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GAS CHROMATOGRAPHIC ANALYSIS WITH GLASS CAPILLARY COLUMNS

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SUMMARY

A critical review of the current situation in glass capillary column chromatography with commercial instruments is presented preferably in the light of experience and experiments in our laboratories. Progress in production, connection and advanced applications of glass capillary columns is surveyed. Various sampling methods (splitting, splitless injection, selective sampling) with regard to different types of samples have been studied. The use of selective detectors (nitrogen and phosphorus flame ionization, electron capture, gas chromatography–mass spectrometry) is discussed and a simple automated double-column set-up for back-flushing in routine capillary chromatography is considered.

INTRODUCTION

Although successful and high-efficiency capillary column separations were achieved more than 15 years ago, discussion still continues on the versatility and usefulness of capillary columns in practical, and especially in routine gas chromatographic (GC) analysis. Some workers argue that they do not need the resolving power of capillary columns for the simple quantitative analytical problems that they have to solve, the precision and accuracy of quantitative analyses with capillary columns are not sufficient, mainly owing to unreliable sampling techniques, and capillary column technology, *i.e.*, production of good columns and their connection to and installation in commercial instruments is sophisticated.

In the last five years, considerable progress has been made especially in glass capillary column technology[1–10] and advanced methods for their application. The commercial GC instruments that are now available, however, have been developed several years ago mainly for packed columns, *i.e.*, for columns that are operated at much higher carrier gas flow-rates, at higher sample loads and therefore much lower resolution. It can easily be appreciated that the components of the instruments (sampling devices, ovens, detectors), including the facilities for parameter control and measurement (temperatures, flow-rates and pressures) have to be re-optimized when high-resolution and/or high-performance analyses in general are to be performed*. The

* The progress in the electronic design of modern GC instruments, for example with regard to the incorporation of microprocessors and computer techniques, is not discussed here.

chemistry of the surfaces of the materials used in which the solute molecules come into contact during the separation and also those surfaces outside the column itself are important for the design of GC instruments.

In discussing the advantages of the application of capillary columns in the various areas of GC analysis, the following main requirements can be considered:

(1) Rapid separation with a just sufficient resolution (the latter achieved either by selecting a stationary liquid with optimal polarity or by using a column with adequate separation efficiency in terms of theoretical plate numbers). Short chromatographic run times save time and improve the standard of the analytical control, for example in process chromatography (Fig. 1).

(2) Good separation efficiency even for compounds of very low volatility at high column temperatures and with temperature programming[11] (Fig. 2).

(3) Maximum resolution at the expense of long chromatograms (for mixtures that contain numerous compounds from various chemical classes of compounds or for the separation of isomers, *i.e.*, of compounds with similar structures) (Fig. 3).

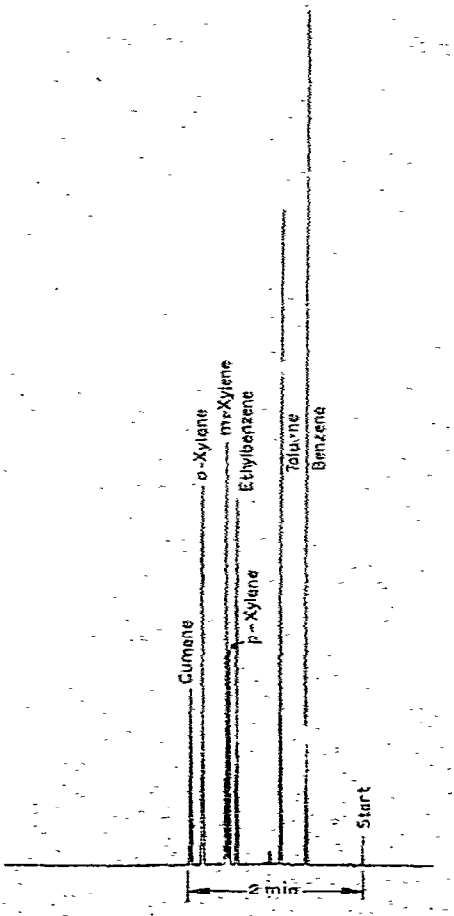

Fig. 1. Rapid separation of aromatic hydrocarbons including xylene isomers. Column, 20 m PPG (0.1 mm I.D.), temperature, 80°; carrier gas, H_2 (2.4 bar).

