.

2.6.3 Incomplete Separation.

Incomplete separation of two compounds will result in a shift of the position of the peak maxima. This shift depends on the ratio of the peak areas of the peaks, the distance (Δt) of the two original peak maxima and the width (expressed as the standard deviation) of the peaks.

The magnitude of the shift of the peak maxima can be computed assuming:

- the shape of a peak follows a normal distribution,
- the standarddeviation of two neighbouring peaks is equal and independent of sample size.

In practice these assumptions will be justified for high-resolution columns, which allow the introduction of small samples.

The peak shape, being the result of superposition of two original peaks at a distance $R\sigma$, can be described with the following equation (23):

$$f(t) = \frac{A_1}{\sigma\sqrt{2\pi}}\, e^{-\frac{1}{2}\left(\frac{t}{\sigma}\right)^2} + \frac{A_2}{\sigma\sqrt{2\pi}}\, e^{-\frac{1}{2}\left(\frac{t-R\sigma}{\sigma}\right)^2} \qquad (2.7)$$

Where A_1, A_2 = area of the peaks.

σ = standard deviation of the peaks.

R = resolution.

t = retention time.

Differentiating this equation and substituting $u = \frac{t}{\sigma}$, the peak maxima and minimum can be calculated by numerical methods from:

$$F(u) = \gamma u e^{-\frac{1}{2}u^2} + (u-R)\ e^{-\frac{1}{2}(u-R)^2} = 0 \qquad (2.8)$$

with $\gamma = \frac{A_1}{A_2}$.

In this equation γ is the ratio of the peak areas (e.g. for $\gamma = 100$, the area of the second peak is 1% of the area of the first).

It can be seen from the computer calculated peak shifts, given in Table 2.6, that the minimum resolution required to find two peak maxima is increasing with increasing γ. For two peaks with equal peak areas the minimum resolution required is 2.5. When the area of the second peak is 1% of the first a minimum resolution of 4.5 is needed.

Since the peak shift is calculated in $\frac{t}{\sigma}$ units, it also depends on the plate number of the column and the capacity ratio.

The plate number (n), the retention time (t_R) and the capacity ratio (k) can be expressed as:

$$n = \left(\frac{t_R}{\sigma}\right)^2, \quad t_R = t_M + t_R' \quad \text{and} \quad k = \frac{t_R'}{t_M} \qquad (2.9\text{-}2.11)$$

From this equation it follows:

$$\sigma = \frac{t_R}{\sqrt{n}} \cdot \frac{k+1}{k} \qquad (2.12)$$

If the shift of the peak top is $u\sigma$, the relative error in the adjusted retention time (t_R') will be:

$$\frac{\delta t_R'}{t_R'} = \frac{u}{\sqrt{n}} \cdot \frac{k+1}{k} \qquad (2.13)$$

Table 2.6 Peak shift of maxima and minima of incompletely resolved peaks for different peak area ratios.

Peak shift in $\frac{t}{\sigma}$ units

R	$1/\gamma=1$	$1/\gamma=0.5$	$1/\gamma=0.1$	$1/\gamma=0.05$	$1/\gamma=0.01$
			first maximum		
0.5	0.25	0.16	0.04	0.02	-
1.0	0.50	0.29	0.06	0.03	0.01
1.5	0.75	0.31	0.05	0.02	0.01
2.0	1.00	0.18	0.03	0.01	-
2.5	0.15	0.06	0.01	0.01	-
3.0	0.04	0.02	-	-	-
3.5	0.01	-	-	-	-
4.0	-	-	-	-	-
4.5	-	-	-	-	-
5.0	-	-	-	-	-
			minimum		
0.5	-	-	-	-	-
1.0	-	-	-	-	-
1.5	-	-	-	-	-
2.0	-	-	-	-	-
2.5	1.25	-	-	-	-
3.0	1.50	1.92	-	-	-
3.5	1.75	2.04	2.81	-	-
4.0	2.00	2.23	2.78	3.04	-
4.5	2.25	2.44	2.89	3.09	3.57
5.0	2.50	2.66	3.04	3.22	3.61
			second maximum		
0.5	0.25	-	-	-	-
1.0	0.50	-	-	-	-
1.5	0.75	-	-	-	-
2.0	1.00	-	-	-	-
2.5	2.35	-	-	-	-
3.0	2.96	2.92	-	-	-
3.5	3.49	3.48	3.39	-	-
4.0	4.00	4.00	4.00	3.90	-
4.5	4.50	4.50	4.50	4.49	4.48
5.0	5.00	5.00	5.00	5.00	5.00

The dependence of the retention index on the peak shift can be calculated in the following way:
The retention index is defined as:

$$I_x = \frac{\ln t'_x - \ln t'_z}{\ln t'_{z+1} - \ln t'_z} \cdot 100 + 100z. \qquad (2.14)$$

Where t'_x is the adjusted retention time of a component x and t'_{z+1} and t'_z are the adjusted retention times of the bracketing n-alkanes, z being the number of carbon atoms.

Since only t'_x is influenced by the peak shift, $\ln t'_{z+1}$ and $\ln t'_z$ can be considered as constant. Differentiation of equation 2.14 gives:

$$\frac{\delta I_x}{\delta t'_x} = \frac{100}{\ln t'_{z+1} - \ln t'_z} \cdot \frac{1}{t'_x} \qquad (2.15)$$

Since $\ln t'_{z+1} - \ln t'_z \approx 0,4$ (Table 2.11) for a squalane column between 50^o and 70^{o}C, this results in:

$$\delta I_x \approx 250 \frac{\delta t'_x}{t'_x} \qquad (2.16)$$

The maximum peak shift will be found for $\gamma = 1$ and $R = 2$. In this case the error in retention time and retention index, calculated by equation 2.13 and 2.15 is presented in Fig. 2.8 as a function of the capacity ratio for different plate numbers. These plate numbers can be considered to be representative for packed and open hole tubular columns of moderate length.

These results stress the importance of the use of high resolution columns for the determination of accurate retention data.

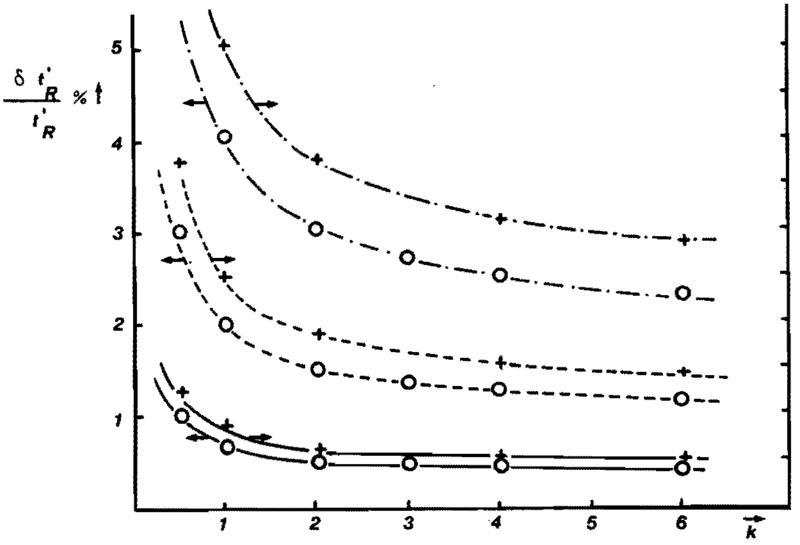

Fig. 2.8 Maximum errors in retention data caused by incomplete separation as a function of capacity ratio (k) and plate number (n).
—— $n = 9.10^4$, ······ $n = 10^4$, —·—·— $n = 2500$. $R = 2$, $\gamma = 1$.

For columns used in this work ($n \simeq 5.10^5$ and $0,5 < k < 1$ for n-hexane, at 50°C) the influence of the peak shift is demonstrated in Fig. 2.9. The maximum

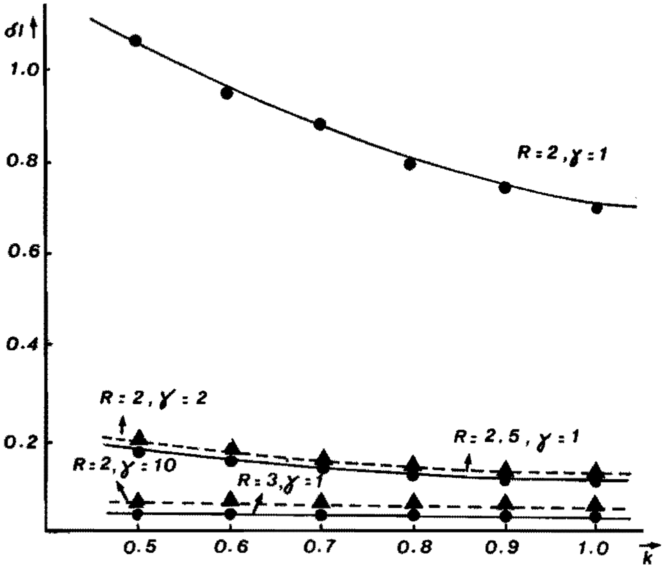

Fig. 2.9 Dependence of the peak shift on resolution, peak area ratio and the capacity ratio of n-hexane ($n_{n\text{-hexane}} \simeq 5.10^5$).

peak shift ($R=2$, $\gamma=1$) is between 0.7 and 1.1 index units for capacity ratios for n-hexane between 0.5 and 1.0. For a change in resolution from 2 to 2.5 or a change in γ from 1 to 2 the maximum peak shift of the first peak will be between 0.1 and 0.2 index units.

2.6.4 Non Ideality of Carrier Gas.

According to Desty (15) small variations of solute retention due to the choice of carrier gas and mean column pressure have been examined for a number of hydrocarbons.

Because of the variation of solute retention with the carrier gas and mean column pressure these parameters should be included when retention data are reported.

To explain these variations in solute retention, caused by gas-phase imperfection, various equations have been suggested, as reported by Young (24). According to Cruickshank, Windsor and Young (25) the relation between the capacity ratio (k) and the mean absolute column pressure ($P_oJ_3^4$), can be given with sufficient accuracy for carrier gases, which have small gas phase imperfections, by the equation:

$$\ln k = \ln \frac{nRT}{V_1' p_2^o \gamma_{2,0}^\infty} - \frac{p_2^o}{RT}(B_{22} - V_2') + \frac{P_oJ_3^4}{RT}(2B_{12} - V_2') \qquad (2.17)$$

where

$$J_3^4 = \frac{3\{(P_i/P_o)^4 - 1\}}{4\{(P_i/P_o)^3 - 1\}}$$

n = number of moles of the stationary phase.

V_1' = volume of the stationary liquid at T°K.

p_2^o = saturated vapour pressure at T°K.

$\gamma_{2,0}^\infty$ = activity coefficient at infinite dilution.

B_{22} = second virial coefficient, characterizing the interactions between solute molecules in the gas phase.

B_{12} = second virial coefficient, characterizing the interaction between solute and carriergas molecules.

V_2' = molar volume of the solute at $T^{o}K$.

P_i = column inlet pressure.

P_o = column outlet pressure.

For a particular solute and stationary phase at constant temperature equation 2.17 can be written in the more simple form:

$$\ln k = \ln k_0 + \beta \qquad (2.18)$$

where

$$\beta = \frac{P_o J_3^4}{RT} (2B_{12} - V_2')$$

By definition the retention index can be expressed:

$$I = 100 \left(z + \frac{\ln r_{x,z}}{\ln r_{z+1,z}} \right) \qquad (2.19)$$

where $r_{x,z}$ = the relative retention time of a component x, using a standard n-alkane with z-carbon atoms.

It can be derived from equations 2.18 and 2.19

$$\delta I = \frac{100}{\ln r_{z+1,z}} \cdot \Delta (\beta_x - \beta_z) \qquad (2.20)$$

For a squalane column at 50°C, according to Table 2.11, the pressure dependence of the retention index can be calculated by the equation:

$$\delta I \approx 230 \, \Delta(\beta_x - \beta_z) \qquad (2.21)$$

Only for a limited number of hydrocarbons B_{12} values for the system nitrogen/squalane are published. The dependence of the retention index on column pressure is calculated for some compounds and presented in Table 2.7. Experimental results for the same hydrocarbons are also given in this Table.

Table 2.7 Dependence of the retention index on mean column pressure ($P_oJ_3^4$). Stationary phase:squalane. Carrier gas: N_2. Temperature: 50°C. B_{12} values: Reference 26.

Component	$B_{1,2}$	$\beta \times 10^2$		δI	
	$cm^3/mole$	$P_oJ_3^4=2.31$	$P_oJ_3^4=3.78$	Calc.	Exp.
n-Pentane	- 85	- 2.52	- 4.11		
n-Hexane	- 113	- 3.16	- 5.16		
2,3 dim.Butane	- 98	- 2.91	- 4.77	- 0.27	0.02
Hexene-1	- 110	- 3.05	- 4.97	0.80	0.05
Cyclohexane	- 122	- 3.05	- 4.97	0.18	0.23
Benzene	- 104	- 2.77	- 4.52	0.76	0.28

The agreement between calculated and experimental results is rather poor, although, the order of magnitude of calculated values lines up with experimental data. Considering the precision of available B_{12} values and their questionable accuracy, a better agreement can not be expected.

The influence of pressure and nature of the carrier gas on the retention index, as measured in our laboratory, is presented in Table 2.8.

Remembering the precision of the retention index, corresponding with a standard deviation of < 0.05 index units, it can be said that the retention index depends on mean column pressure, nature of carrier gas and the structure of hydrocarbons.

Table 2.8 Retention indices of different types of hydrocarbons for different carrier gases and their dependence on mean absolute column pressure. Stationary phase: squalane, column length: 100m, i.d: 0.25 mm, temperature: 50°C ± 0.01°C.

Carrier gas	$P_oJ_3^4$	Compound number										
		1	2	3	4	5	6	7	8	9	10	12
Helium	2.305	500	549.20	567.39	582.03	600	606.00	627.61	636.59	662.37	676.23	700
	3.034	"	549.24	567.39	582.04	"	606.00	627.61	636.61	662.36	676.23	"
	3.774	"	549.20	567.37	582.04	"	605.99	627.62	636.60	662.37	676.23	"
Nitrogen	2.308	"	549.19	567.39	582.05	"	606.24	627.87	637.07	662.73	676.23	"
	3.034	"	549.19	567.40	582.08	"	606.32	627.95	637.20	662.83	676.23	"
	3.773	"	549.21	567.41	582.10	"	606.41	628.04	637.35	662.96	676.23	"
Hydrogen	2.303	"	549.18	567.38	582.02	"	606.08	627.69	636.73	662.48	676.23	"
	3.037	"	549.18	567.40	582.04	"	606.11	627.75	636.79	662.54	676.25	"
	3.770	"	549.15	567.39	582.05	"	606.15	627.76	636.83	662.57	676.24	"

Compound numbers agree with Table 2.9.

Little can be said about the magnitude of this structural effect, since only a limited number of hydrocarbons of different structural type have been measured.

For helium the variation of mean column pressure does not influence the retention index, for any type of hydrocarbons. The same applies to hydrogen and nitrogen only for alkanes. For hydrogen also no effect for alkenes can be observed. For nitrogen the effect is increasing in the order alkenes < cyclic compounds.

The value of pressure and the nature of carrier gas should be carefully noted when using published retention data.

Comparing retention data of different origin, correction factors for column pressure and nature of carrier gas can be derived from Table 2.8.

2.6.5 Interfacial Adsorption.

In most practical cases solute retention will mainly depend on the bulk-liquid partition coefficient. However, more often than is generally recognised the solute may also be retained as a result of adsorption at one or more interfaces in the system.

Solid supports for the stationary phase have long been known to interact with several types of solutes and preventive measures (e.g. acid washing, silanisation, addition of tail reducers) are widely taken.

According to Urone and Parcher (27), support effects can be observed even in columns with 20% or more stationary phase.

Interactions of the solute with the gas-liquid interface received little theoretical and no practical consideration until Martin (28,29) demonstrated that solute adsorption on the bulk-liquid surface (Gibbs adsorption) exists for polar phases.

This result was reproduced and extended by Pecsok (30) and confirmed by static measurements (31-33).

According to Martire (34) the effect of adsorption on the liquid surface is small compared to solution effects, except in those instances when a highly polar liquid coating is used and the solute is only slightly soluble.

In practice, however, it appears difficult to determine the separate contributions of bulk-liquid partition and liquid-interfacial and solid support adsorption.

Assuming that all these retention mechanisms operate essentially independently, the contribution to the adjusted retention volume (V'_x) of a compound is given by equation (35, 36):

$$V'_x = K_l V_l + K_{g,l} A_l + K_s A_s \qquad (2.22)$$

In this equation K_l, $K_{g,l}$ and K_s are respectively the gradients of the distribution isotherm for the retention mechanism in the bulk-liquid, gas-liquid interface and the solid support. A_l and A_s are the active surface areas of the liquid and the support. V_l is the total volume of the liquid phase in the column.

The bulk-liquid partition coefficient can be obtained by plotting V'_x/V_l vs. $1/V_l$ and extrapolating $\frac{1}{V_l} \longrightarrow 0$.

For asymmetrical peaks, assuming that the asymmetry is not caused by extra column factors, V'_x becomes concentration dependent. Adjusting the sample size to maintain constant inlet concentration, the variation of the distribution coefficients can be eliminated experimentally as described previously (37).

Even at constant solute concentration in gas and liquid phase, the plot of V'_x/V_l vs $1/V_l$ cannot be expected to be linear, since both A_l and A_s are functions of V_l.

These problems can be reduced by taking the following measures:

- reduction of sample size so that retention times become concentration independent (constant distribution coefficients, symmetrical peaks).
- decrease of liquid- and support surface.
- improvement of the precision of the measurements.

These conditions are fulfilled to a great extent in open-hole tubular columns and also partly in efficiently packed columns with an internal diameter less than 1 mm (e.g. micropacked columns). Considering as a first approximation, $K_{g,l}A_l$ and K_sA_s to be constant for these column types equation 2.22 can be rewritten:

$$V'_x = K_lV_l + A_x \qquad (2.23)$$

$A_x = K_{g,l}A_l + K_sA_s$ for a component x.

At effective infinite dilution (symmetrical peaks) a linear relationship can be expected, plotting V_x'/V_l vs. $1/V_l$.

Relative retention data derived from bulk liquid partition coefficients,obtained from these plots, will be not very precise, because of variation of V_x' and V_l.

To improve the precision of calculated relative retention data, corrected for adsorption effects, equation 2.23 can be rearranged. The relative retention time can be expressed as :

$$r_{x,s} = \frac{V'_x}{V'_s} \tag{2.24}$$

where V'_x and V'_s are the adjusted retention volumes of the component and the standard.

Assuming that for the selected standard $A_x = 0$, it follows from equation 2.23:

$$V'_s = V_l \cdot K_{l,s} \tag{2.25}$$

$K_{l,s}$ being the bulk-liquid partition coefficient of the standard.

Substituting eqn. 2.23 and 2.25 in eqn. 2.24, yields:

$$r_{x,s} = \frac{V'_x}{V'_s} = \frac{K_{l,x}}{K_{l,s}} + \frac{A_x}{K_{l,s}} \cdot \frac{1}{V_l} \tag{2.26}$$

From the relation between partition coefficient ($K_{l,s}$), capacity ratio ($k_{l,s}$) and phase ratio $\left(\frac{V_l}{V_g}\right)$ of the standard it follows:

$$V_l = \frac{k_{l,s}\, V_g}{K_{l,s}} \tag{2.27}$$

Finally substituting eqn. 2.27 in 2.26, yields:

$$r_{x,s} = \frac{K_{1,x}}{K_{1,s}} + \frac{A_x}{V_g} \cdot \frac{1}{k_{1,s}} \qquad (2.28a)$$

Plotting $r_{x,s}$ vs. $\frac{1}{k_{1,s}}$, relative retention times corrected for adsorption effects ("ideal" relative retention times), can be obtained as the intercept at $1/k_{1,s} = 0$ (Fig. 2.10

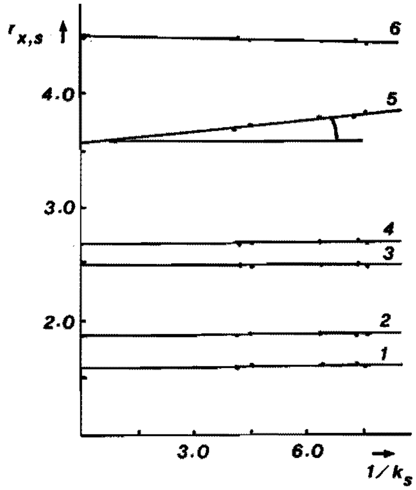

Fig. 2.10 Dependence of the relative retention time on film thickness (expressed as 1/k for n-pentane) Columns: stainless steel, length 100m, i.d. 0.25 mm, stationary phase: squalane, temperature 70°C ± 0.01°C.

The slope of this plot is a measure for the adsorption effect. Equation 2.28a can be rearranged as following:

$$k_x = \frac{A_x}{V_g} + \frac{K_{1,x}}{K_{1,s}} \cdot k_{st} \qquad (2.28b)$$

Plotting k_x vs. k_{st}, the intercept at the k_x axis is a measure for the contribution of adsorption (Fig.2.11). In this case the "ideal" relative retention time can be calculated from the slope of the plot.

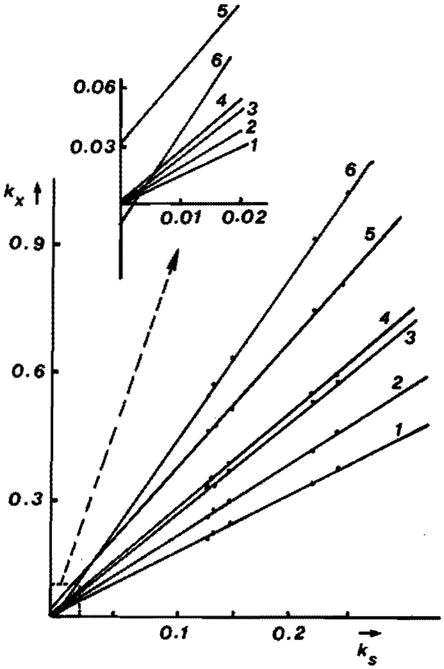

Fig. 2.11 Dependence of the capacity ratio of different hydrocarbons (k_x) on liquid phase content (expressed as k of n- pentane). Columns: see Fig. 2.10

Because relative retentions and capacity ratios can be determined more precisely than V'_x and V_l, corrections for the contributions of adsorption to solute retention will be more reliable.

In Fig. 2.12 as an example the retention index for different types of hydrocarbons is presented as a function of the percentage of stationary phase

According to Condor (37) the product $K_{g,l}A_l$, for liquid coatings above 5%, changes only slightly. Therefore the large deviations, especially for unsaturated compounds, below 5%, liquid coating must be caused mainly by support adsorption. For instance for benzene, between 2 and 10% stationary phase a difference of about 10 index units is observed.

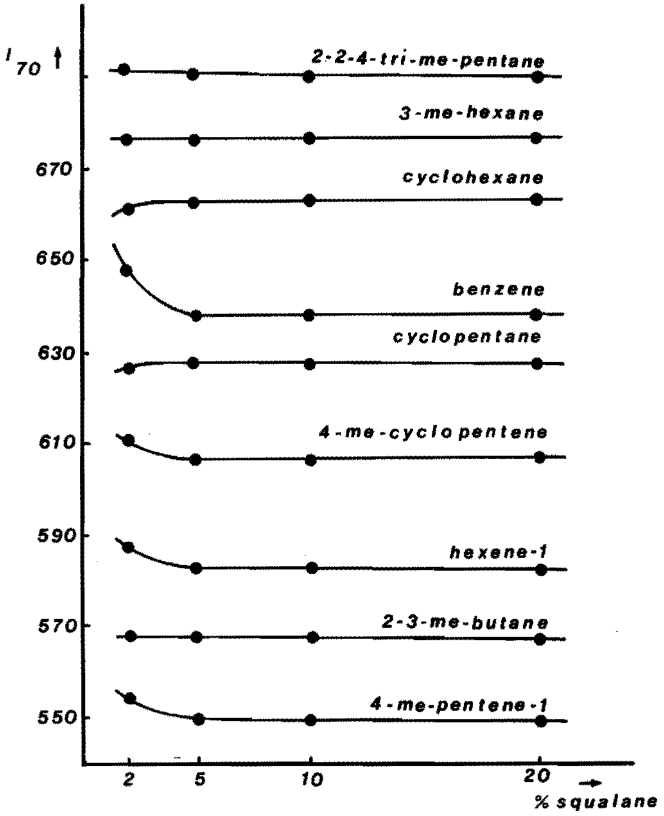

Fig. 2.12 Retention index as a function of percent (w/w) stationary phase (squalane) at 50°C. Support: Chromosorb W, acid washed.

Although, only a limited mumber of hydrocarbons of different structural type have been measured, it seems that, except for cycloparaffins, the retention index is decreasing with increasing degree of loading.

For benzene and cyclohexane, which give the largest deviations, it was experimentally proved (38) that the adsorption isotherms are of the same type. For these reasons and because all peaks were nearly symmetrical, it seems that the contribution of adsorption to solute retention is increasing in the order cylco-paraffins < alkanes < alkenes < aromatics.

Table 2.9 Comparison of "ideal" and measured relative retention times of hydrocarbons on squalane, at 70°C.

Comp. n°.	Solute	$r_{\text{"ideal"}}$			$r_{\text{meas.}}$
		Micropacked		Open-hole tubular	Open-hole tubular
		Chrom.W n.a.w.	Chrom.W.A.W.		
1	n-pentane	0.402	0.402	0.401	0.402
2	4m,pentene-1	0.634	0.634	0.635	0.637
3	2,3 dim butane	0.754	0.753	0.752	0.753
4	hexene-1	0.848	0.850	0.851	0.855
5	n-hexane	1	1	1	1
6	4m.cyclopentene-1	1.082	1.082	1.079	1.084
7	m.cyclopentane	1.318	1.318	1.312	1.318
8	benzene	1.430	1.422	1.425	1.452
9	cyclohexane	1.818	1.819	1.812	1.819
10	3m.hexane	1.982	1.980	1.985	1.985
11	2.2.4 tri-m pentane	2.266	2.264	2.277	2.271
12	n.heptane	2.430	2.430	2.440	2.436

For different types of hydrocarbons, relative retention times corrected for adsorption effects, calculated by eqn, 2,28 a and b, are presented in Table 2.9. The intercepts at $1/k_{1,s} = 0$ are calculated with a computer (method of least squares) for open-hole tubular columns and micropacked columns. The deviations from linearity of the $r_{x,s}$ vs $1/k_{1,s}$ and k_x vs. $k_{1,s}$ plots were within experimental error.

To perform this extrapolation different $k_{1,s}$ values are required. For the open-hole tubular columns (length 100 m, int. diam. 0,25mm) these $k_{1,s}$ values were obtained

by recoating the column several times ($0.3 < k_{l,s} < 0.8$ for n-hexane, at 50°C). For the micropacked columns (length 15m, int. diam 0.8 mm) two different supports, (chromosorb W a.w. and n.a.w.) coated with 2, 5, 10,and 20 % The stationary phase was squalane (MERCK, Darmstadt).
The column temperature was 70 ± 0.01°C and the carrier gas nitrogen.

Because calculated ("ideal") values are obtained by extrapolation, they can be expected to be less precise than the values experimentally obtained. Within experimental and calculation errors the accuracy of relative retention data of alkanes and cycloparaffins is not influenced by adsorption effects for the open-hole tubular column considered. ($k_{n\text{-hexane}} = 0.7$ at 50°C). For benzene and also for unsaturated hydrocarbons the influence of adsorption cannot be neglected. Comparing the difference between experimental and "ideal" relative retention (adsorption shift) and remembering Fig. 2.11, it can be concluded that benzene is most strongly adsorbed.

In the following an attempt is made to decide if the observed adsorption effect is due to either support adsorption or adsorption at the gas-liquid interface.

Assuming that adsorption at the gas-liquid interface can be neglected, the adsorption shift will depend on the nature of the support and/or column wall, the degree of liquid loading and the regularity of the liquid film. In this case it may be expected that the adsorption shift will decrease with increasing film-thickness. Plotting k_x vs $k_{l,s}$ these plots will pass the origin for all hydrocarbons if the liquid film is homogeneously distributed on support and/or column wall. For an irregular liquid film the adsorption shift can be expected to be different for various supports and column walls, which have dissimilar surfaces.

For benzene, having the largest adsorption shift, it can be concluded from experimental results, presented in Table 2.10, that neither the first nor the second possibility is observed in practice. The relative retention data, experimentally obtained with different supports and column types, are constant within experimental error. The difference between the experimental and "ideal" values is significant.

Table 2.10 Experimental and "ideal" relative retention data of benzene for different types of squalane columns, at 70°C.

Column	Support or column material	% stationary phase (w/w)	Experimental		"Ideal"	
			r	I	r	I
micropacked	Chrom.W.a.w.	20	1.451	641.4	1.425	639.3
micropacked	Chrom W n.a.w.	20	1.453	641.5		
micropacked	glass-beads	0.1	1.451	641.4		
open-hole	stainless-steel	-	1.452	641.4		
open-hole	glass	-	1.456	641.7		

Therefore the results can be considered as an indication that at high liquid loading, the contribution of adsorption to solute retention for benzene on squalane is mainly caused by adsorption at the gas-liquid interface. For low liquid loadings adsorption at the support plays a major role.

The retention indices, presented in Table 2.10, are calculated by the equation:

$$I_x = \frac{\ln r_{x,z}}{\ln r_{z+1,z}} \cdot 100 + 100\, z \tag{2.29}$$

where $\ln r_{z+1,z}$ is the separation factor of the bracketing n-alkanes, having z respectively z+1 carbon atoms.

For the calculation of the "ideal" retention index of benzene the apparent separation factor of benzene is used. This factor is obtained by interpolation of the separation factors of n-alkanes, presented in Table 2.11.

The procedure described before can also be applied to study the aging of the column. As is generally known the quantity of stationary phase is decreasing during life-time. For a 100m open-hole tubular squalane column experimentally a decrease of the capacity ratio of n-hexane at 50 °C from 0.80 to 0.56 was found, during 18 months. At the same time the retention index of benzene increased from 641.7 to 642.3.

With this column, plotting $r_{x,s}$ vs $1/k_{1,s}$ for benzene an "ideal" relative retention time of 1.426 was obtained as the intercept, $1/k_{1,s} = 0$.

This can be seen as an indication that the influence of the chemical change of the stationary phase (e.g.oxidation) is neglegibly small. The change of retention data, as a result of aging of the column, is most likely mainly caused by the "stripping" effect.

As a final result of the foregoing considerations the adsorption shift of benzene is proposed as a measure for the interlaboratory agreement of relative retention data, for hydrocarbons.

2.6.6 The Gas Hold-up Time.

The retention time of an inert compound (t_m) which passes through the column without being retarded (usually helium or air) must be known for the calculation of relative retention data.

Direct measurement is not always possible e.g. the flame ionisation detector, under normal operating conditions does not respond to helium or air. Therefore methane is often used, its retention time being taken as t_m. Methane, however, is soluble to some extent in almost every stationary phase. This may introduce a systematic error in that way.

Table 2.11 Separation factors of n- alkanes. z = the number of carbon atoms.

z	Log $r_{z+1,z}$				
	Our results		Results obtained by Martire (39)		
	T = 50°C	T = 70°C	T = 53.2°C	T = 74.1°C	T = 93.9°C
4	0.4562	0.4144			
5	0.4366	0.3945	0.4360	0.3934	0.3504
6	0.4287	0.3861	0.4275	0.3823	0.3512
7	0.4250	0.3823			
8	0.4215	0.3785			

Many suggestions have been made for the calculation of the "dead time". All these calculation methods utilise the linear relationship that would exist, between the logaritm of the adjusted or relative retention times and the number of carbon atoms in a homologuous series.
In fact this relationship is not linear. For the higher members of the series, the non linearity is decreasing, as shown in Table 2.11 and Fig. 2.13 for the separation factors (ln $r_{z+1,z}$) of consecutive n-alkanes.

The separation factor of the n-alkanes is an important parameter for the recalculation of retention data presented in a different way (retention index, relative retention, n-nonane values etc), to enable a comparison of these data.

The consequence of the continuous decrease of the separation factor, suggested by Fig. 2.13 is the introduction of an apparent separation factor.

This factor can be obtained by interpolation of the separation factors of the n-alkanes bracketing the compound considered.

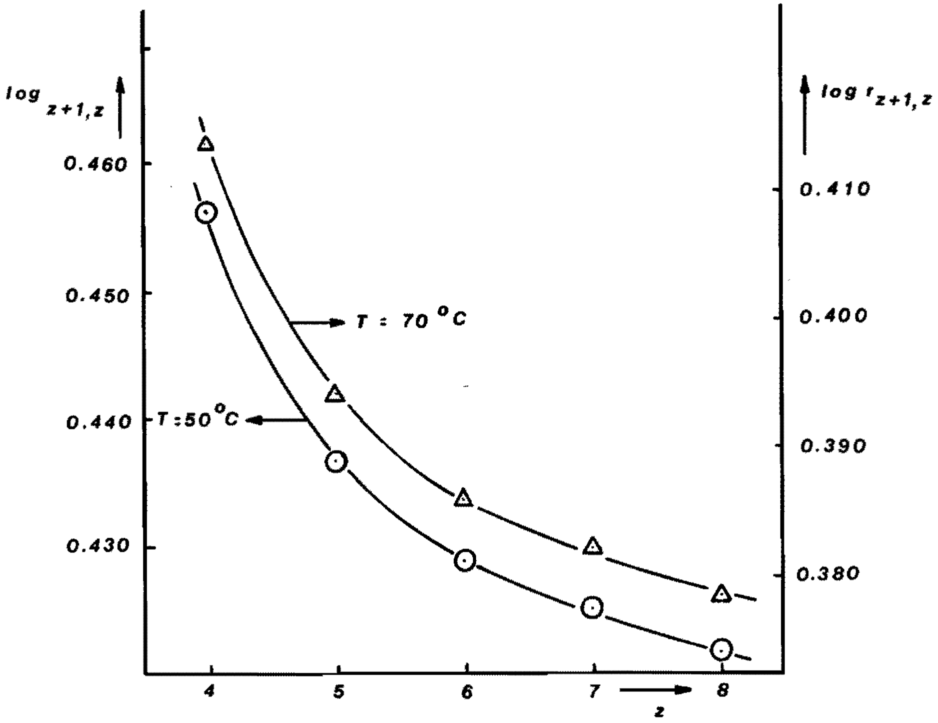

Fig. 2.13 Relation between separation factor (log $r_{z+1,z}$) and carbon number (z), for n-alkanes.

It may be imagined that the introduction of an apparent separation factor will enhance the correlation of retention indices and physical properties.

Recently, several methods of "dead time" determination discussed in the literature, have been summarised by Kaiser (40).

According to a suggestion of Halasz a procedure for the direct measurement of t_m for capillary columns with a flame ionisation detector has been developed in our laboratory.

The F.I.D. gives a response for inert gases when operating at reduced hydrogen and air flow rates. Different from the method proposed by Versino (42) no hydrocarbon traces, which might slightly change the properties of the stationary phase, have to be added to the carrier gas. The measurements are made with a high quality equipment as described in section 2.5. The pressure drop across the column could be kept constant within 2.10^{-3} atm., with a TEXAS INSTRUMENTS pressure controller. Time measurements are made with a stopwatch.

Column conditions :	length 100 m; diam. 0.25 mm; temp. 70.00 ± 0.01°C; inlet pressure 2.430 ± 0.001 atm.; carrier gas nitrogen;
Flame conditions:	temp. 120°C; hydrogen flow 15 ml/min; (60% of the optimal value); air flow 200 ml/min. (60% of the optimal value).
Sample introduction system:	HAMILTON splitter inlet; temp. 120°C; split ratio 1:300.

Under these conditions injections of large samples, about 0.5 ml of inert gas give negative peaks, even for nitrogen from the same tank as the carrier gas. Signals from hydrocarbons remain positive (see Fig. 2.14). The small differences in elution times between gases and methane prevent the direct measurement of the adjusted retention time of methane.

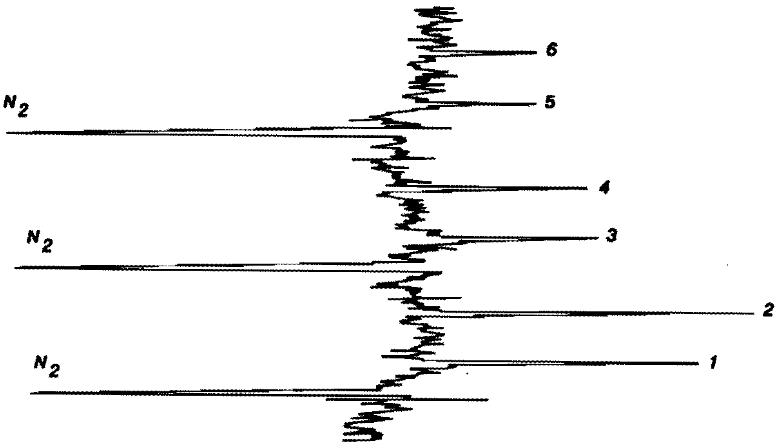

Fig. 2.14 Chromatogram of 0.5 cm^3 of nitrogen containing approximately 5 p.p.m. propane and n-butane 1,3,5 =propane, 2,4,6 = n- butane. Column: length 100m, i.d. 0.25 mm, stainless steel. Carrier gas: nitrogen. Stationary phase: squalane. Temperature: 70°C. Full scale deflection: $5. 10^{-12}$A.

Therefore two different samples have been prepared:

Sample I) Inert gas plus approximately 5 ppm of propane and n-butane.

Sample II) Approximately equal amounts of methane, propane and n-butane.

The sample size for sample I) is 0.5 ml (electrometer setting 5.10^{-12} A f.s.d.). During the introduction of these fairly large samples an increase of inlet pressure of 0.005 atm is observed at the pressure recorder, however the return to preset pressure is instantaneous. Of sample II) about 5 microliter is injected (electrometer setting 5.10^{-10} A f.s.d.).

$t_{CH4} - t_{inert\ gas}$ is calculated from:

$$(t_{n-C_4} - t_{inert\ gas})\ I - (t_{n-\ C_4} - t_{CH_4})\ II$$

During the measurements change in experimental conditions did not occur as could be checked by comparing:

$(t_{n-C_4} - t_{C_3})$ I and $(t_{n-C_4} - t_{C_3})$ II and also

by the absolute retention times of n-butane and propane. From Table 2.12 it follows that no significant differences in "dead time" obtained from different inert gases are found.

Table 2.12 Retention time differences between methane and some inert gases (inlet pressure 2.430 atm.).

	t_R(sec)	standard deviation	number of experiments
CH_4 - O_2	1.6	0.29	12
CH_4 - He	1.7	0.36	11
CH_4 - air	1.5	0.27	11
CH_4 - Ar	1.5	0.18	11
CH_4 - H_2	1.3	0.28	8
CH_4 - N_2	1.5	0.28	12

"Dead times" calculated from the log-plot of n-alkanes with experimental inert gas and methane elution times are compared in Table 2.13. It may be concluded that on wall coated open hole tubular columns at moderate temperature the methane time is closer to the real "dead time" (assuming that helium is not retained) than calculated "dead times" from the log-plot of n-alkanes.

From Table 2.13 it can be seen that "dead times" calculated from the log-plot of n-alkanes differ considerably from the experimental inert gas and methane elution times. The effect of these different "dead times" on the calculation of retention indices is also shown.

The method of retention index calculation from three n-alkanes as proposed by Groenendijk and van Kemenade

Table 2.13 The effect of the "dead" time on the calculation of retention indices.

Compound	Index	Index	Index	Index	Index
2 Me Butane	475.47	475.37	474.82	474.80	474.66
2 Me Pentane	570.01	569.97	569.71	569.70	569.64
2,2 di Me Pentane	626.95	626.93	626.82	626.82	626.79
1 trans 3 di Me Cyclopentane	690.10	690.09	690.05	690.05	690.04
1 trans 2 cis 3 tri Me Cyclopentane	751.11	751.09	751.04	751.04	751.03
1 cis 3 di Me Cyclohexane	789.89	789.88	789.87	789.87	789.87
Dead time	965.2sec.	963.6sec.	955.8sec.	953.8sec.	950.7sec.
	methane	inert gas	extrapolated from $C_4C_5C_6$	extrapolated from $C_5C_6C_7$	extrapolated from $C_6C_7C_8$

(9.10,43) remains advantageous from the point of view of standardisation and also in those cases where a large solvent peak may mask the signal of an inert gas peak.

For precision measurements which enable the determination of the numerical value of physical constants the real "dead time" should be used.

2.6.7 Influence of the operator.

Taking into account differences in reaction time between operators an additional random error may be expected, measuring retention times with a stopwatch.

The delay between the injection of sample and the start of the time measurement and estimation of the right peak maximum can be dependent on the operator.

Therefore retention indices and standard deviations measured by three different skilled operators have been compared for a synthetic mixture of more than 20 compounds for a 100 m open tubular squalane column.

No significant differences in retention index and standard deviations could be observed between different operators.