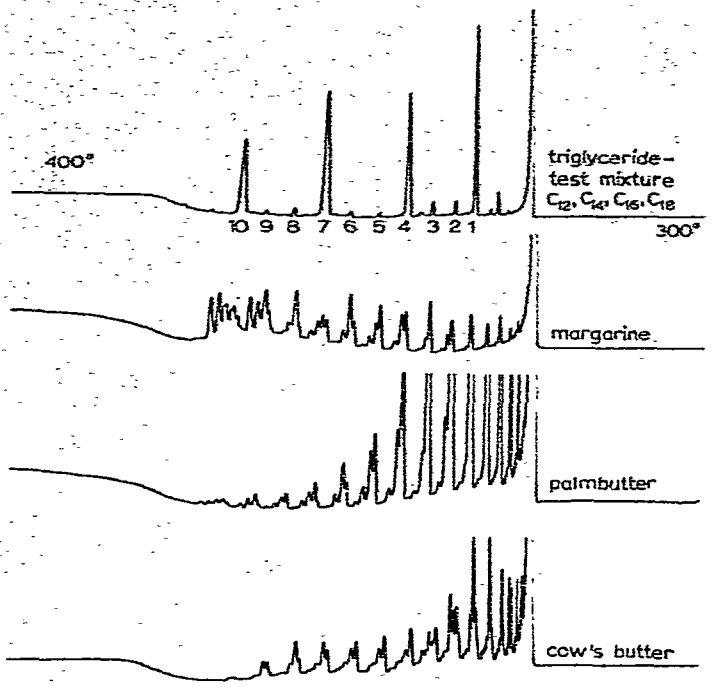

Fig. 2. Separation of C_{12}, C_{14}, C_{16} and C_{18} triglycerides with a capillary column. Column: 20-m Poly-S179. Temperature: 300–400° programmed at 4°/min. Carrier gas: H_2 (2 bar). Peaks: 1 = tri-C_{12}-(lauryl)-triglyceride; 2 = di-C_{12}-mono-C_{14}-triglyceride (?); 3 = mono-C_{12}-di-C_{14}-triglyceride (?); 4 = tri-C_{14}-(myristyl)-triglyceride; 5 = di-C_{14}-mono-C_{16}-triglyceride; 6 = mono-C_{14}-di-C_{16}-triglyceride (?); 7 = tri-C_{16}-(palmityl)-triglyceride; 8 = di-C_{16}-mono-C_{18}-triglyceride (?); 9 = mono-C_{16}-di-C_{18}-triglyceride (?); 10 = tri-C_{18}-(stearyl)-triglyceride.

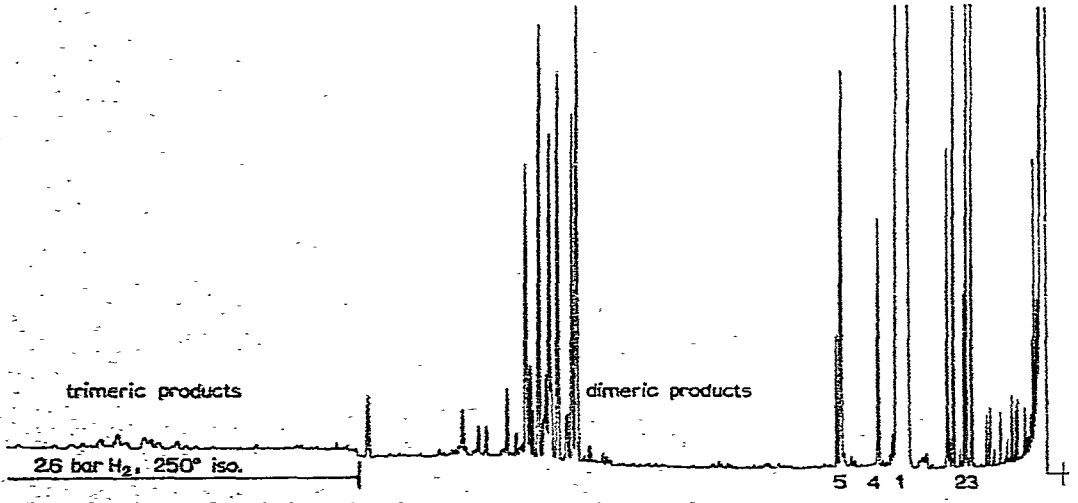

Fig. 3. Products of radiation of 2-deoxyribose (sample by courtesy of Dizdaroglu a. v. Sonntag). Derivatization: reduction with $NaBH_4$ and silylation. Column: 50-m SP 400 silicone oil. Carrier gas: hydrogen (1 bar). Temperature programme: 150–250° at 2°/min. Peaks: 1 = 2-deoxyribitol; 2 = 2,3-dideoxypentitol; 3 = 2,4-dideoxypentitol; 4 = 2-deoxyribonic acid; 5 = ribitol.

(4) Precise and accurate quantitative analyses at maximum detectability.

The advantages and disadvantages that become evident when considering the methods and techniques of capillary column GC have to be discussed in terms of different aspects that depend on the characteristics of the complex mixtures to be analyzed. Complex mixtures may contain components (a) with a wide range of concentrations and especially with large amounts of solvents or excess of derivatization reagents (see Fig. 3); (b) with a wide range of polarities; (c) with a wide range of chromatographic volatilities (see Fig. 3); (d) with different thermal and catalytic stabilities; (e) that appear in the chromatogram with numerous random overlappings (see Fig. 3); and (f) that belong to the same group of isomers (see Fig. 3). Which of these aspects is the most important is dependent on whether partial or total quantitative or qualitative analysis is required. No simple chromatographic procedure is conceivable by which most of the above properties can be dealt with satisfactorily. Capillary columns, with their high separating efficiency, may facilitate the analysis of complex mixtures but require the application and further development of modified and/or new methods and techniques. The latter can be realized only in new chromatographic systems because of the characteristic parameters of capillary columns such as carrier gas flow-rate, sample capacity and pressure drop. In this paper, we report on our experience with known and new methods and techniques of capillary column GC, including:

(1) Sampling procedures for standard and trace analysis with quantitative and qualitative purposes.

(2) Improvement of column preparation with regard to temperature stability and tailing behaviour; comparison of various procedures of surface pre-treatment and coating.

(3) Selective detection with capillary columns [nitrogen and phosphorus flame-ionization detectors (N- and P-FID), electron-capture detector (ECD), gas chromatography–mass spectrometry (GC–MS)].

(4) Multidimensional chromatography involving valveless flow switching and intermediate trapping to perform back-flushing, cutting, enrichment of trace components and multidimensional identification by retention.

As pointed out previously[10], the characteristics of ideal columns are:

	Important for:
High separation efficiency in terms of effective theoretical plates per metre	Rapid analysis
High total separation efficiency of long columns, preferably with high permeability	Difficult separations that require high resolution
Small amount of stationary liquid in the column with excellent film homogeneity	Low retentions and/or column temperatures. Minor risk of decomposition
Optimal tailing behaviour, especially for strongly polar compounds in various stationary liquids	Unsophisticated selection of a suitable column, precise determination of peak position, undisturbed quantitative evaluation

High long-term temperature stability, low baseline noise and drift	High detectability of trace components, optimal evaluation, wide temperature range of application
High sample capacity and wide range of sample load with regard to detection and/or retention determination	High concentration of component in carrier gas and reliable identification by retention
Preparation of reproducible columns with regard to separation efficiency and polarity must be easy	Easy operation of columns in general. Time saving and low-cost execution of routine analyses

With the exception of the sample capacity aspect, capillary columns, even of the thin-film type, meet all of these requirements better than other types of columns. The requirement of a small amount of stationary liquid in the column and high sample capacity are contradictory because the sample capacity decreases proportionally with the amount of stationary liquid in the column. Assuming that at the same temperature and sensitivity of the detection system and with the same stationary liquid the baseline noise and drift of a capillary and a packed column are of the same magnitude, the minimum detectable amount of a trace component is lower for capillary columns, because much higher solute concentrations at the peak maximum are obtained with capillary columns for peaks of the same amount of solute owing to much lower peak spreading during the chromatographic separation.

SAMPLE INTRODUCTION ON TO CAPILLARY COLUMNS

In terms of the minimum detectable amount, capillary columns are superior to packed columns because of their higher resolution, *i.e.*, they give peaks with a much higher ratio of height to width at half-height. Sample introduction, however, is easier and can be carried out with less care with packed columns because of their much higher sample capacity. Using special sampling techniques with capillary columns for the various kinds of samples, the superiority of capillary columns can effectively be applied in routine analysis.

A typical sample load per component for a capillary column with average solubility of the solute in the stationary liquid is in the ng range. The sample load for typical packed columns may be more than 200 times greater without a decrease in their much lower resolution. Direct sampling of nanograms of a certain component is not possible with the classical syringe technique, even when the sample consists of a large number of constituents, but may be attained with dilute solutions of the sample. In practice, samples have to be analyzed that frequently contain trace components (<0.1%), main components (1–50%) and solvents (>90%). The decision on the amount of sample to be introduced has to be a compromise and depends, of course, on the aim of the analysis, *i.e.*, which of the components are to be determined and what resolution may be required for the kind of sample to be separated.

If trace amounts are to be determined with a sufficiently high signal-to-noise ratio and under suitable drift conditions, the size of the sample injected has to be so large that the system may be overloaded for other components that are present at

higher concentrations. Deterioration of resolution and/or a departure from linearity of detection for the main components may be the consequence. At a given sample size a sample load which is optimal with regard to the required detectability and resolution can only be achieved in a single chromatogram for components that are contained in the mixture in a certain but limited range of concentrations. In other instances, several successive chromatograms can be measured with various sample sizes in order to utilize ideal sample loads for each constituent of interest.