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CHAPTER III

PERFORMANCE AND PREPARATION OF HIGH RESOLUTION COLUMNS

3.1 INTRODUCTION

For the characterization of a substance by purely gaschromatographic means, the separation power of the column, is at least as important as the factors discussed in Chapter 2.

The column performance sets limits to the separation attainable and determines the time of analysis. Therefore the use of high resolutions columns may become necessary, especially for complex mixtures (section 2.1).

In high resolution gaschromatography the following basic types of columns are in use:

- open hole tubular columns, Golay 1957 (1)
- porous-layer open tubular columns (2)
- packed capillary columns, introduced by Halasz and Heine (3,4).

Theoretically, open hole tubular columns give the best separations. They combine high plate numbers with high permeability and a high production of plates per unit time.

In practice however difficulties with injection technique, sample size and in the choice of stationary phases are encountered. Stream splitters are unsuitable for high boiling compounds, on account of the desirable fast injection.

The quantitative results are unsatisfactory for samples with a wide boiling range. Also, the loss of sample associated with stream splitters is unacceptable in a number of cases (e.g. trace analysis). Direct injection is still in the development stage (5-7).

Fortunately the above considerations mostly do not apply to the work dealt with in this thesis, which confines itself to hydrocarbons below C_9.

Reproducible coating of openhole tubular columns, especially for glass columns, remains difficult (7-10). The formation of a film of sufficient homogeneity on the wall is the critical step.

To find out if there is an optimal column type for a particular separation problem, the performance of high resolution columns will be compared in the first part of this chapter.

"Micropacked columns", prepared according to a new technique, compare favourable in some cases with the column types mentioned before.

The preparation of "micropacked columns" - which combine to a large extent the advantages of packed and capillary columns - is discussed in the second part of this chapter.

The preparation of open hole-tubular columns, with homogeneous liquid layers, for the separation of hydrocarbons, is also discussed in the last part.

3.2 PERFORMANCE PARAMETERS.

To compare the performance of different column types, a great number of characteristic parameters may be used. In an attempt to clarify the confusion which still exists in comparing the performance of columns of different design, a survey of the most important column characteristics will be given below.

As a first approximation according to chromatographic theory, three main characteristics of column performance may be distinguished:

a). efficiency b). resolution c). speed of resolution.

a). Efficiency.

The efficiency may be expressed as the number of theoretical plates n, which can be derived from the chromatogram by the equation:

$$n = \left(\frac{t_R}{\sigma_p} \right)^2 \qquad (3.1)$$

The standard deviation σ_p (in time units) is equal to half the peak width at 0.607 of the height of the Gaussian peak.

The theoretical plate number is related to column length by the height equivalent to a theoretical plate:

$$H = \frac{L}{n} \qquad (3.2)$$

H is dependent on a number of experimental parameters and is a function of the carriergas velocity (u). At a certain flowrate H is minimal, which means the plate number n is optimal.

b). Resolution.

The resolution (R) expresses the degree of separation of two components, leaving the column shortly after each other.

$$R = \frac{\Delta t_R}{\sigma_p} \qquad (3.3)$$

Using the relation $t_R = t_m(1+k)$ and equation 3.1 it follows:

$$R = (r-1) \frac{k}{k+1} \sqrt{n} \qquad (3.4)$$

r being the ratio of the partition coefficients.

With this equation, the number of plates required for the separation of two components with a relative retention r, can be calculated. The separation is almost complete for R = 6 and just acceptable for R = 4.

The effective plate number (N), introduced by Desty, is obtained from the chromatogram by the equation:

$$N = \frac{(t_R - t_m)^2}{\sigma_p^2} \qquad (3.5)$$

This is a good parameter describing the resolving power of the column, because it is proportional to the extent by which two peak maxima will be separated.

This may be simply derived from e.q. (3.4), using the relation:

$$N = n \left(\frac{k}{k+1} \right)^2 \qquad (3.6)$$

This relation simply follows from equation 3.1, 3.5 and the definition of retention time.
Substituting (3.6) in (3.4) it follows:

$$R = (r-1)\sqrt{N} \qquad (3.7)$$

The third characteristic may be defined as the rate of production of effective plates N/t. It takes into account the often neglected time factor and allows the overall performance of columns to be compared.

In practice factors like permeability and pressure drop, detector sensitivity, velocity of sample introduction limit the efficiency, resolution and speed of analysis attainable.

3.3 PRACTICAL LIMITATIONS OF COLUMN PERFORMANCE.

Permeability and pressure drop.

The permeability is a very important property of a gaschromatographic column, because of its direct and indirect influence on a number of separation parameters. It sets a practical maximum for the column length, i.e. the maximum separation power of a column type and plays a major role in the final value of the retention time. Unfortunately it is not mentioned very often.

Experimentally the permeability of the column may be obtained from a plot of the column outlet flow-rate (u_o) versus the corresponding values of P^2-1. P is the ratio of the column in- and outlet pressure.

This relation may be derived from the relation between the gas velocity at any point in the column u(x) and the pressure gradient (dp/dx):

$$u(x) = \frac{\kappa}{\eta} \cdot \left(\frac{dp}{dx}\right) \qquad (3.8)$$

This is the Darcy equation, which is valid at low flow velocities (less than 100 cm/sec. for argon and 300 cm/sec. for hydrogen), normally encountered in linear gas chromatography (11).

Integrating equation 3.8, it follows:

$$u_o = \frac{\kappa P_o}{2\eta L}(P^2-1) \qquad (3.9)$$

Theoretically the permeability of an open-hole tubular column only depends on its diameter. In practice the permeability is somewhat less (10-30%) than the theoretical value $r_o^2/8$(12), which is probably because of the wall roughness or fluctuations in the column diameter. Typical values of the permeability of various types of columns are given in literature (11,13).

In packed- and packed capillary columns the permeability may be increased by an increase in particle size or a decrease in packing density. The permeability for this column types also depends on the particle diameter/column diameter (d_p/d_c) ratio, and the roughness of the support.

According to the Kozeny-Carman equation (14), which applies to the ideal case of smooth sperical particles, the permeability (κ) is proportional to the square of the average diameter of the support particles (d_p):

$$\kappa = \frac{{d_p}^2}{180} \cdot \frac{\varepsilon^3}{1-\varepsilon^2} \qquad (3.10)$$

In this equation, the porosity ε , is the complementary value of the packing density and is defined as the ratio of the mobile gas space and the column volume.For non spherical materials a suitable apparent particle diameter may be introduced.

For conventional packed columns ($d_p/d_c < 0,1$) the permeability is independant of the column diameter.

The influence of particle size on permeability for packed capillary columns is studied by Guiochon (14) in the d_p/d_c range of 0.1 to 0.3, with columns of an internal diameter of 0.5 mm.

The values for different packed capillary columns range from $\kappa = 6\text{-}10 \cdot 10^{-7}$ cm^2.

In fig. 3.1 the permeability of packed capillary columns and micropacked columns (described in section 3.5) is compared with the permeability of packed and open-hole tubular columns.

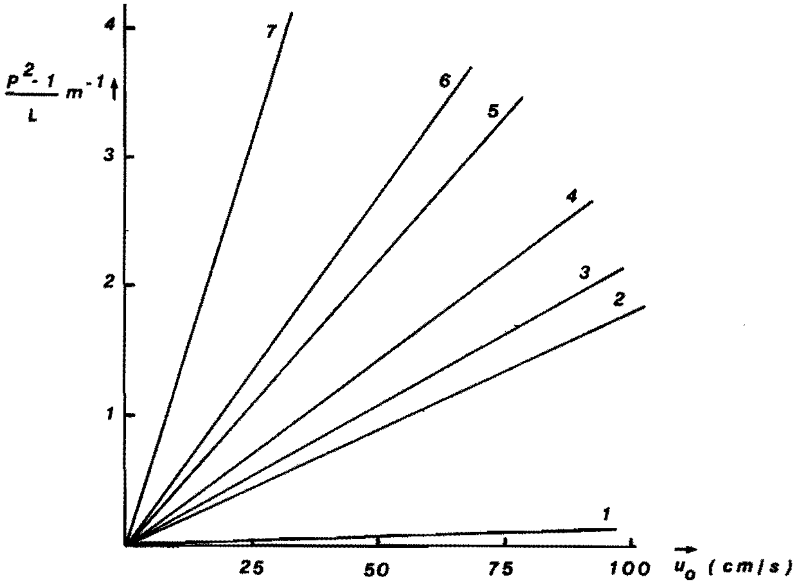

Fig. 3.1 Plots of carrier gas outlet velocity vs.values of $\frac{p^2-1}{L}$ for various types of columns.

1. open-hole tubular column (i.d. 0.25 mm).

2 and 4. packed capillary columns, d_p = 100-125 μ , d_c = respectively 0.45 and 0.55 mm, support Chromosorb P.

3 and 5. micro packed colums, d_p = 140-160 μ , d_c =respectively 0.64 and 1.26 mm, support Glass beads.

6 and 7. conventional packed columns, d_p = 200-250 μ , d_c = 4 mm, support Glass beads respectively Chromosorb P

Comparing the plots 2 and 4, 3 and 5 it can be seen that for constant particle diameter range the permeability decreases with increasing column diameter. The influence of the nature of support is demonstrated in plot 6 and 7, for glass beads the permeability is better than for the porous support. The permeability for packed capillaries and micropacked columns is between open-hole and packed columns.

The permeability which sets limits to the column length, also influences the performance index, introduced by Golay (1), as a measure for column performance. At optimum carriergas velocity (u_{opt}) the performance index is given by equation:

$$PI = 0.12 \frac{256.\sigma_p^4.t_m}{t_R^4(t_R-15/16t_m)} . t_R. p \qquad (3.11)$$

where σ_p = peak width at 0.607 of the total height
t_R = retention time
t_m = retention time of a component which is not retarded.
p = pressure drop over the column.

The performance index has the dimensions of viscosity g/cmsec (pressure in dynes/cm^2). The performance index has a minimum value of 0.1 when the carriergas is helium. Values greater than this are usually obtained although PI's approaching 0.2 have been reported for open-hole tubular columns. Packed columns range from 20-1000 poises.

The performance index shows a large dependence on the product t_R. p, which indicates the price to be paid in waiting time and pressure drop, to obtain maximum performance of the column.

Rewriting eqn. 3.11 in terms of u_{opt} yields:

$$PI = 31.2 \frac{H_{min}^2}{u_{opt}} . \frac{1+k}{k.} \frac{p}{L.} \qquad (3.12)$$

The latter equation shows the importance of pressure drop/unit length.

Speed of sample introduction.

Up till now one important aspect has been left out of consideration, the capacity of the column, which determines the most practical parameter, the sample size.

Schematically, a given quantity of sample can be introduced:

(a) as a narrow band of high concentration or (b) as a plug of correspondingly lower concentration.

(a) If the sample is fed into the column as a band of high concentration, the local conditions at least in the front of the column, will be such that the partition coefficient K becomes concentration dependent. Each part of the solute band will move at different speed. The result will be a band broadening, additional to the broadening effects discussed before (and expressed in the plate number n). The peaks become distinctly a-symmetric.

(b) The sample is diluted with carrier gas before it enters the column. This causes additional symmetric band broadening. The variance σ_p^2(sec^2) of an eluting peak may be considered to be composed of the variance of the sample at the inlet σ_i^2 and the variances σ_c^2 due to column processes (Fig. 3.2).

$$\sigma_p^2 = \sigma_i^2 + \sigma_c^2 \qquad (3.13)$$

It is a trivial observation that the best resolu-

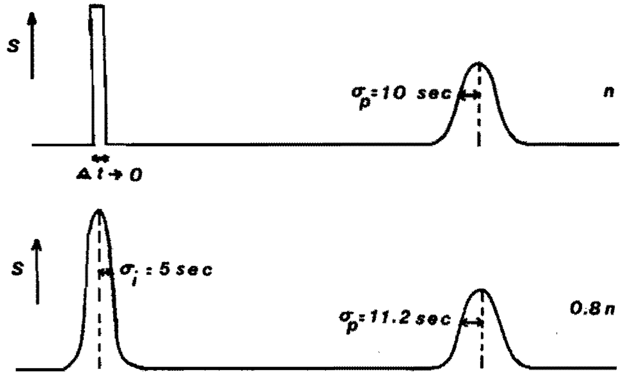

Fig. 3.2 Speed of sample introduction and column efficiency.

tion will be obtained at $\sigma_i^2 = 0$ (infinitely narrow sample band) $R_{max} = \Delta t_R/\sigma_c$. For finite sample volumes R will be smaller than R_{max}. However, from the eqn. above and the definition of resolution it follows that for $\sigma_i = 0.5\ \sigma_c$ the resulting decrease in resolution will be only 10%. From this the permissible input width of the input peak σ_i can be calculated.

From the definition of plate number, n, and the equation for retention time $t_R = t_m(1+k)$, where $t_m = L/u_o$ it can be derived that:

$$\sigma_c = \frac{1+k}{u_o}\sqrt{LH} \qquad (3.14)$$

Time constants.

According to Schmauch (1) the time constant of the detection system must be less than one fifth of standard deviation σ_p if the peak is to be truly recorded. Only for very fast analysis this will become a limiting factor.

Detector sensitivity.

The signal of ionization detectors, commonly used, are proportional to the mass flow rates. This means the minimum amount of sample that can be detected is strongly dependent on the speed of passage of a peak through the detector and therefore on column dimensions. Columns of small diameter and accordingly higher optimal gas-velocity enable the detection of smaller quantities.

Assuming an ionisation efficiency, λ, of 0.25 Coulomb/g at C and a noise level of 10^{-13}A for a flame ionisation detector (as reported in literature) it can be calculated that the signal h (⟶ Signal to noise ratio), to be obtained from a column, is given by:

$$h = \frac{o}{\sigma_c \sqrt{2\pi}} = \frac{M.(\%C).\lambda}{12.\ \sqrt{2\pi}} \ \frac{u_o}{(1+k)\ \sqrt{LH}} \qquad (3.15)$$

M = weight of injected sample; h and o respectively are the height and area of a gaussian peak; u_o = the carrier gas velocity at the column outlet.

3.4 COMPARISON OF COLUMN TYPES.

Considering above mentioned factors, it appears that open-hole tubular columns are theoretically the most favourable ones and that the packed capillaries according to Halasz and the micro packed columns prepared in our laboratory take an intermediate position. The micro packed columns will be discussed in section 3.5. Besides these theoretical considerations there are, however still many practical factors which anyhow do turn the scales to the packed columns, which in practice are used in 90% of the cases. The open-hole tubular columns are used in most other cases, the packed capillaries according to Halasz are, for the major part still in the developing stage.

With packed columns any support material can be used, these supports can be coated or impregnated previously with stationary liquid or used as such (e.g. porapak, mol.sieves). Packing of the columns as well as coating can be done in a reproducible way. Column overloading does not likely occur and therefore sample introduction offers no serious problems.

The choice of open hole tubular (Golay) columns, however is fairly restricted, especially glass columns, for the analysis of sensitive biological materials, are hardly commercially available. The coating of the column with a uniform layer of stationary phase is difficult to achieve.

Therefore, only a very limited number of stationary phases, suitable for work at elevated temperatures, is available. Stream splitting sample introduction systems, as commonly used in connection with open hole tubular columns, cannot be employed for high boiling compounds. Direct sample introduction systems still are very much in the developing stage.

Packed capillary columns according to Halasz are manufactured by heating a packed glass column until the glass softens, then the column is drawn to a capillary tube and if desired wound up to a helix, of course the use of metals for this type of columns is prohibitive. Usually the packing of the drawn capillary is very irregular.

Then a solution of stationary phase in a volatile solvent is forced through the column, the solvent is evaporated and the stationary phase remains in the column impregnating the support and covering the inner glass wall.

Table 3.1 Comparison of column types (L = 5 m, relative retention time = 1.10, capacity ratio = 3, quantity of sample = 10^{-9}g).

	Golay		Halasz	Packed	Micropacked
1.D mm	0.07	0.25	0.50	4.0	0.8
n	20.000	10.000	5.000	7.700	15.500
$H_o$mm	0.25	0.50	1.00	0.65	0.32
$u_o$cm/sec	102	40	40	9	11
R	10.7	7.5	5.4	6.6	9.3
$t_R$sec	20	50	50	220	182
p atm	4	0.10	2.0	2.6	1.4
PI g/cm.sec	2	0.5	42	100	11
$\kappa \cdot 10^{-7} cm^2$	8	100	6	1	2
n/t sec^{-1}	1000	200	100	35	85
σ_c sec	0.14	0.50	0.70	2.50	1.46
σ_i sec	0.07	0.25	0.35	1.25	0.73
s/noise	570	160	114	32	55

The amount of stationary phase as well as the film formation on the inner wall of the capillary tube cannot be controlled sufficiently well. This makes the choice of available liquid phases smaller as in the case of open-hole tubular columns. Another drawback is that the support material must be resistant to the heating, necessary to soften the glass. Therefore the choice of support material is restricted e.g. silanized supports or glass beads can not be used. The permissible sample size is somewhat larger than with open-hole tubular columns, therefore sample introduction gives less difficulties.

In Table 3.1 literature data together with results obtained in our laboratory on a new micro type of packed columns are summarized. For the sake of easier comparison all column lengths are chosen to be 5 m; H_2 is the carrier gas and squalane the stationary liquid phase. For the calculation of resolution a solute pair with relative retention 1.10 is selected; the capacity ratio of the first eluting component is chosen to be 3.

The signal to noise ratio is calculated for a flame ionization detector assuming λ = 0.25 Coulomb/g. at.C and a noise level of the detector system of 10^{-13}A. The injected amount of sample is fixed on 10^{-9}g.C.

In Table 3.2 micropacked columns, packed capillary columns conventional packed and open-hole tubular columns all prepared in our laboratory are compared. The packed columns are prepared following the same packing procedure (see section 3.5). For the open-hole tubular glass column, prepared according to a coating procedure of Tesarik (15), a direct sample introduction system described by Kuppens (6) is used.

Table 3.2 Comparison of column types used in the authors laboratory. Solutes: TMSi-derivatives of steroids or n-hydrocarbons; Carrier gas: hydrogen.

	p atm.	k	H_o mm	u_o $cm.s^{-1}$	n	n/t s^{-1}	P.I.	T
Micro packed column L = 14.3 m.i.d. 0.8 mm Support GLC-110 glass beads (140-160 μ)								
A 0.05% PMPE (Dehydroepiandrosterone)	4	4.8	0.32	11.5	44500	61	9.4	240°
B 0.05% SE-30 (Pregnanediol)	4	7.4	0.34	11.8	42600	44	9.7	220°
Packed capillary (Halasz) L = 21 m, i.d. 0.55 mm Support Chromosorb W (140-160 μ). 0.2 SE-30 (n C-24)	1	2.5	1.19	4.8	17600	11.5	61.3	260°
Packed column L = 2.8 m, i.d. 2 mm Support GLC 110 (140-160 μ) 0.1% PMPE (Estradiol)	0.25	50	0.43	3.0	6450	1.4	15	182°
Support Chromosorb W (140-160) 0.5% PMPE (Estradiol)	0.33	20	0.46	4.9	6000	5.0	16.6	215°
Open hole tubular column L = 23 m i.d. 0.25 mm SE-30 (n C-24).	0.30	1.6	0.46	16.4	50000	137	0.9	254°

3.5 MICROPACKED COLUMNS.

Introduction:

The advantages of ordinary packed columns and to a large extent those of capillary columns may be combined in a new type of column, that we shall call, "micro-packed" columns (24,25). According to Halasz and Heine (3) micro-packed columns are columns with a column diameter less than one mm, if d_p/d_c ratio is less than 0.2 and/or if the packing density is almost as high as in the conventional packed columns.

The column type described below is in agreement with this definition in sofar that the d_p/d_c ratio is between 0.2 and 0.4.

Micropacked columns have the following advantages

- Any support may be impregnated with any stationary phase in the desired quantity.
- Packing may be prepared in large batches to ensure reproducible properties.
- The pressure drop is not excessive.
- The number of theoretical plates is high.
- Direct injection presents no difficulties.
- The small volume allows the use of expensive packing (e.g. Durapak, Dexil).
- Any column material may be used.

Packing Material.

In practice the length of packed columns is limited by the pressure drop. As discussed in section 3.3,1, the permeability of the column can be increased by a reduction of the column diameter, at constant particle size.