With capillary columns, so-called "splitters" (Fig. 4) are normally used, the injected sample being divided into a small portion that enters the column and a larger portion that is vented. Splitting ratios between 1:20 and 1:1000 are common. In most instances, these splitters allow only the optimal sample load for the main components and not for the trace components. For trace analyses, very large sample volumes have to be injected and vaporized. The standard splitter design was described, for example, by Halász and Schneider[12] (see Fig. 4), and several similar and improved versions have since been published. Slight modifications have also been made by ourselves[8].

In the present work we investigated the performance of our splitter with regard to the precision and accuracy of the chromatographic analysis of artificial but typical samples. Some minor improvements in design and a careful selection of certain operational parameters are proposed as a consequence of the results in Table II.

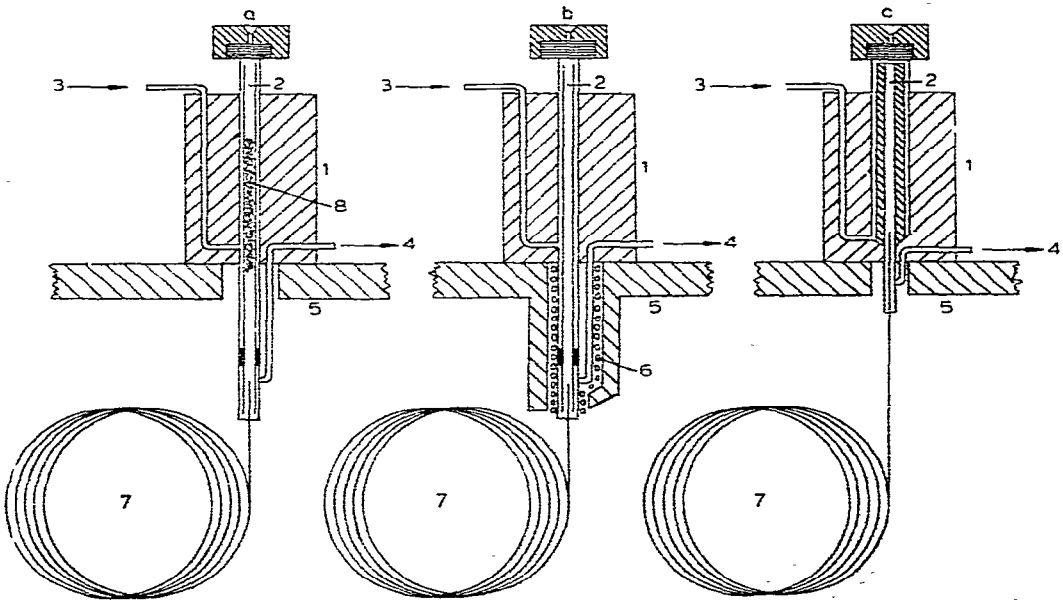

Fig. 4. Sampling devices for capillary columns. (a) Splitter for isothermal and temperature-programmed analyses, insufficient heating of splitting region for high-boiling mixtures, easy connection of capillary, straightening of column inlet not necessary. (b) Extra heating of splitter: suitable for high-boiling compounds, still easy connection of capillary. (c) Grob-type "splitter": splitting region inside well heated injection block, suitable for high-boiling compounds, difficult connection of capillary because of long straightened end of capillary. 1 = Heated injection block; 2 = glass tube; 3 = carrier gas inlet; 4 = to needle valve; 5 = insulation; 6 = extra heating; 7 = capillary column; 8 = glass-wool.

Quantitative analyses of three different artificial mixtures containing homologous *n*-alkanes, fatty acid esters and alcohols have been performed isothermally, the splitter being at a temperature close to that of the column oven. The splitter was tested in the usual manner[13] by determination of the sample composition and the deviation from the expected values of the peak areas caused by discrimination according to molecular weight, polarity and concentration of the constituents and its independence of sample size, splitting ratio, temperature of the injection port and splitting region, and whether the column operation was isothermal or temperature programmed. Results for the repeatability and reproducibility of peak area percentages are given, together with the errors involved. The same mixtures of known composition were also analyzed with a packed column, *i.e.*, without splitting and with the same detector.

Conclusion

The following conclusions can be drawn on the basis of the results in Tables I–III.

(1) The average normalized peak areas determined with isothermal column operation and with the splitter at the column temperature were found to be in excellent agreement with the values obtained without splitting in a packed column system.

(2) The variance of repeatability of normalized peak areas was dependent on the following factors. (a) The type and condition of the syringe used: lower errors

TABLE I

COMPARISON OF REPEATABILITY OF RELATIEVE PEAK AREA DETERMINATION WITH DIFFERENT SYRINGES

Sample: 0.1 μl (splitting ratio 1:200) of even-numbered C_6–C_{18} fatty acid esters. Results given are relative standard deviations (%) from 12 measurements.