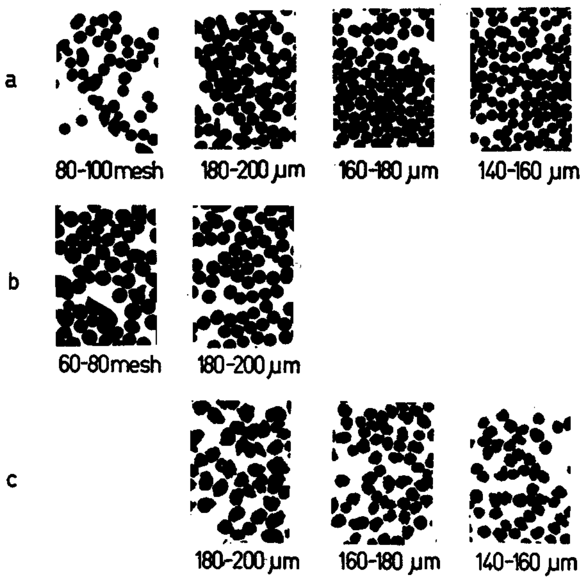

Fig. 3.3 Effect of sieving on particle size and shape a, b: glass beads, GLC-110; c: Chromosorb W-acid washed.

For columns with a diameter less than 1 mm, the introduction of packing material is a serious problem, especially when the length exceeds 3 to 4 m. We have found that significant improvements are obtained if carefully sieved fractions of support are used. The parcticle size distribution must be smaller than 20 µm. A comparison of sieved and non-sieved Chromosorb W (acid-washed) and silanized glass beads is given in Fig. 3.3. The sieved support shows a marked improvement in the regularity of packing, and the regularity increases with decreasing particle size.

Sieving also removes large particles that might block the capillary during packing. It was found that a ratio of particle diameter to column diameter of about 0.2-0.3 is the most favourable. Elimination of fines is achieved by sieving in a vacuum. Instead of sieving, flotation or sedimentation may be used depending on the density of the support. The latter techniques yield a very narrow range of particle size. It was found that specified mesh-size ranges of commercial supports were smaller than those obtained after sieving.

Preparation of micropacked columns.

An empty glass or metal tube of the desired dimensions is coiled and one end of the tube is plugged with 1-2 cm of glass wool. The other end of the tube is connected to a cylindrical container. The axis of the coil is horizontal and that of the container is vertical. The packing is introduced into the container and the latter is connected to a pressure line. The lower part of the container and almost the complete coil are placed in an ultrasonic bath so that the plugged end of the column is above the level of the bath. Vibration and pressure will transport the packing continuously into the column. Care is taken to keep the pressure gradient across the packed part of the column about constant, to ensure a homogeneous packing density along the column.

At the start of the procedure, the increase in pressure should be very gradual so as not to blow out the plug. The final pressure depends on the material used but will be in the order of 0.4-2 atm per m of column length. The lower value applies to glass beads and the higher to Chromosorb. With glass columns, the packing can be followed visually. In the case of metal columns, packing up to a constant weight should be carried out. The time necessary for packing is 1-2 min per m of column length. Table 3.3 shows the reproducibility of packing density.

Table 3.3 Reproducibility of packing density.

Column	I.D. (mm)	Length (m)	Particle size of glass beads support (μm)	Packing on support	Weight of packing per m of column length (g/m).
SS	1.0	6.0	140-160	PMPE	1.35
SS	1.0	6.0	140-160	PMPE	1.35
SS	1.0	6.0	140-160	PMPE	1.34
SS	1.0	6.0	140-160	PMPE	1.33
SS	1.0	6.0	160-180	PMPE	1.29
SS	1.0	6.0	160-180	PMPE	1.29
Glass	0.8	14.6	140-160	PMPE	0.66
Glass	0.8	14.6	140-160	SB-30	0.65
Glass	0.8	10.0	140-160	SE-30	0.65
Glass	0.6	26.0	140-160	PMPE	0.33
Glass	0.6	7.6	140-160	PMPE	0.33

Apparatus.

A home-made gas chromatograph equipped with means for direct sample introduction and a stream splitter (Hamilton) was used in our measurements. Direct injection is illustrated in Fig. 3.4.

The inlet port was provided with a glass insert tube of 11 cm length and 0.8 mm i.d; One end of the insert for the connection to the column was wider and conically shaped. The column was inserted about 2 mm into the cone.

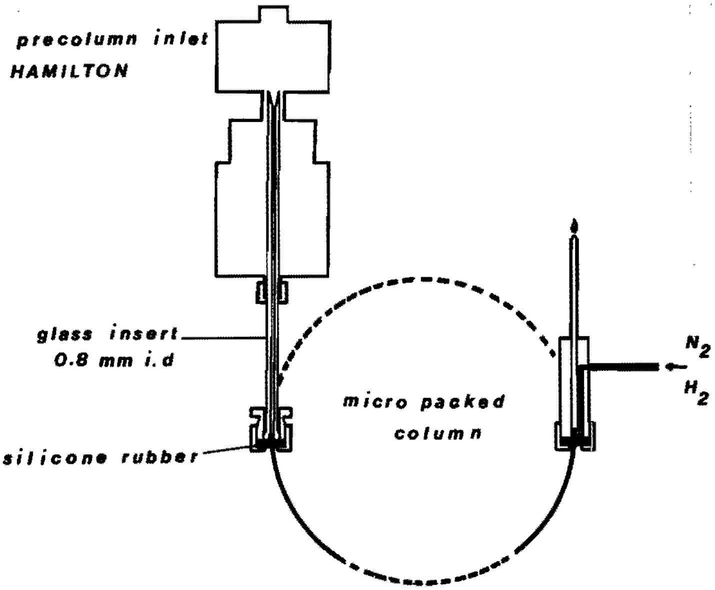

Fig. 3.4 Connection of micro-packed column to gas-chromatographic system.

The seal was made by means of a silicone rubber ring. The outlet of the column protruded into the nitrogen + hydrogen stream for the flame-ionization detector. Since the flow rate was high (1 cm^3/sec), the effective dead volume between column and detector was very small.

Comparison of packed capillary columns and micro-packed columns.

The packed capillary columns according to Halasz and Heine (3,4) require a support of sufficient mechanical strength and great thermal stability to withstand the glass-drawing operation. This support, in most cases, cannot be coated in advance.

These micro-packed columns make no special demand on the support (except for homogeneity of particle size) and the latter can be coated in advance. The great difference in regularity and density of both types of column can be seen in Fig. 3.5. A soft packing in the case of packed capillary

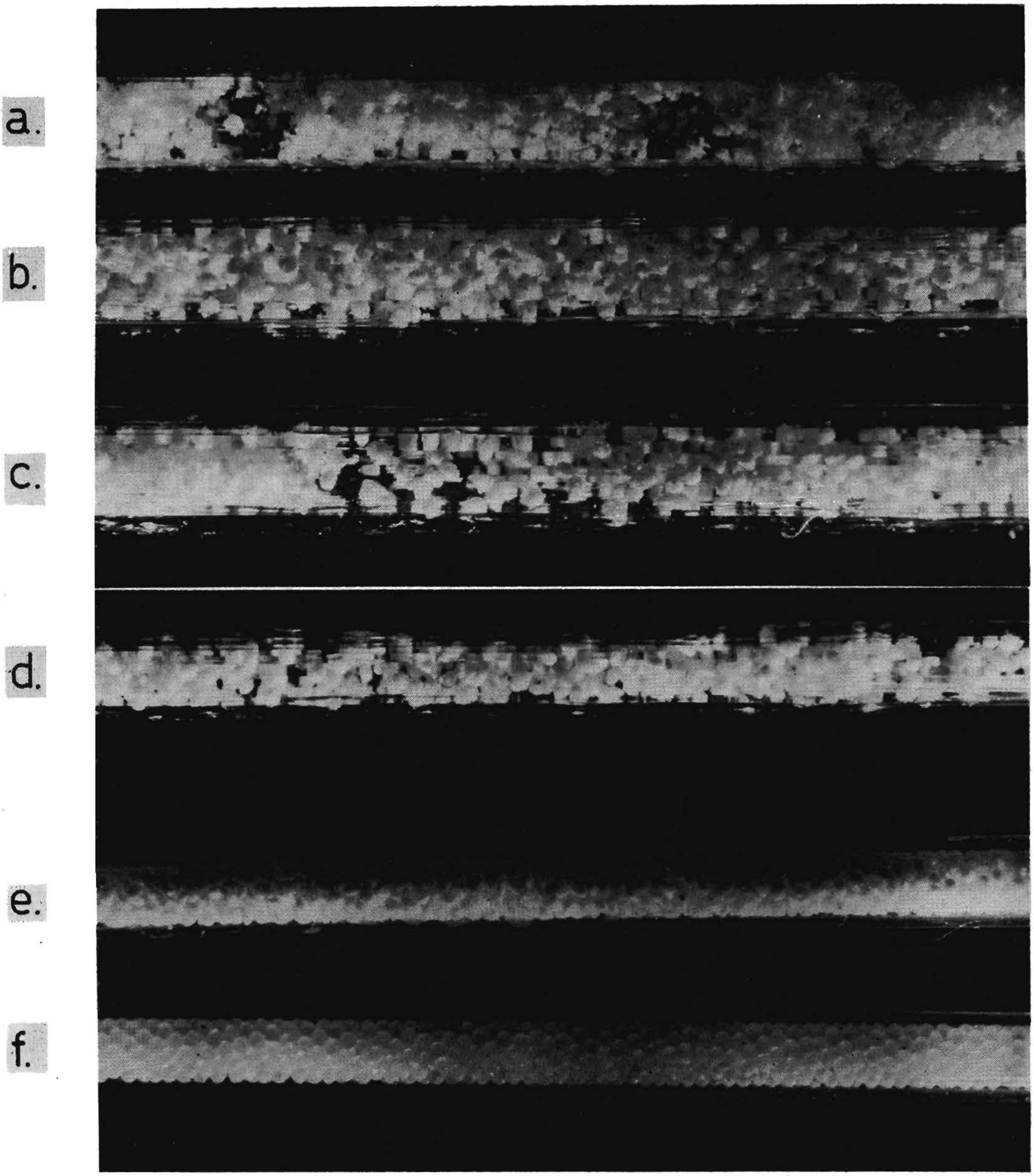

Fig. 3.5 Comparison of packed capillary (a, b, c, d) and micro-packed columns (e, f). a: Gas-Chrom S, 80-100 mesh; b, c, e: Chromosorb W-AW, 180-200 µm; d: Chromosorb W-AW, 80-100 mesh; f: glass beads, GLC 110, 180-200 µm.

columns gives rise to holes (Fig. 3.5.a). Also in the case of packed capillary columns, sieving improves the quality of the packing (Fig. 3.5.b and d). The use of a glass rod inside the tube during packing before the drawing operation results in a looser packing (compare Fig. 3.5.b and c). The use of a narrow sieve fraction in micro-packed columns results in very homogeneous packings (Fig. 3.5.e and f).

Optimum plate numbers were 3500 per m for glass beads and 3000 for Chromosorb W even for columns of 15 m length.

Some applications.

Some separations obtained with micropacked columns are applied to mixtures of low boiling saturated and unsaturated hydrocarbons, amphetamines and steroids as illustrated in Fig. 3.6-3.10.

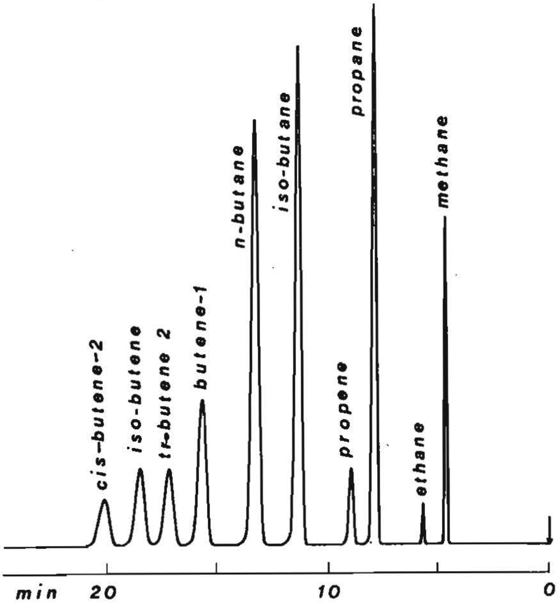

Fig. 3.6 Analysis of hydrocarbon mixture with micro-packed column. Spherosil (66 m^2/g)/10%squalane, 100-200 μm, L = 5 m, I.D. = 0.8 mm, T = 70°C, P_i = 3.2 atm. $n_{opt.}$ (at 1.7 atm.) = 10,200 for n-butane (k = 2.9).

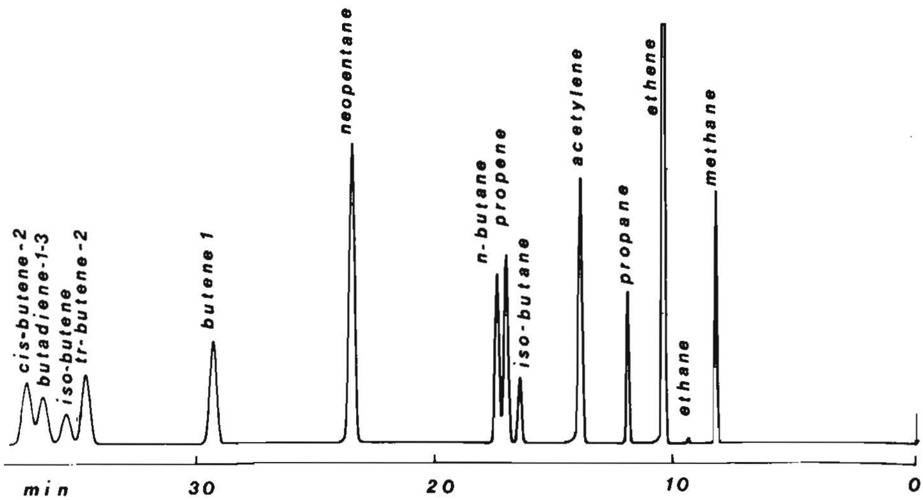

Fig. 3.7 Analysis of hydrocarbon mixture with micro-packed column. Porasil C/phenyl isocyanate, 180-200 μm, L = 15 m, I.D. = 0.8 mm, T = 70°C, P_i = 3.5 atm., $n_{opt.}$ (at 3.6 atm.) = 46,000 for butadiene-1-3 (k = 4.4).

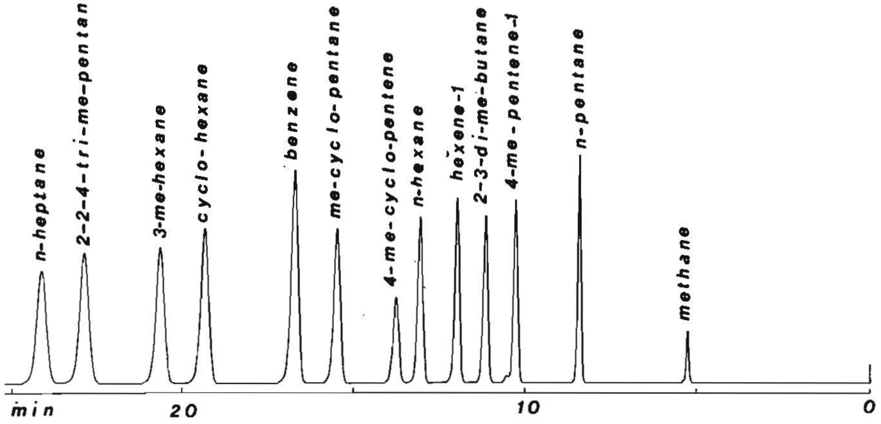

Fig. 3.8 Analysis of hydrocarbon mixture with micro-packed column. Chromosorb W-AW/5 % squalane, 180-200 μm, L = 15 m, I.D. = 0.8 mm, T = 70°C, P_i = 3.5 atm., $n_{opt.}$ (at 3.9 atm.) = 33,500 for n-heptane (k =4.6).

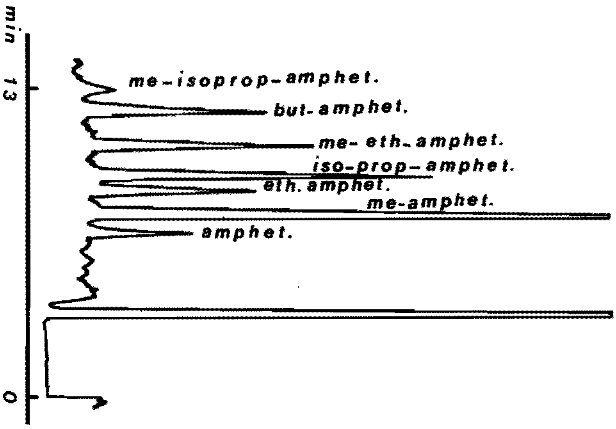

Fig. 3.9 Analysis of amphetamines with micro packed column. Chromosorb W-AW/5 % KOH/10 % OV 101, 180-200 µm, L = 10 m, I.D. = 0.8 mm, T = 125°C, P_i = 4.0 atm., $n_{opt.}$ (at 4.0 atm.) = 25,000.

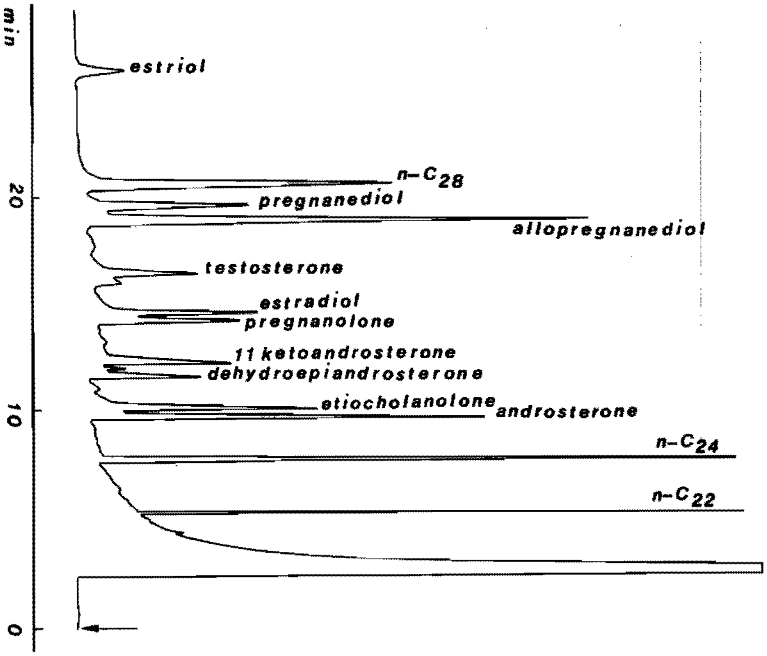

Fig. 3.10 Analysis of steroids with micro-packed column. Glass beads GLC 110 (DOW CORNING)/ 0.05 % SE-30, 180-200 µm, L = 15 m, I.D. = 0.8 mm, T = 220°C, P_i = 3.5 atm., $n_{opt.}$ = 45,500.

3.6 OPEN-HOLE TUBULAR COLUMNS.

3.6.1 Introduction

The outstanding advantage of open-hole tubular columns, as can be concluded from section 3.4, is the low resistance to gas-flow and the consequent low pressure-drop.The resulting high permeability of such columns permits the use of very long columns, which may have a large number of theoretical plates.

Therefore this column type is extremely suitable for the analysis of complex mixtures of hydrocarbons, which may consist of a great number of closely related isomers.

In addition the following advantages of this column type must be mentioned. Since peak area is proportional to the amount of sample, the peaks will also be correspondingly taller compared with peaks obtained with packed columns for the same sample size. That is why the detection limit is lower for open-hole tubular columns. For several reasons, discussed in chapter 2, index systems which can be applied to the characterization of compounds by purely GC means, are much more reliable for open-hole tubular columns.

The application of this column type requires a detection system which combines high sensitivity, low dead volume and fast response. Therefore the flame ionisation detector which exhibits all these characteristics is the detector of choice.

To enable the application of tabulated retention data for the characterization of compounds by purely GC means, the preparation of high-resolution columns must be reproducible.

Some problems encountered in the preparation of open-hole tubular columns will be discussed below.

A simple procedure is described which guarantees a constant column quality for qualitive analysis of hydrocarbons.

3.6.2 Column efficiency and film thickness.

Column efficiency is usually expressed as the number of theoretical plates or the height equivalent to a theoretical plate, H,(16). According to Giddings et al (26) the column efficiency of open-hole tubular columns can be expressed by:

$$H = (\frac{B}{v} + C_g v)\ f_1 + C_1\ v f_2 \qquad (3.16)$$

B, C_g and C_1 are given by:

$$B = 2\ D_g \qquad (3.17)$$

$$Cg = \frac{1+6k+11k^2}{24(1+k)^2} \cdot \frac{r^2}{D_g} \qquad (3.18)$$

$$C_1 = \frac{k^3}{6(1+k)^2} \cdot \frac{r^2}{K^2 D_1} \qquad (3.19)$$

In these equations,

f_1, f_2 = correction factors for non ideal gas phase

v = linear velocity of a not retarded component.

r = radius of the column.