Volume of syringe (µl)	*Concentration of sample (wt.%)*						
	0.92	*1.46*	*2.29*	*51.51*	*22.28*	*13.61*	*7.94*
10 (Hamilton)	4.1	2.7	1.5	0.4	0.5	1.4	1.2
10 (other)	6.9	5.0	3.2	1.2	0.8	2.4	3.9
5 (other)	8.8	5.2	4.5	0.8	0.7	2.1	2.7

TABLE II

COMPARISON OF THREE DIFFERENT TYPES OF VAPORIZATION TUBES

Sample: 0.1 μl (splitting ratio 1:200) of even-numbered C_6–C_{18} fatty acid esters. Results given are mean concentrations found (wt.%), with relative standard deviations of repeatability in parentheses, from 7 measurements. Similar results were obtained with splitting ratios between 1:70 and 1:700.

Tube	*Concentration of artificial mixture (wt.%)*						
	0.92	*1.46*	*2.29*	*51.51*	*22.28*	*13.61*	*7.94*
Empty glass tube	1.03	1.55	2.36	52.16	22.43	12.66	7.81
	(4.1)	(2.7)	(1.5)	(0.4)	(0.5)	(1.4)	(1.2)
With silanized	0.94	1.45	2.31	51.69	22.36	13.55	7.71
glass-wool plug	(0.9)	(0.3)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)
Tube with deformation	0.99	1.48	2.31	51.59	22.40	13.52	7.70
of cross-section	(4.1)	(1.9)	(0.6)	(0.2)	(0.2)	(0.4)	(0.8)

TABLE III

PRECISION AND ACCURACY OF GAS CHROMATOGRAPHIC ANALYSES OF THREE ARTIFICIAL MIXTURES CONTAINING ALKANES, FATTY ACID METHYL ESTERS AND ALCOHOLS AT VARIOUS SPLITTING RATIOS

Parameter	*Alkanes*					*Fatty acid methyl esters*			
	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_6	C_8	C_{10}	C_{12}
Known wt.% concentration in mixture	31.92	29.13	12.64	14.16	12.15	0.92	1.46	2.29	51.51
Found mean value of peak areas at splitting ratio									
1:700						0.75	1.30	2.15	50.94
1:500	32.42	29.19	12.41	13.81	11.81				
1:250									
1:200						0.74	1.31	2.19	50.85
1:70						0.74	1.30	2.18	51.04
1:50									
Found mean value of peak areas corrected with response factors*	32.13	28.93	12.33	13.88	12.08	0.94	1.45	2.31	51.69
Relative standard deviation**,*** (a)	1.1	1.3	1.6	1.6	2.6				
(b)	0.3	0.2	0.3	0.3	0.6				
Relative systematic error (accuracy)	2.4	0.7	2.4	1.9	0.6	2.1	0.7	0.9	0.4
Response factor (relative to *n*-decane)	1.00	1.01	1.02	1.04	1.06	1.60	1.41	1.34	1.29

* Fatty acid methyl esters: splitting ratio 1:200; alcohols: splitting ratio 1:250, empty vaporization tube.

** Alkanes: $n = 6$, fatty acid methyl esters: see Table II; alcohols: $n = 5$.

*** For alkanes two values are given, the first of which is for an empty vaporization tube (a), while the value below (b) is for a vaporization tube with a silanized glass-wool plug. For alcohols, the values are for a vaporization tube with a silanized glass-wool plug, treated with OV-101.

were found, for example, with a Hamilton syringe (see Table I). (b) The homogeneity of the sample–carrier gas mixture: optimal homogeneity was achieved with a short plug of glass-wool inside the vaporization glass tube of the splitter. The variance of repeatability using a specially shaped vaporization tube (see Fig. 4) was also lower than with the standard vaporization tube[14] but not as good as with the glass-wool plug (see Table II). (c) The temperature of the injection port and the vaporization tube in relation to the volatility of the high-boiling components in the mixture. (d) The splitting ratios, but only beyond the range between 1:75 and 1:1000 when 0.1 μl of sample was injected.

(3) By coating the surfaces of the vaporization tube, the peak symmetry could be improved, especially for more polar sample components. Improved repeatability and reproducibility (dependent on splitting ratio) of peak area determination was observed when OV-101-coated glass-wool was used for the plug in the vaporization tube. Coating with the polar Carbowax, however, had a negative effect on precision and accuracy. We also expect that the catalytic decomposition of sensitive components is reduced by this provision (see Table III).