D_g = diffusion coefficient of the solute in the gas phase.

D_1 = diffusion coefficient of the solute in the liquid phase.

k = capacity ratio.

Substituting the relation,

$$K = \frac{V_g}{V_1}\ k = \beta k \qquad (3.20)$$

in eqn. 3.19, yields:

$$C_1 = \frac{k}{(1+k)^2} \cdot \frac{r^2}{6\beta^2 D_1} \qquad (3.21)$$

where

V_g = volume of the gas phase.
V_1 = volume of the stationary phase.

The relation between β and the film thickness (d_f) is given by:

$$\beta = \frac{V_g}{V_1} = \frac{(r-d_f)^2}{r^2-(r-d_f)^2} \cong \frac{r}{2d_f} \qquad (3.22)$$

for $r \gg d_f$.

Substituting eqn. 3.22 in 3.21 it follows:

$$C_1 = \frac{2k}{3(1+k)^2} \cdot \frac{d_f^2}{D_1} \qquad (3.23)$$

since

$$K = \frac{r}{2\ d_f}\ k \qquad (3.24)$$

Since C_1 and C_g decrease with decreasing film thickness the plate number will increase with decreasing film thickness.

A second reason to strive after a thin film is the analysis time, which increases with increasing film thickness.

There are also some arguments, however, for the application of a thick film. Firstly, the capacity ratio is directly proportional to film thickness, so that the number of theoretical plates required for complete separation (n_n) of two peaks, is increasing with decreasing capacity ratio (k), as can be seen from the equation:

$$n_n = \frac{R^2}{(r-1)^2} \cdot \left(\frac{k+1}{k} \right)^2 \qquad (3.25)$$

Where R = resolution required for complete separation.
r = relative retention of the compounds to be separated.

At the same time the sample capacity will decrease with decreasing film thickness, which may result in overloading of the column.

The third argument concerns the adsorption effect of the column wall.

At low liquid loadings the column wall will be not sufficiently desactivated and interact with the solute which will result in concentration dependent retention times, as discussed in section 2.6.5.

Weighing these factors one against another it can be concluded that a minimum film thickness is required, so that the adsorption effect of the column wall can be neglected. For an optimal analysis time in addition the liquid film should also be homogeneous.

Generally it can be said from personal experience that with a mean film thickness between 0.5 and 1.5 μ, the best results are obtained in practice.

3.6.3. Preparation of Open-hole Tubular Columns.

Column cleaning.

Prior to coating metal column tubings must be cleaned in order to eliminate the material that may have been left inside as a result of the manufacturing process. Open-hole tubular columns as received appear to have capacity ratios for n-hexane at 25°C of more than 1.5.

Several cleaning procedures reported in literature (17-19) were tried during this investigation. The metal capillaries are subjected to a number of cleaning steps, forcing subsequently different organic solvents through the tubing. After washing the tube is dried in a stream of inert gas prior to coating.

In order to control the cleaning procedure the column is subjected to an intermediate test. This test indicates whether the column allows a successful coating or the pre-treatment must be repeated.

At 25°C a mixture of methane and n-hexane and benzene vapours, is injected to the column. As discussed in section 2.6.5., n-hexane and benzene have different adsorption properties; benzene being the most strongly adsorped hydrocarbon in the studied range, C_4-C_9.

Benzene and n-hexane appeared to be separated in all cases. It was observed that the more intensive the cleaning the more asymmetric the benzene peaks. For thouroughly cleaned columns even the n-hexane peaks were asymmetrical.

Columns with a very asymmetrical benzene peak give rise to serious coating problems if squalane was used as the stationary phase. The reproducibility of retention indices for unsaturated hydrocarbons was rather poor (1-3 index units) and the life time of the columns was short (about 3 weeks). This problem could not be solved by recoating the column several times.

Such columns, which are unemployable for coating with squalane, appeared to be particularly useful for coating with polar stationary phases, e.g. dimethylsulfolane and acetyltributylcitrate. The reproducibility of retention indices for all types of hydrocarbons for different columns coated with acetyltributylcitrate, at 50° and 70°C, was within 0.1 index unit. In all cases the first coating was successful. The quality of the columns was excellent ($n > 3000$, life time > 1 year, the constancy of retention indices during 1 year better than 0.2 index units).

With a "soft" cleaning procedure, using only n-hexane and benzene, the symmetry of the benzene peak was much better. After continuing the flushing with these two solvents until the capacity ratio of n-hexane is about 0,05, squalane columns can be prepared which permit reproducibility of retention indices for all hydrocarbons within 0.2 index units, for peaks which are completely separated.

As an example, the influence of the quantity of n-hexane and benzene used for the cleaning of a 100 m column, is demonstrated in Fig. 3.11.

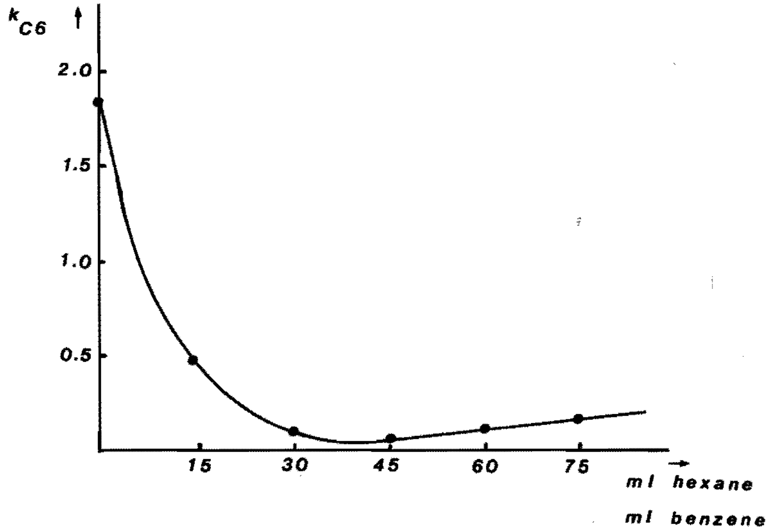

Fig. 3.11 Dependence of the capacity ratio of n-hexane on the quantity of organic solvents during the cleaning of a stainless steel column.

It can be observed that in this case, after flushing 3 times with 15 ml of n-hexane and 15 ml of benzene, a minimum capacity ratio for n-hexane is obtained of about 0.05 at 25°C. If the cleaning is continued the capacity ratio of n-hexane is increasing as is the asymmetry of the benzene peak.

Following the cleaning and test procedure described before, it can be expected that the reproducibility of retention data between laboratories can be within 0.2 index units, for hydrocarbons.

Column Coating.

There are two different methods to deposite the stationary phase on the column wall, the static method, developed by Golay (1) and modified by Bouche and Verzele (20) and the dynamic method introduced by Dijkstra and de Goey (21).

The last method is used in this investigation. In this method, a solution of the stationary phase in a volatile organic solvent is forced through the tubing, with the aid of an inert gas. The inside wall of the column is wetted by the solution. Subsequently the solvent is evaporated by a small flow of the inert gas through the column for a few hours.

The advantages of this method are that it does not requires special equipment and that it can be carried out relatively quickly and easily.

The volume of the coating solution can be smaller or larger than the column volume. The first method is called the "plug method".

As discussed in section 3.6.2.the quality of the column is determined by the thickness and uniformity of the liquid layer on the inside column wall. In the case of a thin layer adsorption on the column wall predominates.

Thick films on the other hand are not stable and will clog the end of the column. Mean film thicknesses between 0.5 and 1.5 are therefore applied in this investigation.

The thickness of the film will mainly depend on the roughness of the wall, the viscosity and polarity of the solvent and the linear speed of the coating solution. According to Desty and Kaiser (22,23) the best results will be obtained with 5-15% concentrations of stationary phase in the solvent and linear velocities between 2 and 10 cm.sec^{-1}. To obtain a uniform film the velocity of the coating solution through the column must be constant.

At constant pressure drop across the column, using the plug method, the length of the plug will decrease by wetting of the column wall, so that the linear velocity of the plug will increase. When the volume of the coating solution is larger than the column volume the linear velocity will also be not constant in the beginning and at the end of the coating.

To prevent the decrease of the length of the plug a combination of both these methods can be used. In first instance a coating solution of three times the column volume is forced through the column at constant inlet pressure. Immediately after the total quantity of this solution has passed the column, a plug of about 10% of column volume is forced through the column, also at constant inlet pressure. After drying with a gentle stream of inert gas, during 8 hours, the column is placed in the gaschromatograph for conditioning.

In all experiments the concentration of stationary phase in the solvent was 10%, the linear velocity of the coating solution was always between 5 and 10 cm.sec^{-1}.

The regularity of the film along the column, for the three variants of the dynamic method mentioned before, is investigated as follows:

The quantity of stationary phase was determined for a 100 m stainless steel column by weighing the column before and after coating. The mean film thickness was calculated by the equation:

$$d_f = \frac{g}{2\pi . d . l . \rho} \qquad (3.26)$$

where g = weight of stationary phase (g)

ρ = density of stationary phase (g.cm^{-3})
l = length of the column (cm)
d = diameter of the column (cm)
d_f= film thickness (cm).

The mean film thickness of this column was 0.7 μ, which corresponds with a capacity ratio of 1.20 for n-hexane at 50°C. Since the film thickness is directly proportional to the capacity ratio, these results allow the comparison of the mean film thickness of different columns, independent of column length.

Cutting up the column into pieces of known length and comparing the mean film thickness of the fragments, the uniformity of the liquid film can be determined. In this way the uniformity of the distribution of stationary phase along the column for the three variants of the dynamic method can be compared.

The results of this comparison, presented in Table 3.4 are obtained with 30 m columns at 50°C, measuring the capacity ratio for n-hexane of fragments each 5 m long.

Table 3.4 Dependence of the uniformity of the film thickness on the coating method. Method 1: coating solution 3x column volume. Method 2: coating solution 10 % of column volume. Method 3: Combination of method 1 and 2. Length of column: 30 m. Length of fragments: 5 m.

	Mean film thickness in μ		
	Method I	Method 2	Method 3
0-5 m	0.4	0.5	0.6
5-10 m	0.7	0.6	0.6
10-15 m	1.0	0.6	0.7
15-20 m	1.4	0.6	0.7
20-25 m	1.8	0.8	0.7
25-30 m	2.1	1.0	0.7

It can be seen from Table 3.4 that the uniformity of the film obtained by the combination of method 1 and 2 gives the best results. Therefore this method, which is used in all cases in this investigation, has to be recommended for the preparation of long open-hole tubular columns for the separation of hydrocarbons.

Column Conditioning.

After the freshly prepared column is dried, it has to be conditioned. To achieve this, the column is placed in the gaschromatograph and the temperature is raised in steps of 15°C to a maximum operating temperature, 70°C in this case. The temperature is kept constant for all the steps during about 4 hours, under a small flow of carrier-gas (0.5 - 1.0 $ml.min^{-1}$).

After the last step the conditioning of the column is continued about 100 hours at the maximum operating temperature, after which the column is ready for use. Conditioning the column in this way improves its stability and results in a lower background signal as compared with a freshly prepared column.

Quality Control of the Column.

For all columns, cleaned, prepared and conditioned as described before, the reproducibility of retention indices of hydrocarbons was better than 0.2 index units. The capacity ratio of n-hexane, at 50°C, for squalane columns was between 0.8 and 2.5. For the acetyltributylcitrate columns this value was between 0.4 and 0.8. The ratio of the front-side and the back-side peak width at half the height of the peak, which is a measure for the peak symmetry, was larger than 0.9 for benzene, at 50°C, in all cases. The sample quantity was always less than 10^{-8}g.

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CHAPTER IV

CHARACTERIZATION OF HYDROCARBONS BY MEANS OF ACCURATE RETENTION DATA

4.1 INTRODUCTION.

Although gas chromatography is essentially a method of separating a mixture into its constituents, qualitative and quantitative information can be obtained for each component. A gas chromatogram yields, for each component, one characteristic retention value and one typical detector response factor.

For qualitative analysis use can be made of the different selectivities of various detectors. A mass spectrometer used as a detector will give a highly characteristic response for each compound in the form of its mass spectrum. There are however cases, where different substances, e.g. hydrocarbon isomers, exhibit almost identical mass spectra. In those cases the retention value remains important even if a coupled mass spectrometer is available.

Retention values will become more characteristic with increasing accuracy and precision. At the same time the separation power of the column should be made as high as technically possible (section 2.1).

The use of different temperatures and of different columns adds considerably to the final characterization. The possibility of coincidence of various retention data can be greatly reduced in this way.

Since in many laboratories collections of standard substances are incomplete or not available, sets of collected accurate retention data will be essential for characterization purposes. Apart from this it will save very much time if tables of accurate retention data are available.

To enable the exchange of retention data within and between laboratories, all possible sources of error in the gas chromatographic system have to be eliminated to such an extent that the accuracy of the retention data keeps step with the separation power of the column. With a high quality equipment (2.5) and high resolution open-hole tubular columns (3.6.3), retention indices of some 170 hydrocarbons have been measured at $50^{o}C$ and $70^{o}C$, with squalane and acetyltributylcitrate as the column liquids.

The agreement between the results presented in this thesis and retention indices given in the literature and the reproducibility within our laboratory will be discussed in the following sections.

The application of precise retention data and the temperature dependence of the retention index for type classification will be demonstrated by two examples.

Finally the potentialities of precise retention data for the characterization of compounds in complex mixtures of hydrocarbons is shown. For this purpose a system of coupled high resolution columns of different polarity, without valves in the sample path, was developed. This system enables the transfer of a part of a selected peak, eluted from the first column, on to a second column of different polarity. In this way compounds which are not or not completely resolved on the first column, can be separated and characterized on the second column. The accuracy of retention data obtained with this system is as good as the accuracy obtained with single columns.

4.2 REPRODUCIBILITY OF RETENTION INDICES WITHIN OUR LABORATORY.

The reproducibility of retention indices within our laboratory is shown in Table 4.1 (stationary phase: squalane) and Table 4.2 (stationary phase: acetyltributylcitrate). The measurements were done on two different instruments with different stainless steel columns and by different operators. The time difference between the measurements was about one year for both phases. The internal diameter of the the columns was 0.25 mm. The length of the squalane columns was 100 m. The lengths of the acetyltributylcitrate columns were 50 and 75 m. The age of all the columns was less than 6 months.

Table 4.1 Reproducibility of retention indices and their temperature coefficients, within our laboratory, for apolar columns. Operating conditions and system design: section 2.5. Quantity of sample: 10^{-8}g. Carrier gas: nitrogen. Pressure drop over the column: 2.500 atm. Stationary phase: squalane. Method of time measurement: instrument 1, stopwatch; instrument 2: digitizer-computer system. Number of measurements: > 4.

Component	Instrument 1, Column 1 Operator 1				Instrument 2, Column 2 Operator 2				I_{1-2}
	I_{70}	s.d.	dI/dT	s.d	I_{70}	s.d.	dI/dT	s.d.	
2-me-hexane	666.96	0.035	0.017	0.003	666.98	0.005	0.018	0.001	- 0.02
2-2-di-me-hexane	720.47	0.028	0.052	0.002	720.47	0.006	0.051	0.002	0.00
2-2-3-tri-me-hexane	824.30	0.024	0.137	0.004	824.32	0.005	0.136	0.001	- 0.02
2-2-3-3-tetra-me-pentane	857.49	0.026	0.270	0.002	857.48	0.005	0.270	0.001	0.01
cyclohexane	667.12	0.030	0.220	0.002	667.14	0.005	0.222	0.001	- 0.02
heptene-1	682.33	0.021	0.025	0.002	682.32	0.004	0.023	0.002	0.01
2-me-tr-hexene-3	646.70	0.015	-0.019	0.001	646.70	0.006	-0.019	0.001	0.00
2-2-di-me-cis-hexene-3	718.81	0.018	0.102	0.001	718.81	0.005	0.103	0.001	0.00
2-3-4-tri-me-pentene-2	766.76	0.022	0.041	0.003	766.78	0.006	0.042	0.001	- 0.02
benzene	641.81	0.024	0.232	0.002	641.90	0.005	0.232	0.001	- 0.09

Table 4.1 clearly shows that the reproducibility of retention data, for hydrocarbons on squalane, within our laboratory, is in most cases considerably better than 0.1 units. As discussed in section 2.4.4 the difference of the standard deviations of retention indices and dI/dT values on columns 1 and 2, is caused by the method of time measurement (column 1: stopwatch, column 2: digitizer-computer system).

It might be concluded that the agreement of retention indices on both columns, for alkanes, is independent of the value of dI/dT. This means, the difference of the temperature between both columns is negligibly small. Since the components of the mixture are completely separated, the influence of peak shift can also be neglected. Therefore it seems, considering the reproducibility of the retention indices of benzene (section 2.6.5), that the polarity of the columns is slightly different.

Table 4.2 Reproducibility of retention indices and their temperature coefficients, within our laboratory, for polar columns. Operating conditions: see Table 4.1. Stationary phase: acetyltributylcitrate. Method of time measurement: digitizer-computer system. Pressure drop over the column: column 1, 1.000 atm.; column 2, 1.500 atm. Column length 50 and 75 m. Number of measurements: 4.

Component	Instrument 1, Column 1 Operator 1				Instrument 2, column 2 Operator 2				
	I_{70}	s.d.	dI/dT	s.d.	I_{70}	s.d.	dI/dT	s.d.	I_{1-2}
2-me-hexane	667.20	0.009	0.013	0.003	667.27	0.007	0.012	0.001	- 0.07
2-2-di-me-hexane	721.64	0.009	0.047	0.002	721.69	0.007	0.048	0.001	- 0.05
2-2-3-tri-me-hexane	829.04	0.012	0.139	0.001	829.06	0.006	0.138	0.003	- 0.02
2-2-3-3-tetra-me-pentane	866.40	0.008	0.290	0.002	866.49	0.012	0.290	0.003	- 0.09
cyclohexane	681.55	0.011	0.224	0.002	681.44	0.011	0.234	0.002	0.11
hexene-1	617.05	0.006	0.009	0.004	617.00	0.007	0.005	0.001	0.05
2-me-tr-hexene-3	675.69	0.007	-0.055	0.003	675.69	0.006	-0.056	0.002	0.00
2-2-di-me-cis-hexene-3	748.60	0.008	0.083	0.002	748.65	0.008	0.082	0.002	- 0.05
2-3-4-tri-me-pentene-2	802.24	0.008	0.005	0.002	802.30	0.008	0.005	0.003	- 0.06
benzene	779.40	0.008	0.190	0.002	779.36	0.009	0.187	0.003	0.04

The repeatability of the polar columns is somewhat less than the repeatability of the squalane columns. For the time measurement the digitizer computer system is used. Possibly this difference is caused by the lower pressure drop over the columns (section 2.4.2).

However, the reproducibility of retention indices for the polar columns is also within 0.1 index units, it seems that also the reproducibility is somewhat lower than for the squalane columns.

The excellent reproducibility of the dI/dT values for both phases allows its application to the characterization of hydrocarbons in complex mixtures, as will be discussed in section 4.5.

4.3 TABULATION OF RETENTION DATA.

Under the experimental conditions given in section 2.5 and with columns prepared according to the procedure described in section 3.6.3, retention indices of 170 odd hydrocarbons are given in Table 4.3, at 50°C and 70°C. Squalane and acetyltributylcitrate are the stationary phases.

The measurements were done with synthetic mixtures of about 30 hydrocarbons each, which all were completely separated at both temperatures. The composition of the mixtures was different for both phases. Normally the standard compounds (n-alkanes) were included in these mixtures. In those cases where a compound was not or not completely separated from a standard, this n-alkane was omitted in the mixture. The adjusted retention time of this n-alkane was calculated from its relative retention time in the other mixtures. The retention time of methane was taken as the "dead time" in all the measurements (2.6). The quantity of sample was always less than 10^{-8}g per component. The carrier gas was nitrogen. For the squalane columns the pressure drop across the column was 2.500 atm. ± 0.002 atm. The column length was 100 m, the internal diameter 0.25 mm. The column material was stainless steel. The pressure drop over the acetyltributylcitrate columns was 1.000 respectively 1.500 ± 0.002 atm., for column lengths respectively of 50 and 75 m. The column material and the internal diameter of the columns was the same as for the squalane columns.

Table 4.3 Retention indices of hydrocarbons on squalane and acetyltributylcitrate, at 50 and 70°C and their temperature coefficients.