(4) The average normalized peak areas did not exhibit significant differences in the various series of experiments. In the extended range from 0.1 to 10 μl of injected sample at a constant splitting ratio, only a limited proportionality could be at-

			Alcohols									
4	C_{15}	C_{18}	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{14}	C_{16}	C_{18}
.29	13.61	7.94	9.88	10.34	8.72	10.18	10.04	9.65	10.42	9.51	11.29	9.45
.66	13.88	8.11										
			9.03	9.89	8.64	10.26	10.14	9.65	10.42	9.51	12.19	9.98
			9.06	9.93	8.73	10.42	10.14	9.96	10.73	9.64	11.68	8.96
.66	14.01	8.11										
.71	13.92	8.07										
			9.14	9.96	8.79	10.42	10.22	9.88	10.69	9.74	11.70	9.10
.36	13.55	7.71	9.51	10.33	8.72	10.42	10.19	9.99	10.67	9.24	11.22	9.09
			1.5	1.3	1.4	2.1	0.8	0.2	0.6	0.7	1.6	2.3
.4	0.4	2.9	5.3	1.5	2.5	0.6	2.1	2.7	3.3	0.1	0.2	0.5
.25	1.23	1.21	1.27	1.27	1.21	1.22	1.22	1.22	1.21	1.16	1.17	1.23

tained between the calculated amount of sample and the absolute peak areas obtained. This phenomenon will be investigated in the near future.

For the assessment of these results, one should consider that the column temperature was fairly high in comparison with the volatility of the sample constituents at the various measurements (200° for the *n*-alkanes between C_{10} and C_{20} and 200° for the fatty acid methyl esters between C_6 and C_{18}). The lower the column temperature, the higher the temperature at which the splitter has to be kept in order to avoid discrimination. In general, splitters should be operated at temperatures similar to those of injectors.

Response factors of the components were determined under the same instrument conditions; good agreement was obtained between theoretical and the measured values of concentration.

In the second series of experiments, temperature programming was also applied for the analysis, using the same splitter as before. Because of the low splitter temperature at the start of the chromatogram, insufficient precision and accuracy for peak areas and concentrations were obtained. The injection port was heated as before in order to ensure complete vaporization. As the peak areas and concentrations of the high-molecular-weight components were found to be much too low, the results are not given here and will be published elsewhere. Good results could be achieved, how-

ever, when the splitter was heated to a temperature close to that of the injection port. Therefore, splitters must not be operated at temperatures that are as low as those used with capillary columns (generally lower than with packed columns). These effects also place a severe restriction on the application of column cassettes for isothermal and temperature-programmed work as proposed by ourselves[15] and Kaiser[16], in which the splitter and make-up gas connections to the detector are integrated within the column unit.

For comparison, the performance of a slightly modified Grob-type injection system[17] was investigated, in which the capillary column is inserted into the end of the vaporization tube, which is a part of the injection block. The splitting region is therefore at the same temperature as that of the vaporization tube in the injection block. During the injection, the splitting valve is closed for a period during which the injected sample is transferred from the vaporization chamber into the capillary column. The original reference samples were diluted with a solvent (heptane) and the sample size was chosen such that the same sample load for the diluted sample components was achieved. The absolute peak areas obtained confirmed this assumption.

Precise (*i.e.*, repeatable) and accurate relative peak areas or concentrations could not be obtained for the higher components of a mixture of *n*-alkanes up to C_{34} even at temperatures above 300° in the injector, although no splitting was involved. The discrimination of high-molecular-weight components is caused by the incomplete transfer of the vaporized sample into the cold inlet of the column by the carrier gas flow, which is, of course, low for capillary columns in comparison with the volume of the injector. If the solvent is very volatile and the trace components of interest are involatile, the column can be held at an initial temperature at which the solvent can easily migrate through the column. In this way, the solvent is removed before the temperature programme is initiated. With this procedure, deterioration of the coating or film homogeneity in the first part of the column may occur. Uncoated and even activated surfaces will affect the tailing behaviour and the performance of the column in general. The direct insertion of the straightened inlet of the glass capillary into the vaporization tube of the injector provides an optimal temperature profile between the injection port and the column inlet. The necessity for straightening the column inlet is, of course, a disadvantage because decomposition of the stationary liquid in this section of the column is a consequence of the heating procedure in a small flame. Accumulation of volatile decomposition products and incompletely coated column walls may arise, the latter also causing the above difficulties. The method of splitless injection described by Grob and Grob[17] can be used in isothermal chromatography when heavy overloading of the solvent or main component peak does not lead to overlapping with the peaks of significant components with lower retentions. If possible, the solvent can be selected according to the volatility of the constituents to be determined. In isothermal separations, the solvent cannot be removed selectively and thus prevented from entering the main separation without using a double column system, including intermediate trapping. The Grob method, with temperature programming and using a pre-separation in short column lengths, is a special case of what we propose to call "selective" sampling. Concerning the splitless injection technique, it seems to be not very consequent that the sample is evaporated in the injector block, then transferred to the cold column inlet, condensated and vaporized again by initiating the programmed heating of the column. Using a double column system with

intermediate trapping, a sharp "cutting" or pre-separation of significant peaks can be accomplished. Only the selected components of interest, among them an internal standard of known sample concentration, are allowed to enter the main column via a specially constructed double-T connector[10].

CONNECTION TECHNIQUES FOR GLASS CAPILLARIES

The connection of the inlet of the column to the injection block splitter unit and of the column outlet to the detector (preferably mass flow-dependent types) has to be made with minimum dead volume, *i.e.*, considering the low carrier gas flow-rates in such columns, and avoiding unnecessary contact of the sample with bare surfaces of the chromatographic system. If the column inlet itself acts as the high flow resistance part of the splitter, no dead volume of the sampling device influences the broadness and symmetry of the sample plug because of the very high gas flow-rates in the low-resistance part of the splitter. In spite of the short residence times of the sample, the adsorption of high-boiling and polar components may depend on the temperature and surface activity of the vaporization tube. Even more difficulties of this nature can be expected to arise with the splitless injection technique because of the low flow-rates in the injection port when the splitter is closed. Only in temperature-programmed chromatography does partial or complete trapping of the sample in the cool column inlet probably compensate for discrimination of components of low volatility, especially when the injector unit cannot be heated to a sufficiently high temperature because of decomposition of significant components.

Similar but less severe problems arise at the column outlet. By mixing the eluate flow with about a 20-fold flow of make-up gas (which can be done, of course, only when using mass flow-dependent detectors), a decrease in resolution caused by too large a volume and a poor flow geometry of the connections is avoided. The hydrogen flow for the FID has a suitable rate and can also be used as make-up gas, by inserting the column outlet into the jet of the detector. This arrangement, however, is not conducive to rapid and easy column changes. In any event, cold make-up gas connections should be avoided in order to prevent adsorption or condensation. In general, the maximum component concentrations in the carrier gas are much lower at the column outlet than at the inlet of the column. Nevertheless, sufficient heating of the make-up gas unit equivalent to that of the detector is desirable. Furthermore, the above-mentioned properties of bare surfaces of the connection unit between the column outlet and the detector affect the chromatographic performance and should be kept at a minimum.

SELECTIVE DETECTORS (ECD, N- AND P-FID, GC–MS)

Difficulties with connections arise when too high effective detector volumes are involved in relation to the carrier gas flow-rates in capillary columns. Especially for the ECD[18–21], the effective detector volume is much larger than in the FID, so that high make-up gas flow-rates may be necessary, which, however, cannot be applied because the ionization characteristics of the detector are adversely affected. The geometry of the detector cell probably has to be changed in order to achieve a better compromise between sensitivity and effective detector volume. This conclusion was a

result of measurements with a Hewlett-Packard 18713A ECD connected with a 0.25-mm I.D. glass capillary column. Varian[22] and Perkin-Elmer[23] report similar experiences. Argon containing 5% of methane was used as the scavenger gas at the column outlet and for the necessary purging of the detector volume (see Fig. 5). A chromatogram of halogenated hydrocarbons shows that the tailing caused by poor cell geometry or by adsorption in the cell could not be removed completely. With the N-FID, no specific problems of this nature have been observed by several workers (*e.g.*, Hartigan *et al.*[24]).