Component	Squalane			Citroflex A_4		
	I_{50}	I_{70}	$\frac{dI}{dT}$	I_{50}	I_{70}	$\frac{dI}{dT}$
tr-butene-2	406.6	406.3	- 0.015	440.9	440.3	- 0.034
2-2-di-me-propane	412.3	412.9	0.030	412.4	414.4	0.102
cis-butene-2	416.9	417.3	0.017	454.5	454.3	- 0.011
3-me-butene-1	450.3	450.8	0.025	-	-	-
2-me-butane	475.3	475.5	0.007	475.2	475.8	0.028
1-tr-2-di-me-cyclopropane	479.2	479.8	0.028	-	-	-
pentene-1	481.8	482.1	0.017	515.5	515.6	0.003
2-me-butene-1	488.0	488.1	0.005	525.2	524.9	- 0.015
tr-pentene-2	500.0	499.8	- 0.010	535.1	534.3	- 0.041
cis-pentene-2	504.9	505.1	0.010	542.2	542.0	- 0.009
3-3-di-me-butene-1	506.8	508.1	0.065	536.7	537.4	0.039
eth-cyclopropane	510.2	511.5	0.066	-	-	-
3-me-butadiene-1-2	510.3	509.9	- 0.020	581.3	579.8	- 0.073
2-me-butene-2	514.3	514.4	0.003	551.5	551.2	- 0.019
1-cis-2-dim-cyclopropane	514.9	516.2	0.057	546.9	547.8	0.037
pentadiene-1-tr-3	515.8	516.5	0.035	595.6	595.1	- 0.024
pentadiene-1-2	525.6	525.9	0.016	598.5	597.2	- 0.062
pentadiene-2-3	530.2	529.5	- 0.032	603.5	601.1	- 0.072
2-2-di-me-butane	536.8	538.5	0.082	536.9	538.6	0.084
1-1-2-tri-me-cyclopropane	549.2	550.3	0.054	-	-	-
4-me-pentene-1	549.4	550.4	0.050	581.4	582.0	0.0
cyclopentene	549.5	552.0	0.128	596.1	-	-
3-me-pentene-1	551.4	553.0	0.079	582.1	583.4	0.063
4-me-cis-pentene-2	556.2	556.6	0.024	589.1	589.1	- 0.002
2-3-di-me-butene-1	558.8	559.6	0.043	593.7	594.3	0.026
4-me-tr-pentene-2	561.9	561.6	- 0.013	593.5	592.7	- 0.043
hexadiene-1-5	562.9	563.7	0.040	631.4	631.7	0.017
cyclopentane	565.7	568.5	0.138	583.2	586.2	0.147
2-3-di-me-butane	567.3	568.9	0.077	568.5	570.2	0.084
2-me-pentane	569.7	570.0	0.017	569.6	570.0	0.020
2-me-pentene-1	580.1	580.6	0.022	616.7	616.7	0.002
hexene-1	582.3	582.7	0.024	616.9	617.1	0.009
3-me-pentane	584.2	585.1	0.045	585.3	586.3	0.048
2-eth-butene-1	592.0	592.1	0.005	628.0	627.9	- 0.009
tr-hexene-3	592.1	591.6	- 0.022	625.0	624.0	- 0.052
cis-hexene-3	592.6	593.0	0.019	627.2	627.4	0.008
tr-hexene-2	596.9	596.7	- 0.007	630.4	629.8	- 0.063
2-me-pentene-2	597.8	597.6	- 0.011	634.5	633.7	0.038
3-me-cis-pentene-2	602.8	603.4	0.033	640.0	640.1	0.030
3-me-cyclopentene	603.2	606.1	0.143	646.3	648.7	0.120
cis-hexene-2	603.6	604.1	0.025	640.1	640.2	0.007
4-4-di-me-pentene-1	604.6	606.5	0.094	634.8	636.4	0.076
3-me-tr-pentene-2	612.7	612.9	0.008	649.4	649.2	- 0.015
4-4-di-me-tr-pentene-2	614.7	614.6	- 0.008	662.8	664.8	0.100
1-1-2-2-tetra-me-cyclopropane	619.9	621.7	0.095	-	-	-
eth-cyclobutane	621.1	623.6	0.122	634.7	637.2	0.125

2-3-di-me-butene-2	625.1	625.7	0.026	663.4	663.4	0.000
2-2-di-me-pentane	625.6	627.0	0.065	625.7	627.0	0.065
3-3-di-me-pentene-1	626.2	628.5	0.117	656.0	658.1	0.106
me-cyclopentane	627.9	631.0	0.152	641.4	645.1	0.188
2-3-3-tri-me-butene-1	628.5	630.7	0.112	645.6	644.9	- 0.036
2-4-di-me-pentane	629.8	630.5	0.035	629.9	630.5	0.032
4-4-di-me-cis-pentene-2	635.5	637.6	0.105	668.0	669.7	0.085
3-4-di-me-pentene-1	636.9	639.2	0.112	667.1	669.2	0.101
benzene	637.2	641.8	0.232	775.6	779.4	0.190
2-4-di-me-pentene-1	637.7	639.0	0.065	671.9	-	-
2-2-3-tri-me-butane	639.7	642.6	0.144	641.9	645.1	0.159
2-4-di-me-pentene-2	640.6	640.0	- 0.030	674.8	673.5	- 0.063
1-me-cyclopentene	644.5	646.8	0.165	689.3	691.2	0.093
3-me-hexene-1	644.7	646.0	0.068	675.6	676.6	0.055
3-eth-pentene-1	646.9	648.9	0.101	676.0	677.8	0.092
2-me-tr-hexene-3	647.1	646.7	- 0.019	676.8	675.7	- 0.055
5-me-hexene-1	650.0	650.9	0.044	685.1	685.5	0.024
2-3-di-me-pentene-1	650.4	652.2	0.099	684.2	685.7	0.075
4-me-cis-hexene-2	654.9	656.1	0.060	686.5	687.4	0.046
4-me-tr-hexene-2	656.7	657.4	0.038	684.2	687.1	0.145
4-me-hexene-1	657.9	659.3	0.063	691.2	692.2	0.052
3-3-di-me-pentane	658.9	661.5	0.132	660.6	663.4	0.144
3-me-2-eth-butene-1	659.1	659.9	0.040	692.3	692.7	0.022
5-me-tr-hexene-2	659.5	659.8	0.015	-	-	-
cyclohexane	662.7	667.2	0.222	677.0	681.6	0.234
2-me-hexane	666.6	667.0	0.017	666.9	667.2	0.013
3-4-di-me-cis-pentene-2	670.6	671.5	0.044	713.0	713.0	0.001
2-3-di-me-pentane	671.7	673.4	0.085	673.9	675.8	0.094
1-1-di-me-cyclopentane	673.5	677.1	0.183	685.9	689.6	0.187
3-me-hexane	676.2	676.9	0.036	677.4	678.1	0.035
2-me-hexene-1	678.1	678.5	0.023	715.3	715.3	0.004
3-4-di-me-tr-pentene-2	678.3	678.8	0.029	712.9	712.9	- 0.001
2-eth-pentene-1	681.8	682.3	0.025	716.6	716.7	0.006
heptene-1	681.8	682.3	0.025	717.2	717.3	0.008
1-cis-3-di-me-cyclopentane	682.7	685.9	0.163	-	-	-
3-me-cis-hexene-3	684.6	685.0	0.020	726.4	725.7	- 0.036
3-eth-pentane	686.0	687.2	0.057	688.9	690.0	0.055
1-tr-3-di-me-cyclopentane	686.8	690.1	0.165	-	-	-
tr-3-heptene	687.5	687.4	- 0.006	719.3	718.6	- 0.032
1-tr-2-di-me-cyclopentane	689.2	692.4	0.159	699.7	702.8	0.152
2-2-4-tri-me-pentane	689.9	692.0	0.109	690.6	692.0	0.068
cis-3-heptene	690.4	691.1	0.034	724.7	724.9	0.012
2-me-hexene-2	691.2	691.2	0.002	727.2	726.7	- 0.025
3-me-tr-hexene-3	691.2	691.1	- 0.009	720.7	720.5	- 0.010
2-2-di-me-tr-hexene-3	692.8	692.4	- 0.020	720.7	719.5	- 0.057
3-me-cis-hexene-2	693.3	694.1	0.045	729.4	729.8	0.023
2-5-di-me-tr-hexene-3	695.1	694.7	- 0.021	720.6	719.5	- 0.054
3-eth-pentene-2	697.2	697.7	0.030	733.4	733.4	0.002
tr-heptene-2	698.4	698.5	0.001	732.1	731.7	- 0.022
2-3-di-me-pentene-2	703.4	704.2	0.038	741.4	741.8	0.020
2-4-4-tri-me-pentene-1	704.3	706.8	0.045	736.0	738.3	0.117
3-eth-cyclopentene	712.4	715.8	0.171	754.9	757.9	0.166

2-4-4-tri-me-pentene-2	715.4	716.3	0.044	749.7	749.9	0.013
2-2-di-me-cis-hexene-3	716.8	718.8	0.103	746.9	748.6	0.083
2-2-di-me-hexane	719.4	720.5	0.052	720.7	721.64	0.047
1-cis-2-di-me-cyclopentane	720.9	725.1	0.209	734.6	738.9	0.212
1-1-3-tri-me-cyclopentane	723.6	727.5	0.191	731.3	735.1	0.190
me-cyclohexane	725.8	730.6	0.243	736.3	741.5	0.257
2-5-di-me-hexane	728.4	729.0	0.030	729.5	729.7	0.010
2-4-di-me-hexane	731.9	733.0	0.051	733.5	734.6	0.053
eth-cyclopentane	733.8	737.5	0.183	746.9	750.8	0.196
2-2-3-tri-me-pentene-1	734.6	738.3	0.189	768.3	772.0	0.183
2-me-3-eth-pentene-1	735.0	737.4	0.118	766.2	768.5	0.103
2-2-3-tri-me-pentane	737.1	740.2	0.152	740.7	743.9	0.164
2-3-di-me-hexene-1	739.3	740.9	0.081	773.2	774.5	0.067
1-tr-2-cis-4-tri-me-cyclopentane	741.1	744.5	0.169	746.5	750.1	0.178
2-me-tr-heptene-3	741.1	741.2	0.003	769.4	768.8	- 0.030
3-3-di-me-hexane	743.5	745.9	0.121	745.2	747.8	0.129
toluene	745.4	750.2	0.240	876.8	881.1	0.215
3-4-4-tri-me-cis-pentene-2	747.1	748.8	0.084	782.0	783.1	0.053
1-tr-2-cis-3-tri-me-cyclopentane	747.8	751.1	0.165	755.4	758.8	0.167
2-5-di-me-hexene-2	749.9	750.6	0.034	783.8	783.7	- 0.002
2-3-4-tri-me-pentane	752.4	755.1	0.138	756.3	759.2	0.146
2-3-3-tri-me-pentane	759.4	763.4	0.202	763.6	767.9	0.218
2-3-di-me-hexane	760.1	761.5	0.072	762.8	764.3	0.072
2-me-3-eth-pentane	761.4	763.5	0.107	-	-	-
1-1-2-tri-me-cyclopentane	763.2	767.9	0.232	573.4	573.9	0.026
2-me-heptane	764.9	765.2	0.015	765.1	765.4	0.013
2-3-4-tri-me-pentene-2	765.9	766.8	0.041	802.2	802.2	0.005
4-me-heptane	767.2	767.7	0.026	768.6	768.7	0.003
3-4-di-me-hexane	770.6	772.6	0.099	773.9	776.0	0.104
3-me-heptane	772.3	772.9	0.031	774.0	774.5	0.029
2-2-4-4-tetra-me-pentane	772.7	776.4	0.187	-	-	-
1-cis-2-tr-4-tri-me-cyclopentane	773.1	777.4	0.218	781.7	786.1	0.223
3-me-3-eth-pentane	774.0	777.5	0.189	777.5	781.6	0.203
2-2-5-tri-me-hexane	776.3	777.5	0.062	778.7	779.7	0.051
2-me-3-eth-pentene-2	778.4	780.0	0.082	765.5	767.6	0.109
octene-1	781.2	781.7	0.028	816.8	817.0	0.011
tr-octene-4	783.6	783.9	0.014	814.3	814.1	- 0.012
1-cis-3-di-me-cyclohexane	785.0	789.9	0.242	791.6	796.7	0.255
1-1-di-me-cyclohexane	787.0	792.8	0.291	799.0	805.0	0.252
2-3-di-me-hexene-2	788.8	790.2	0.072	825.9	826.4	0.025
2-2-4-tri-me-hexane	789.1	791.7	0.132	791.3	794.0	0.138
1-me-cis-3-eth-cyclopentane	790.3	794.1	0.192	-	-	-
tr-octene-2	797.7	797.7	0.000	831.9	831.4	- 0.024
1-tr-2-di-me-cyclohexane	801.8	807.5	0.287	810.2	816.3	0.307
1-cis-2-cis-3-tr-me-cyclopentane	802.2	807.7	0.275	812.8	818.5	0.288
1-cis-4-di-me-cyclohexane	805.2	810.5	0.267	816.8	822.2	0.272
1-tr-3-di-me-cyclohexane	805.6	810.8	0.261	817.8	823.0	0.260
2-4-4-tri-me-hexane	807.7	810.8	0.157	810.1	813.4	0.166
2-3-5-tri-me-hexane	812.0	813.7	0.084	815.4	817.0	0.081
iso-prop-cyclopentane	812.1	817.1	0.247	823.4	828.6	0.264
2-2-di-me-heptane	815.4	816.5	0.051	816.7	817.6	0.044

2-2-3-4-tetra-me-pentane	819.6	824.0	0.218	826.0	830.6	0.231
1-me-cis-2-eth-cyclopentane	821.0	825.6	0.233	834.0	838.8	0.240
2-2-3-tri-me-hexane	821.6	824.3	0.138	826.3	829.0	0.139
2-2-di-me-3-eth-pentane	822.2	826.2	0.200	826.8	831.0	0.210
1-cis-2-di-me-cyclohexane	829.3	835.4	0.307	842.2	-	-
n-prop-cyclopentane	830.3	834.0	0.184	843.2	847.0	0.193
eth-cyclohexane	834.3	839.7	0.269	845.7	851.3	0.278
eth-benzene	834.6	839.8	0.262	-	-	-
3-3-di-me-heptane	835.8	838.0	0.110	839.1	841.3	0.109
2-4-di-me-3-eth-pentane	836.5	839.9	0.171	841.7	845.4	0.183
1-1-3-tri-me-cyclohexane	840.4	846.0	0.282	850.2	855.9	0.286
2-3-4-tri-me-hexane	849.1	852.0	0.146	854.8	857.9	0.156
1-4-di-me-benzene	852.1	854.0	0.247	-	-	-
2-2-3-3-tetra-me-pentane	852.1	857.5	0.272	860.6	866.4	0.290
2-3-3-4-tetra-me-pentane	858.0	863.2	0.257	865.7	871.3	0.278
1-2-di-me-benzene	869.6	875.3	0.285	-	-	-
3-me-octane	870.2	870.7	0.030	871.7	872.3	0.028
3-3-di-eth-pentane	877.2	882.4	0.262	882.5	888.3	0.286

4.4 INTERLABORATORY AGREEMENT OF RETENTION DATA.

The possibility of the efficient exchange of retention data, as a tool for the characterization of compounds by gas-chromatography, greatly depends on the accuracy of these data.

Previous publications (1,2) comparing retention data of different laboratories (ring tests) show that for hydrocarbons the agreement is only 1 to 10 index units.

In some cases it has been claimed that retention data can be acquired with a precision of 0.1 index units (3-6) or even better (7-10). Results obtained by these authors are compared with our results in Table 4.4, for hydrocarbons differing in structural type and degree of branching. Retention indices measured at a different temperature (Sojak: 86^{o}C) were converted to 70^{o}C, using our dI/dT values.

Retention indices of Matukuma (6) and Hiveley and Hinton (5) are omitted. Their values for unsaturated compounds are up to 3 index units higher than the mean values of the other laboratories.

Table 4.4 Comparison of retention indices of C_6-C_9 hydrocarbons measured in different laboratories. Temperature: 70°C. Stationary phase: squalane.

	I_{70}						
	Tourres	Sojak	Schomburg (Ref.18) a	Rijks b	ΔI_{max}	ΔI_{a-b}	dI/dT
3-me-hexane	677.3	-	676.95	676.94	0.4	0.01	0.036
2-3-di-me-pentane	673.7	-	673.37	673.41	0.3	-0.04	0.085
2-3-3-tri-me-pentane	764.1	-	763.22	763.42	0.9	-0.20	0.202
2-2-3-3-tetra-me-pentane	858.1	-	857.21	857.51	0.9	-0.30	0.272
cyclopentane	568.2	-	568.18	568.49	0.3	-0.31	0.138
eth-cyclopentane	738.2	-	737.24	737.49	1.0	-0.25	0.183
cyclohexane	667.5	-	666.75	667.15	0.7	-0.40	0.222
eth-cyclohexane	840.3	-	838.95	839.66	1.3	-0.71	0.269
hexene-1	582.2	582.6	582.71	582.73	0.5	-0.02	0.024
heptene-1	682.3	682.4	682.30	682.33	0.1	-0.03	0.025
octene-1	781.5	781.9	781.65	781.71	0.4	-0.06	0.028
tr-hexene-2	597.8	596.8	596.78	596.71	1.1	0.07	-0.007
tr-hexene-3	591.6	591.7	591.66	591.61	0.1	0.05	-0.022
tr-octene-4	783.7	784.0	783.84	783.86	0.3	-0.02	0.014
3-me-cis-hexene-3	684.5	-	685.04	685.04	0.5	0.00	0.020
2-3-di-me-pentene-1	652.0	-	652.18	652.23	0.2	-0.05	0.099
2-3-3-tri-me-butene-1	630.7	-	630.67	630.71	0.0	-0.04	0.112
2-4-4-tri-me-pentene-2	716.3	-	716.33	716.28	0.0	0.05	0.044
cyclopentene	551.1	-	551.57	552.03	0.9	-0.46	0.128
pentadiene-2-3	530.2	-	529.26	529.51	0.9	-0.25	-0.032
hexadiene-1-5	563.3	-	563.48	563.74	0.4	-0.26	0.040
benzene	-	642.9	641.56	641.88	1.3	-0.32	0.232
toluene	-	750.2	748.93	750.15	1.3	-1.22	0.240
eth-benzene	-	840.1	838.69	839.81	1.4	-1.12	0.262
p-xylene	-	854.1	852.95	854.00	1.1	-1.05	0.247
o-xylene	-	875.5	874.05	875.31	1.4	-1.26	0.285

The deviations (ΔI_{max}) are larger than can be explained by the confidence limits (0.2 index units) of the respective measurements. They can only be partly explained by systematic differences in temperature or by different methods of estimating the dead time (t_M). Most likely the main factors contributing to these discrepancies are the adsorption by the column wall in combination with sample size (asymmetric peaks) and the non ideality of the carrier gas (chapter 2).

This result does not come up to the expectations given rise to in section 4.2, an agreement of retention data between laboratories better than 0.2 index units.

However, with exception of benzene the retention indices given by Sojak at 86°C, after conversion to 70°C with our dI/dT, agree with our data within 0.3 index units even for aromatic compounds, which are most strongly influenced by adsorption and non ideality of the carrier gas.

To enable a discussion of systematic deviations between laboratories, it was agreed with Schomburg to exchange some mixtures of hydrocarbons for measurement of retention indices on open-hole tubular squalane columns, at 70°C. The choice of all other experimental conditions was free. The repeatability in both laboratories corresponds with a standard deviation of < 0.05 index units. The column material was glass in Schomburgs laboratory and stainless steel in our laboratory.

Considering the results of this exchange, given in Table 4.4, it can be concluded that the difference in retention index (ΔI_{a-b}), which is considerable for aromatic compounds, is systematic. This difference can be explained for the main part in the following way.

There is no reason to doubt that the stationary phase, which is a pure compound, will cause systematic deviations. In both laboratories special attention has been paid to the effect of adsorption (retention index and symmetry of the benzene peak), as discussed in section 2.6.5 and the quantity of sample (section 2.4.5). In first instance the influence of these parameters can be neglected. The composition of the mixtures was chosen in such a way that all components are completely separated. The influence of the peak shift can also be neglected.

Considering compounds which are not (alkanes) or hardly (alkenes) influenced by the non ideality of the carrier gas it can be concluded that the column temperature in Schomburgs laboratory was about 1°C lower than our column temperature. In this way 0.1 to 0.3 index units of difference can be explained for alkanes, cycloalkanes and aromatics, dependent on the dI/dT of the solutes.

The influence of the non ideality of the carrier gas was discussed in section 2.6.4. For different carrier gases and different inlet pressures correction factors can be derived from Table 2.8, dependent on the structural type. In Schomburgs laboratory helium was used as the carrier gas, so that their retention indices are independent of inlet pressure. In our laboratory nitrogen was used. The pressure drop over the column was 3 atm. However in Table 2.8 only representatives of different structural types have been measured, most likely the corrections for the non ideality of the carrier gas will also be valid for their homologs. In this way for cyclopentanes a difference of about 0.3 index units can be explained. For cyclohexanes the correction is 0.4 index units. For aromatics the correction is about 0.6 index units.

It can be concluded that the retention indices obtained in both laboratories would agree within 0.2 index units if the carrier gas and the column temperature were equal.

From the foregoing it can be concluded that to obtain an interlaboratory agreement of retention data for hydrocarbons better than 0.2 index units, the following points have to be considered very carefully:

1 Column temperature.
2 Definition and stability of the stationary phase.
3 Carrier gas and mean column pressure.
4 Retention index of benzene and the symmetry of the benzene peak.
5 Quantity of sample.
6 Peak shift caused by incomplete separation.
7 Method of estimating "dead time".

Only in this way data are interconvertible.

4.5 CHARACTERIZATION OF TYPES OF HYDROCARBONS BY MEANS OF ACCURATE RETENTION DATA.

One of the reasons favouring the use of the retention index in the collection of retention data is its weak temperature dependence. However, if extreme care is taken in the measurement of retention indices at two not too distant temperatures, the value of dI/dT appears to give some additional analytical information. This offers promising possibilities (3,12), particularly in the analysis of complex mixtures, e.g. petroleum fractions. It was experimentally proved that the temperature dependence of retention indices, for hydrocarbons, in the range considered (50^{o}C to 70^{o}C), is linear for squalane columns, within experimental error. Peak inversion in this temperature range is unlikely to occur. dI/dT values are relatively easy to collect.

By definition dI/dT for n-alkanes is zero. The numerical value of dI/dT increases with the degree of branching. The largest values are found for cyclic compounds. The effects of branching and cyclisation, on dI/dT, are much stronger than the effect of unsaturation.

From dI/dT values alone it does not appear to be possible to distinguish between cyclopentanes and highly branched alkanes (more than 2 tertiairy or more than one quarternary carbon atoms) or between alkanes and alkenes.

The use of ΔI (the difference in retention index on polar and apolar phases) has already been introduced by Kovats (11) as an aid to recognizing types of compounds. ΔI depends on both the polarity of the compound and the polarity of the stationary phase.

Classes of hydrocarbons having similar ΔI values cannot be distinguished in this way. If, however, dI/dT is plotted against ΔI values, for the same components, a distinction can be made between components having a similar ΔI and different dI/dT or components having a similar dI/dT and a different ΔI. In fact this is a three- dimensional plot.

Components having a similar dI/dT and a different ΔI.

For hydrocarbons, dI/dT values measured in our laboratory on squalane, are plotted against ΔI values ($\Delta I = I_{\text{acetyltri butylcitrate}} - I_{\text{squalane}}$, T = 70°C, Table 4.3) in Fig. 4.1.

Although this method offers the possibility of characterizing different types of hydrocarbons in complex mixtures, further experiments have to be done, especially in the higher molecular weight range and with phases which are more polar.

Accurate dI/dT values can also be applied to distinguish cis and trans alkenes as shown in Table 4.5.

Table 4.5 Temperature dependence of retention indices of cis and trans alkenes on squalane.

Component	dI/dT	
	cis	trans
butene-2	0.017	- 0.015
pentene-2	0.010	- 0.010
hexene-2	0.025	- 0.007
heptene-2	0.030	0.001
octene-2	0.029	0.000
hexene-3	0.019	- 0.022
heptene-3	0.034	- 0.006
octene-3	0.020	- 0.010
3-me-pentene-2	0.033	0.008
3-me-hexene-2 1)	0.045	0.008
3-me-hexene-3 1)	0.020	- 0.009
4-me-pentene-2	0.024	- 0.013
4-me-hexene-2	0.060	0.028
2-2-di-me-hexene-3	0.103	- 0.020
3-4-di-me-pentene-2	0.044	0.029
4-4-di-me-pentene-2	0.105	- 0.008

1) The nomenclature assigned by the American Petroleum Institute has been reversed in this Table.

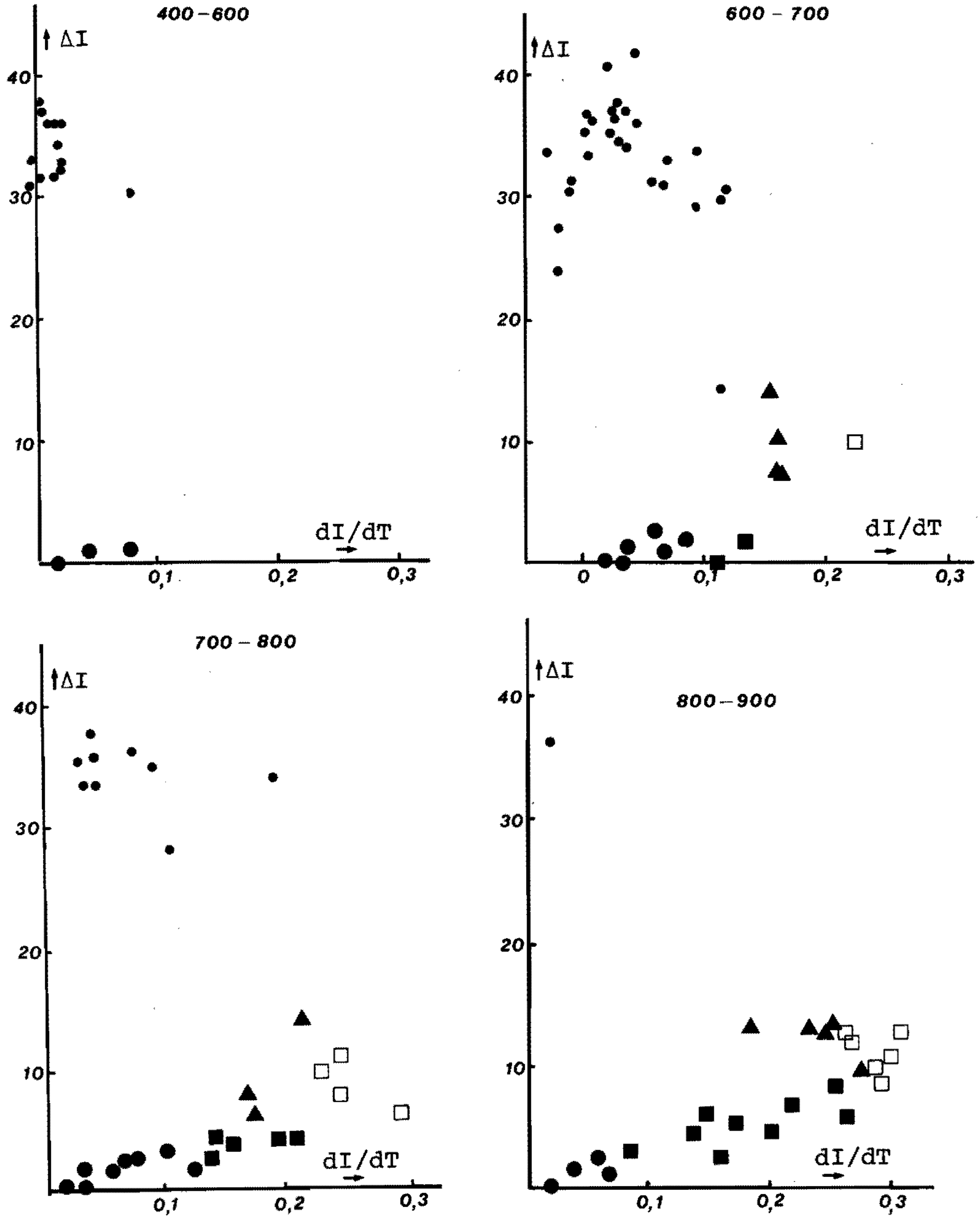

Fig. 4.1 Plot of the temperature coefficient of the retention index (on squalane) against ΔI ($I^{70}_{acetyltributylcitrate} - I^{70}_{squalane}$).
● = slightly branched alkanes; ■ = highly branched alkanes;
● = alkenes; ▲ = cyclopentanes; □ = cylohexanes.

It can be concluded from these results that dI/dT of cis alkenes is higher than of the corresponding trans isomers, independent of the elution order. For 2 and 3 alkenes the trans configuration is eluted first, for mono and dimethyl alkenes the elution order is reversed. In this way it was found that the nomenclature assigned by the American Petroleum Institute should be reversed for 3-me-hexene-2 and 3-me-hexene-3. This conclusion is confirmed by several authors in a different way (5,6,12-14).

4.6 POTENTIALITIES OF PRECISION GAS CHROMATOGRAPHY FOR THE QUALITATIVE ANALYSIS OF COMPLEX MIXTURES OF HYDROCARBONS.

4.6.1 Introduction.

The use of different temperatures and of different columns adds considerably to the final characterization. The possibility of coincidence of various retention data can be greatly reduced in this way.

A difficulty in the application of columns of different polarity, for the analysis of complex mixtures, is the recognition of corresponding peaks, which is complicated because of peak inversion.

To solve this problem a system was developed, consisting of two open-hole tubular columns (length > 75 m, i.d. 0.25 mm) of different polarity, placed in series. The components eluted from the first column may be selectively transferred to the second column of different polarity. The system has no valves in the sample path, so that the dead volume associated with the use of valves in the sample path is eliminated. The possibility of absorption of sample components in lubricants of low friction plastics is also eliminated.

The system is based on the principle of counter pressure and on venting between the columns (15-17). The magnitude of the counter pressure and the resistance of the vent have to be carefully adjusted to the resistance of both columns and the resistance of the connections. In the case of complex mixtures, compounds which show a poor resolution or which are not resolved at all on the first column, can be separated and characterized on the second column.

The precision of the retention data obtained with the system is of the same order as the precision obtained with single columns (s.d. < 0.05 index units), as will be demonstrated on some examples in the following. The system can also be applied for qualitative analysis of trace components.

4.6.2 System design.

The system is schematically given in Fig. 4.2.

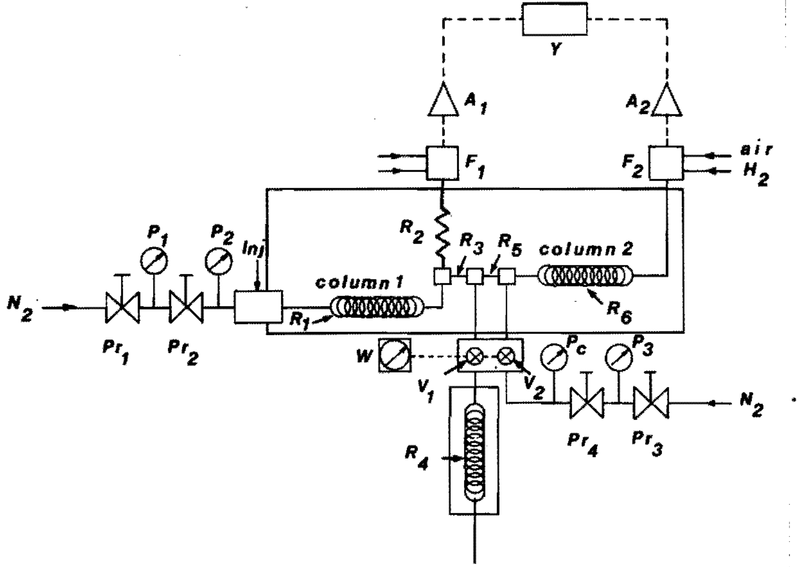

Fig. 4.2 Schematic of the system.

Pr_1 - Pr_4 = Pressure controllers (BECKER, Delft, MB-19936).

P_1 = Column inlet pressure (WALLACE AND TIERNAN, FA 145).

P_c = Counter pressure (WALLACE AND TIERNAN, FA 145).

Column 1 = Length: 100 m, i.d.: 0.25 mm, stationary phase: squalane, material: stainless steel.

Column 2 = Length: 75 m, Stationary phase: acetyltributylcitrate, i.d. and material: see column 1.

F_1, F_2 = Flame ionisation detector.

A_1, A_2 = Detector amplifier.

Y = Recorder (2 tracks).

R_2, R_3, R_5 = Restrictions (dimensions see Fig. 4.3).

R_4 = Restriction (stainless steel capillary, length: 86 m, i.d.: 0.25 mm).

Inj = Inlet system with splitter (HAMILTON).

V_1, V_2 = Solenoid valves (LUCIFER).

W = Time delay switch (SCHLEICHER).

The connections between the columns, the vent and the detector corresponding with the first column are given in detail in Fig. 4.3.

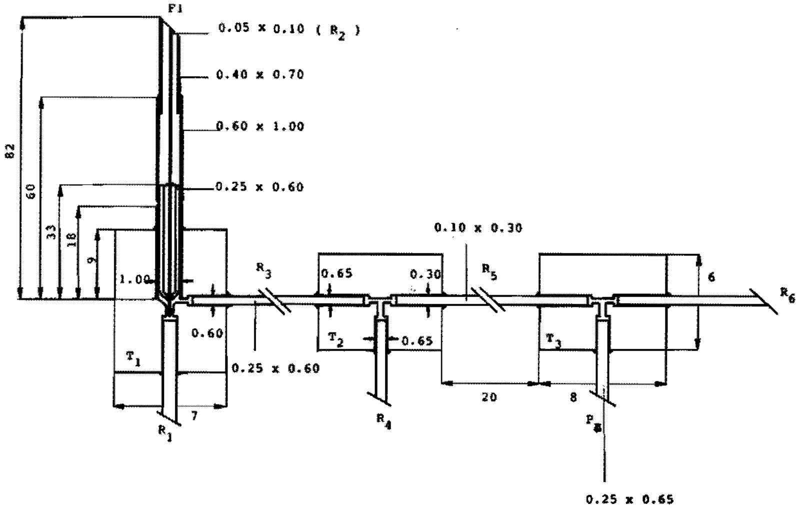

Fig. 4.3 Connections between the columns, the vent and the detector corresponding with the first column.

The sample is injected on the first column with a microsyringe by way of an injection system with splitter (HAMILTON). The carrier gas velocity is controlled by two pressure controllers(BECKER, Delft, MB-19936), placed in series. The pressure drop over the columns (P_2) is registered with a high quality differential manometer (WALLACE AND TIERNAN, FA 145), which can be read out to 0.001 atm. A small part of the gas leaving the first (squalane) column is split off to the first detector (F_1), via a restriction (R_2). The detector signal is registered on a two pen recorder Y (track 1). The rest of the eluent of the first column is sluiced through restriction 4, if the valves V_1 and V_2 are open (normal situation) and is transferred to the second column if $V_1$and V_2 are closed.

The counter pressure (P_c) is controlled and measured in the same way as the inlet pressure (P_2). It has to be carefully adjusted, since to enable precise measurement of retention times on both columns, the carrier gas velocity should be independent of the position of the valves V_1 and V_2. At the same time every compound eluted from the first column should be vented through the restriction R_4, if V_1 and V_2 are open.

To enable a selective injection of a compound on to the second column, the valves V_1 and V_2 which can be actuated together by the time delay switch W, are closed during Δt seconds. A component which is registered at the same time in the detector F_1, will be transferred to the second column during this time. The quantity of sample transferred to the second column can be changed by a variation of Δt. The compounds eluted from the second column are registered on the second track of the recorder Y. The temperature of the part outlined in Fig. 4.2 is kept constant by a water thermostat, within 0.01°C.

4.6.3 Application of the system.

The system described above can be applied to the qualitative analysis of complex mixtures of hydrocarbons, in combination with high-resolution columns and precise retention data in the following way.

Comparison of retention indices of unknown peaks with tabulated retention data (Table 4.3 or accurate retention data given in the literature), at a temperature T_1 (e.g. 50°C) for the first (squalane) column. To every peak in the chromatogram one or more names can be assigned, dependent on the search window. The search window, defined as the interval in retention index within which a compound can be expected, depends on both the separation power of the column and the accuracy of the measured and tabulated retention data.

To be more certain about the name of the component the same procedure can be followed at another temperature, T_2(e.g. 70°C). Comparison of the names obtained at both temperatures possibly will result in the elimination of some of the names assigned to a component at T_1. It must be remembered here that peak inversion is unlikely to occur at two not too distant temperatures (e.g. < 20°C).

The additional information of this second step is dependent on the difference in dI/dT of the compounds in question. Compounds which are not or not completely separated at T_1 (50°C) can be completely or partly separated at T_2 (70°C).

The degree of certainty of the characterization can be raised considerably by application of the dual column system described in section 4.6.2. Compounds which are not or incompletely separated on the first column and which are transferred to the second column of different polarity, will be generally completely or at least partly separated on the second column. The transfer of more than one compound to the second column, during one run, is also possible, although this number is limited. It must be realized, however, that the transfer of a compound to the second column is only necessary when a further elimination of possible names is required.

The potentialities of this procedure will be demonstrated in the following by some examples.

4.6.4 Transfer of one peak per run.

In the first instance the possibilities of the system will be demonstrated by help of a simple synthetic mixture of four hydrocarbons, the bracketing n-alkanes and methane. The composition of the mixture was chosen in such a way that 3 components were not separated on the first column, as shown in Fig. 4.4.

Retention indices measured on both columns, at 50°C, are given in Table 4.6.

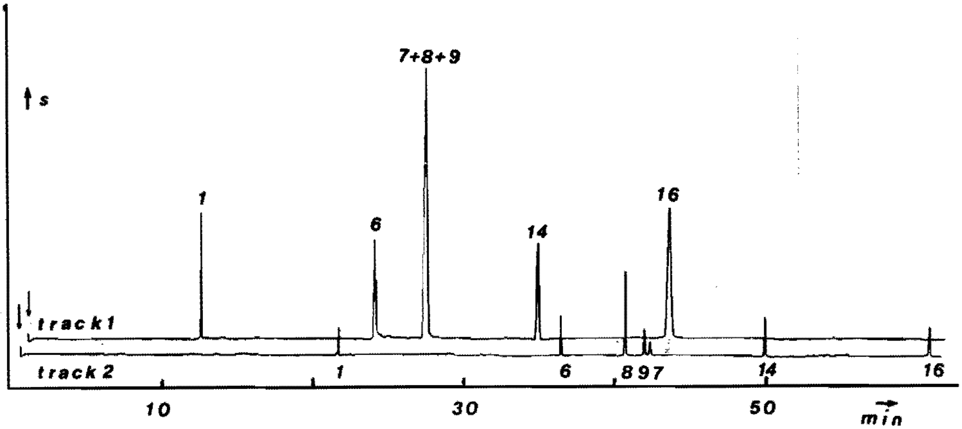

Fig. 4.4 Chromatogram of a mixture of 4 hydrocarbons (7-9, 14), the bracketing n-alkanes (6, 16) and methane (1).
Column 1. Stationary phase: squalane, length: 100 m, i.d.: 0.25 mm, temperature: 50°C ± 0.01°C.
Column 2. Stationary phase: acetyltributylcitrate, length: 75 m, i.d. and temperature: see column 1.

Comparison of measured and tabulated (Table 4.3) retention indices on the squalane column results in the assignment of 3 possible names for the peak 7+8+9 (track 1), with a search window of 1.0 index units which is based on the separation power of the column. From the retention indices on the second column it can be concluded that the peak 7+8+9 consists of 2-3-di-me-butene-2, 2-2-di-me-pentane and 3-3-di-me-pentene-1. It can also be concluded that the precision of the retention indices obtained with the system is of the same order as the precision obtained with single columns.

4.6.5 Transfer of more than one peak per run.

A considerable gain in time will be obtained if more than one compound is transferred to the second column during one run. However, some troubles can be expected in this case. According t the combination of peak inversion and different injection times on the second column, peaks which are completely separated on the first column possibly will be not or not completely separated on the second column or coelute with another component. On the other hand two components which are partly separated on the first column can give a double peak on the second column (2 injections). In both cases a decrease in precision can be expected, as will be demonstrated in the following.

Table 4.6 Comparison of retention indices obtained with the system and tabulated data, at 50°C ± 0.01°C. Abbreviations: s = system, t = tabulated, sq = squalane, ac = acetyltributylcitrate.

Peak number	$I_{sq,s}$	$I_{sq,t}$	Δ_{s-t}	Name	$I_{ac,s}$	$I_{ac,t}$	Δ_{s-t}
1				methane			
6				n-hexane			
7	625.49	625.12	0.37	2-3-di-me-butene-2	663.27	663.35	-0.08
8		625.64	-0.15	2-2-di-me-pentane	625.81	625.67	0.14
9		626.18	-0.69	3-3-di-me-pentene-1	655.92	655.96	-0.04
14	662.62	666.63	-0.01	2-me-hexane	666.97	666.96	0.01
16				n-heptane			

Of a synthetic mixture of 13 hydrocarbons, the bracketing n-alkanes and methane, retention indices were measured with the system at 50°C (Fig. 4.5 and Table 4.7) and 70°C (Fig. 4.6 and Table 4.8). All the components were transferred to the second column during one run.

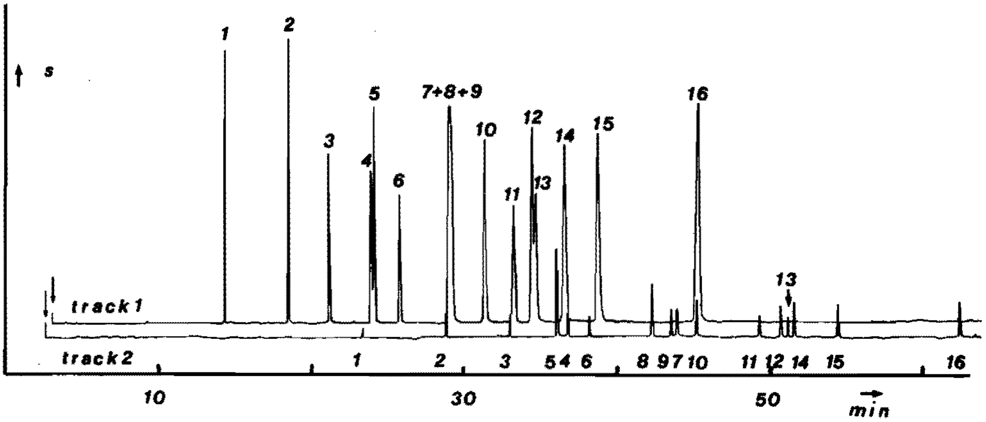

Fig. 4.5 Chromatogram of a mixture of hydrocarbons, at 50°C. Columns: see Fig. 4.4.

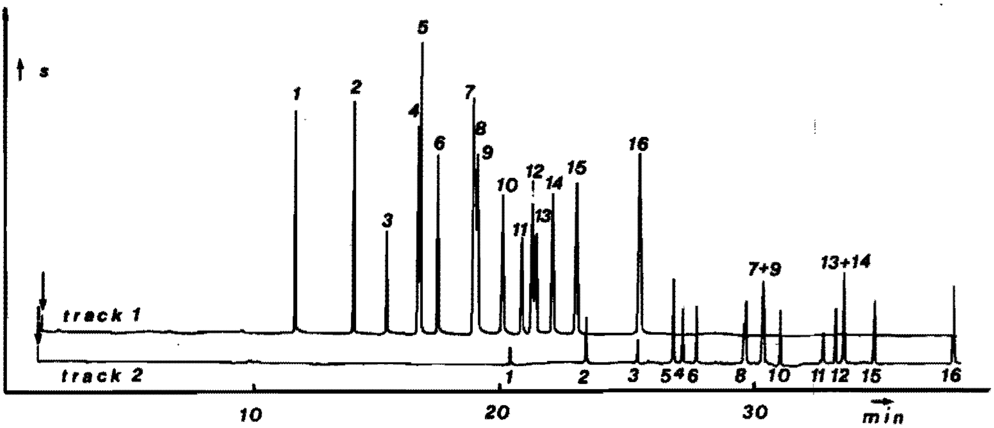

Fig. 4.6 Chromatogram of a mixture of hydrocarbons, at 70°C. Columns: see Fig. 4.4.

Table 4.7 Comparison of retention indices obtained with the system and tabulated values, at 50°C ± 0.01°C. Abbreviations: s = system, t = tabulated, sq = squalane, ac = acetyltributylcitrate, s.d. = standard deviation.

Peak n°	$I_{sq,s}$	s.d.	$I_{sq,t}$	Δ_{s-t}	Name	$I_{ac,s}$	s.d.	$I_{ac,t}$	Δ_{s-t}
1	-	-	-	-	methane	-	-	-	-
2	-	-	-	-	n-pentane	-	-	-	-
3	549.33	<0.01	549.35	-0.02	4-me-pentene-1	581.29	0.03	581.38	-0.09
4	582.23	0.01	582.25	-0.02	hexene-1	616.78	0.04	616.87	-0.09
5	584.22	<0.01	584.24	-0.02	3-me-pentane	585.25	0.04	585.33	-0.08
6	-	-	-	-	n-hexane	-	-	-	-
7	625.49	0.02	625.12	0.37	2-3-di-me-butene-2	663.27	0.01	663.35	-0.08
8			625.64	-0.15	2-2-di-me-pentane	625.81	0.01	625.67	0.14
9			626.18	-0.69	3-3-di-me-pentene-1	655.92	0.03	655.96	-0.04
10	639.67	<0.01	639.70	-0.03	2-2-3-tri-me-butane	641.88	0.03	641.88	0.00
11	650.39	<0.01	650.44	-0.05	2-3-di-me-pentene-1	684.17	0.01	684.18	-0.01
12	656.62	<0.01	656.66	-0.04	4-me-tr-hexene-2	684.18	0.03	684.20	-0.02
13	657.83	<0.01	657.90	-0.07	4-me-hexene-1	691.20	0.04	691.21	-0.01
14	666.62	<0.01	666.63	-0.01	2-me-hexane	666.97	0.02	666.96	0.01
15	676.21	<0.01	676.21	0.00	3-me-hexane	677.39	0.02	677.42	-0.03
16	-	-	-	-	n-heptane	-	-	-	-

number of measurements 5 3

It must be realized that the elution order of the components on the second column (track 2) and the order of size of the retention indices can be reversed, because of interaction of peak inversion and different injection times on the second column. This is clearly demonstrated in Table 4.7 and 4.8.

Table 4.8 Comparison of retention indices obtained with the system and tabulated data, at 70°C ± 0.01°C. Abbreviations: see Table 4.7

Peak n°	$I_{sq,s}$	s.d.	$I_{sq,t}$	Δ_{s-t}	Name	$I_{ac,s}$	s.d.	$I_{ac,t}$	Δ_{s-t}
1	-	-	-	-	methane	-	-	-	-
2	-	-	-	-	n-pentane	-	-	-	-
3	550.37	0.02	550.36	0.01	4-me-pentene-1	582.09	0.05	582.03	0.06
4	582.75	0.02	582.73	0.02	hexene-1	616.95	0.04	617.05	-0.10
5	585.14	0.01	585.14	0.00	3-me-pentane	586.18	0.05	586.29	-0.11
6	-	-	-	-	n-hexane	-	-	-	-
7	625.67	0.01	625.65	0.02	2-3-di-me-butene-2	663.25	0.16	663.35	-0.10
8	-	-	626.95	-	2-2-di-me-pentane	626.57	0.27	626.96	-0.39
9	628.40	0.01	628.52	-0.12	3-3-di-me-pentene-1	656.62	0.35	658.07	1.45
10	642.64	0.01	642.59	0.05	2-2-3-tri-me-butane	644.98	0.07	645.06	-0.08
11	652.19	0.01	652.23	-0.04	2-3-di-me-pentene-1	685.53	0.05	685.68	-0.13
12	657.36	0.01	657.40	-0.04	4-me-tr-hexene-2	687.07	0.04	687.10	-0.03
13	659.19	0.01	659.25	-0.06	4-me-hexene-1	692.09	0.31	692.24	-0.15
14	666.93	0.01	666.96	-0.03	2-me-hexane	667.22	0.10	667.20	0.02
15	676.93	0.01	676.94	-0.01	3-me-hexane	677.96	0.04	678.11	-0.15
16	-	-	-	-	n-heptane	-	-	-	-
number of measurements			4			4			

In spite if the incomplete separation of the components 4,5 and 12,13 on the first column, the influence of peak shift is only slightly noticeable for the last two components, at 50°C. The effect of peak inversion of the components 4 and 5 is so large that they are completely separated on the second column, at both temperatures. The components 13 and 14, however, are coeluted at 70°C, resulting in a decreased precision, especially for component 13.

For the components 7,8 and 9 these problems are much more complicated. At 50°C, only one peak can be observed for these components. The retention index on the squalane column is strongly influenced by the peak shift (section 2.6.3.). Nevertheless these components can be characterized, since they are completely separated on the second column (track 2).

Because of a different dI/dT these components are partly separated on the squalane column, at 70°C. Notwithstanding that they are hardly separated, the influence of peak shift, on this column, is very small for the components 7 and 9. Interaction of peak inversion and different injection times on the second column, at 70°C, result in co- elution of component 7 and 9. For component 8 a double peak is found. In both cases the precision is considerably decreased. This can be explained as follows.

The transfer of a peak to the second column is started at the moment a peak maximum is observed from the first column. Because the peaks 7, 8 and 9 are transferred to the second column during one run, component 8 is injected 2 or 3 times, dependent on the difference in retention time of these components and the width of the injection (Δt, the time during which the valves V_1 and V_2 are closed).

From the foregoing it may be concluded, that a limited number of components can be transferred during one run, if they are completely separated on the first column.

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APPENDIX

Since the beginning of gas chromatography many suggestions for expressing retention data have been proposed by several authors and committees, in order to enable the general use of published retention data. Every method has advantages and disadvantages and will be discussed briefly in the following.

The most direct way to determine the location of a peak in a chromatogram is the retention time (t_R). This is the time elapsed between the injection of a sample and the appearence of the maximum of each peak. The retention time is dependent on most of the experimental parameters (e.g. temperature, carrier gas velocity, column length etc.). It is not suitable for comparison of retention data between laboratories and is even difficult to reproduce in the same laboratory on another instrument.

Adjusted retention time, t_R' is the time elapsed between the appearence of a not retarded peak and the peak in question. It can be obtained subtracting the gas hold up time (t_m) from the retention time. It suffers from the most disadvantages of the retention time, but is suitable to calculate relative retention time, the capacity ratio and other retention data.

The capacity ratio, defined as $k = t_R'/t_m$, has the advantage of being directly related to the partition coefficient, K, by the relation $k = K.V_s/V_m$, where V_s and V_m represent the volumes of the stationary and the mobile phases, respectively. On account of its direct relation to the partition coefficient, this should be a useful method of presentation in all cases. Since the phase-volume ratio is difficult tc control it cannot be expected to be very reproducible.

The specific retention volume, V_g^o, introduced by Littlewood (1) in order to make retention data independent on temperature, amount of stationary phase in the column, carrier gas flow-rate and pressure drop over the column, is given by the equation:

$$V_g^o = \frac{t_R - t_m}{w_s} \cdot F_c \cdot \frac{3}{2} \cdot \frac{P^2-1}{P^3-1} \cdot \frac{273}{T_K} \qquad (1)$$

where w_s = the weight of the stationary phase.
F_c = the volumetric velocity at the column outlet.
P = the ratio of column in- and outlet pressure.
T_K = the column temperature, in ° Kelvin.

Since the temperature dependence of a compound cannot be described by this method (reduction to 0°C) and the quantity of stationary phase is difficult to determine and may change during use, it is not widely applicated.

Therefore the most widespread method used to day is the relative retention, which can be directly calculated from the chromatogram. The use of relative retention data has the advantage that a number of variables are eliminated (column length, carrier gas velocity, quantity of stationary phase and volume of the mobile phase). It is also directly related to the partition coefficient. The precision is dependent on the distance between the peak i and the standard compound s. The relative retention time is defined as the ratio of the adjusted retention time of a compound i and an arbitrarely chosen standard substance s:

$$r_{i,s} = \frac{t_i'}{t_s'} = \frac{K_i}{K_s} \qquad (2)$$

Its wide application is limited since there is no agreement between laboratories about the choice of the standard compound.

In order to overcome this difficulty Evans and Smith(2) introduced the theoretical nonane value ($r_{i,9}$), where 9 stands for n-nonane. The retention is first determined to the closest n-alkane and transferred to a system in which n-nonane is the standard. Although this system has one standard, the precision of the relative retention time depends upon the distance of the primary standard and n-nonane. Another problem in using relative retention times is the temperature dependence of the compounds, which is not linear and relatively large.

To overcome the difficulties previously mentioned, Kovats (3) proposed the retention index, in 1958, defined as following:

$$0.01\ I_i = \frac{\log t'_i - \log t'_z}{\log t'_{z+1} - \log t'_z} + z \qquad (3)$$

By definition $t'_z < t'_i < t'_{z+1}$

where t'_i = the adjusted retention time of a component i.

t'_z = the adjusted retention time of a n-alkane with z carbon atoms.

t'_{z+1} = the adjusted retention time of a n-alkane with z+1 carbon atoms.

The relation between the retention index and relative retention time can be obtained, rewriting equation 3:

$$0.01\ I_i = \frac{\log r_{i,z}}{\log r_{z+1,z}} + z \qquad (4)$$

Since that time the Kovats retention index has been advocated by many authors (e.g. 4-7), as a means for standardisation of retention data. It appears to be widely accepted nowadays.

SUMMARY

Potentially, high resolution gas chromatography, which combines an unsurpassed separation power to minute sample requirements, is extremely suited for the identification of volatile organic compounds in complex mixtures. The quantity of sample ($< 10^{-7}$g), although an advantage in many applications, almost excludes the use of ancillary techniques, except mass spectrometry, for the identification of separated compounds.

Therefore it is highly desirable to have means available for the characterization of GC peaks by retention data only. Sets of collected accurate retention data are essential for this purpose.

A glance at selected recent literature data, however, reveals a lack of agreement between laboratories amounting to one to five Kovats retention index units.

Retention values will become more characteristic with increasing accuracy and separation power of the column.

In this work we have firstly investigated the role of all of the relevant factors which contribute to the random error. The most important factors proved to be, fluctuations of temperature, carrier gas velocity, time measurement and quantity of sample.

It is shown that a precision corresponding to a standard deviation of 0.05 index units is attained.

We also set out to reduce the systematic errors by studying the role of: the nature of the stationary phase, peak shift through incomplete separation, the non ideality of the carrier gas, interfacial adsorption, the gas hold-up time.

It is demonstrated that it is possible to reproduce retention indices to within about 0.2 index units, in other laboratories.

It is also shown that the temperature coefficient of the retention index, which is now also available with a higher accuracy, yields important information both for characterization and stuctural information (e.g. cis-alkenes have a consistently higher dI/dT than the trans-alkenes).

A method is found to prepare high resolution capillary columns in a reproducible way and with a homogeneous film. The effect of adsorption by the column wall is negligible.

A list of retention indices of some 170 hydrocarbons (C_4-C_9) on squalane (non polar) and acetyltributylcitrate (polar) at 50 and 70°C is presented.

To make the utmost use (for the characterization of hydrocarbons in complex mixtures) of the combination of high resolution columns and accurate retention data, a system is described in which two open-hole tubular columns are coupled. This system enables the transfer of one or more selected peaks eluting from (and measured on) the first column to a second column of different polarity. The sample transfer does not affect the accuracy of the retention data.

SAMENVATTING.

Gas chromatografie met kapillaire kolommen is een bij uitstek geschikte methode voor het identificeren van vluchtige organische componenten in complexe mengsels. Het toepassen van andere analyse technieken, met uitzondering van massa spectrometrie, voor het identificeren van gescheiden componenten is practisch uitgesloten door de zeer keine monster grootte ($< 10^{-7}$g), die overigens in de meeste gevallen als een voordeel moet worden beschouwd.

Het karakteriseren van componenten met alleen gas chromatografie is daarom van groot belang.

De beschikbaarheid van tabellen met nauwkeurige retentie grootheden is hierbij essentieel.

Uit recente literatuur gegevens blijkt echter dat de overeenstemming van retentie grootheden, gemeten op verschillende laboratoria, veel te wensen overlaat. Zelfs voor verzadigde koolwaterstoffen liggen de verschillen tussen 1 en 5 Kovats retentie index eenheden.

De retentie grootheden zijn meer karakteristiek naarmate de meetnauwkeurigheid toeneemt en het scheidend vermogen van de kolommen groter is.

Door een betere beheersing van de belangrijkste oorzaken van toevallige fouten (fluctuaties in de kolomtemperatuur, de draaggas snelheid, de tijdmeting en de monster grootte) werd, met één kolom en één instrument, een reproduceerbaarheid bereikt overeenkomend met een standaard afwijking van 0,05 index eenheden.

Dat het mogelijk is de verschillen in retentie index tussen laboratoria terug te brengen tot ongeveer 0,2 index eenheden, kon worden aangetoond na het minimaliseren van de relevante systematische fouten (aard en zuiverheid van de stationaire fase, onvolledige scheiding, niet idealiteit van het draaggas, adsorptie aan de fase grensvlakken en de "dode tijd").

Bij de bereikte meetnauwkeurigheid kan uit de temperatuur coefficient van de retentie index waardevolle informatie worden verkregen voor het vaststellen van de identiteit en de structuur van gescheiden componenten (bijv. de dI/dT van cis-alkenen blijkt systematisch hoger dan de dI/dT van trans-alkenen).

Een reproduceerbare kolom kwaliteit voor stalen kapillaire kolommen werd bereikt door het modificeren van bestaande "coat" methoden. De kolommen hebben een homogeen verdeelde vloeistof film en de invloed van adsorptie aan de kolomwand is verwaarloosbaar.

Om alle mogelijkheden van de combinatie kapillaire kolommen en hoge meetnauwkeurigheid ten volle te benutten werd een systeem ontwikkeld, bestaande uit twee gekoppelde kapillaire kolommen. Met dit systeem kunnen een of meer geselecteerde componenten, na het doorlopen van de eerste kolom (waarop ze ook worden gemeten) worden doorgestuurd naar de tweede kolom, met een andere polariteit. De meetnauwkeurigheid van de retentie indices op beide kolommen wordt hierdoor niet beinvloed.

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LEVENSBERICHT.

Op aanbeveling van het college van Dekanen volgt hier een kort levensbericht van de schrijver.

Hij werd geboren te Brunssum 11 februari 1934. Na het behalen van het diploma H.B.S.-B aan het Bisschoppelijk College te Sittard in 1951, trad hij in dienst van het Stikstofbindingsbedrijf van de Staatsmijnen in Limburg.

Hij was hier werkzaam als analist en begon in september 1962 als werkstudent met de ingenieursstudie aan de Technische Hogeschool te Eindhoven, afdeling Scheikundige Technologie.

Op 22 december 1965 trad hij als laboratorium-assistent in dienst bij de Technische Hogeschool te Eindhoven. In januari 1970 werd het ingenieursexamen afgelegd in de afdeling Technologie.

Hierna volgde de benoeming tot wetenschappelijk medewerker bij de groep Instrumentele Analyse en werd het afstudeerwerk voortgezet met een onderzoek dat leidde tot dit proefschrift.

ERRATA Proefschrift v. J.A. Rijks.

P 57 Fig. 2.10 1= 4-me-pentene-1; 2= 2-3-di-me-butane;
3= n-hexane; 4= 4-me-cyclopentene; 5= benzene;
6= cyclohexane.

P 58 Fig. 2.11 ibid.

P 65 Fig. 2.13 log $_{z+1,z}$ must be log $r_{z+1,z}$.

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STELLINGEN

1 Bij de uitwisseling van retentie gegevens tussen laboratoria dient rekening te worden gehouden met het niet ideale gedrag van het draaggas.

2 De door de "Commission of Analytical Chemistry" van de IUPAC voorgestelde definitie van de retentie index moet, op grond van de niet lineairiteit van de log plot voor n-alkanen, worden afgewezen.

International Union of Pure and Applied Chemistry, Information Bulletin 15, 1973.

3 De door Czeglédy-Janko and Cieleszky gevolgde methode om het opwerkings rendement van gechloreerde pesticiden in bloed te bepalen is onjuist.

G. Czeglédy-Janko and V. Cieleszky, Analyst 93, (1968), 445.

4 De door Weiss en zijn medewerkers gevonden correlatie tussen de roosterconstanten en de biologische werking van kwarts is aanvechtbaar.

Z. Weiss et al., Staub-Reinhalt. Luft, 33, (1973), 10.

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D.N.F. Atkins, R.A. Cox and A.E.J. Eggleton, Nature 235, (1972), 372.

6 Bij konformatie berekeningen, zelfs aan eenvoudige moleculen, met behulp van rekenprogramma's, is het gebruik van moleculaire mechanica te prefereren boven berekeningen gebaseerd op het MO concept.

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7 Bij ziektebeelden, die mogelijk veroorzaakt worden door afwijkingen in de steroid huishouding, verdient het bepalen van de onderlinge verhouding van de individuele steroiden de voorkeur boven het bepalen van absolute hoeveelheden of groepen steroiden.

8 Bij het onderwijs en de examinering in de moderne talen bij het voorgezet onderwijs, is het gebruik van woorden buiten bestaande referentiekaders voor taalkundig onderzoek - voor Frans bijvoorbeeld "Francais Fondamental" en "Dictionaire de Juilland"- alleen als uitzondering aanvaardbaar.

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Eindhoven, 21 september 1973. J.A. Rijks.