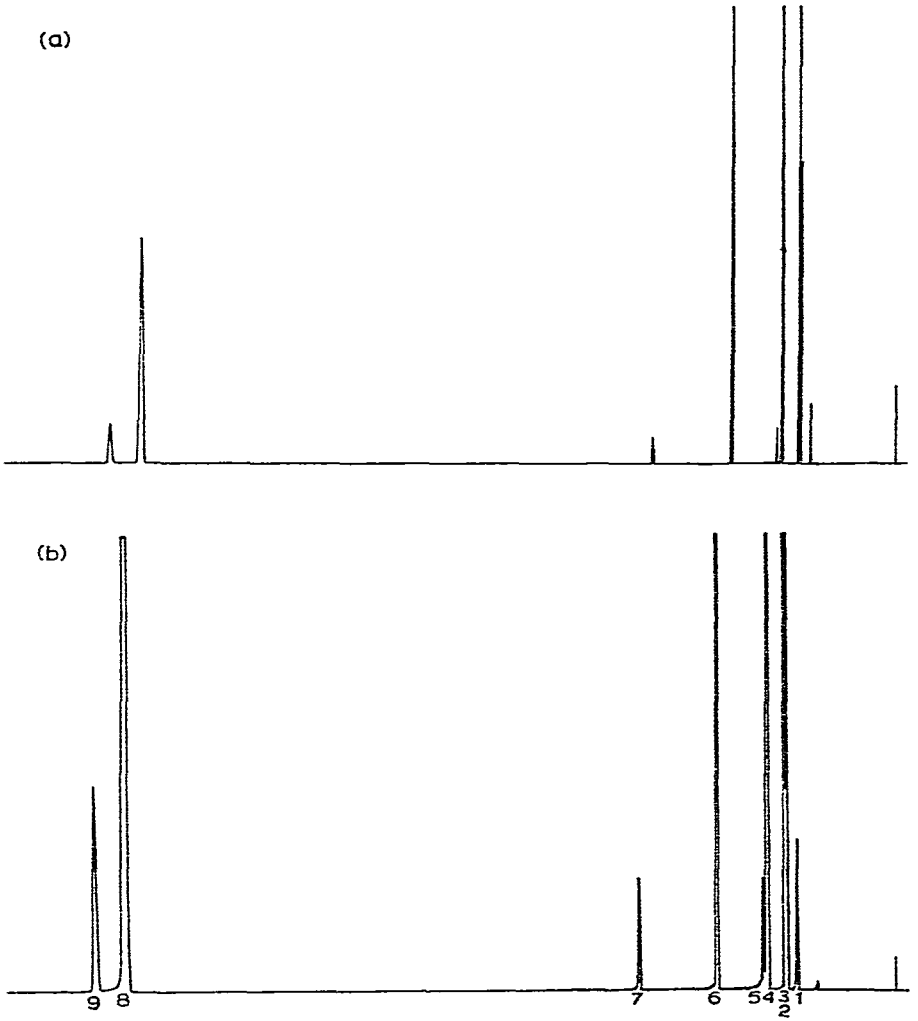

Fig. 5. ECD detection of chlorinated hydrocarbons separated by capillary column. Scavenger gas flow (Ar + 5% CH_4): (a) 100 ml; (b) 10 ml. Column: PPG, 100 m (0.27 mm I.D.). Temperature: 80°. Carrier gas: Ar (1 bar). Sample: 1 μl. Splitting ratio: 1:60. Peaks: 1 = dichloromethane; 2 = 1,1,1-trichloroethane; 3 = tetrachloromethane; 4 = trichloromethane; 5 = 1,2-dichloroethane; 6 = tetrachloroethylene; 7 = 1,1,2-trichloroethane; 8 = 1,1,2,2-tetrachloroethane; 9 = unknown.

PRODUCTION AND STANDARD OF PERFORMANCE OF GLASS CAPILLARY COLUMNS

The procedure for the preparation of glass capillary columns applied in our laboratories is rapid and simple[8,9]. Typical features of the procedure, which in some regards is similar to that described by Alexander and Rutten[25], are:

(1) Soft alkaline glass tubes are drawn to give capillaries of preferably 0.25 mm I.D. and 0.8 mm O.D. using an HP drawing machine, the principle of which was described by Desty *et al.*[26].

(2) Glass capillaries are treated with flowing dry hydrochloric acid at a pressure drop of up to 5 atm at 450° for 2–3 h. Typical surface structures obtained by this procedure can be seen in the scanning electron microscope photograph in Fig. 6. These surfaces are more pronounced than those obtained using the fluorinated ether etching method proposed by Novotný and Tesařic[3]. Therefore, we expect difficulties to occur when coating the latter surfaces with stationary liquids of variable polarity.

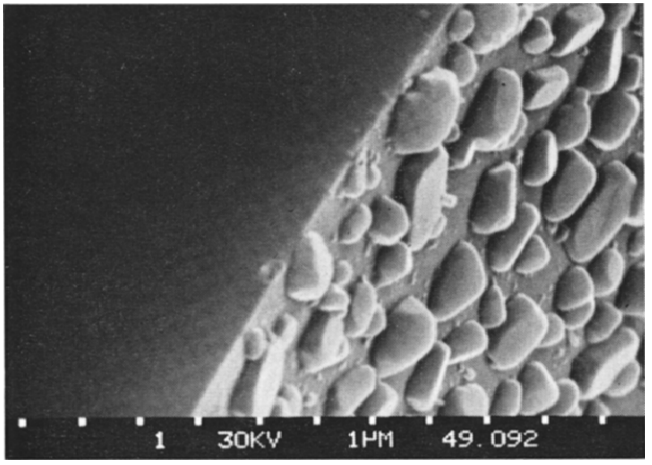

Fig. 6. Scanning electron microscope photograph of HCl-treated soft glass.

(3) The coating is carried out dynamically with column lengths of up to 170 m and with concentrated solutions of the stationary liquids using the "mercury drop method". The coating procedure can easily be executed in 1 day or simply overnight, even with long columns. Before coating with non-polar stationary liquids, the capillary surfaces are deactivated with very dilute solutions of thermally stable, strongly polar stationary liquids such as Carbowax 20M. The excess of, *i.e.*, the non-adsorbed, deactivation reagent is subsequently removed by flushing the column with a large volume of the pure solvent. Deactivation of surfaces for amine separations in Carbowax 20M columns is commonly effected with sodium hydroxide. Fig. 7 illustrates the effect of this procedure in glass capillaries.

(4) The common thermal after-treatment is performed up to temperatures that are slightly higher than the prospective temperature limit for the practical application

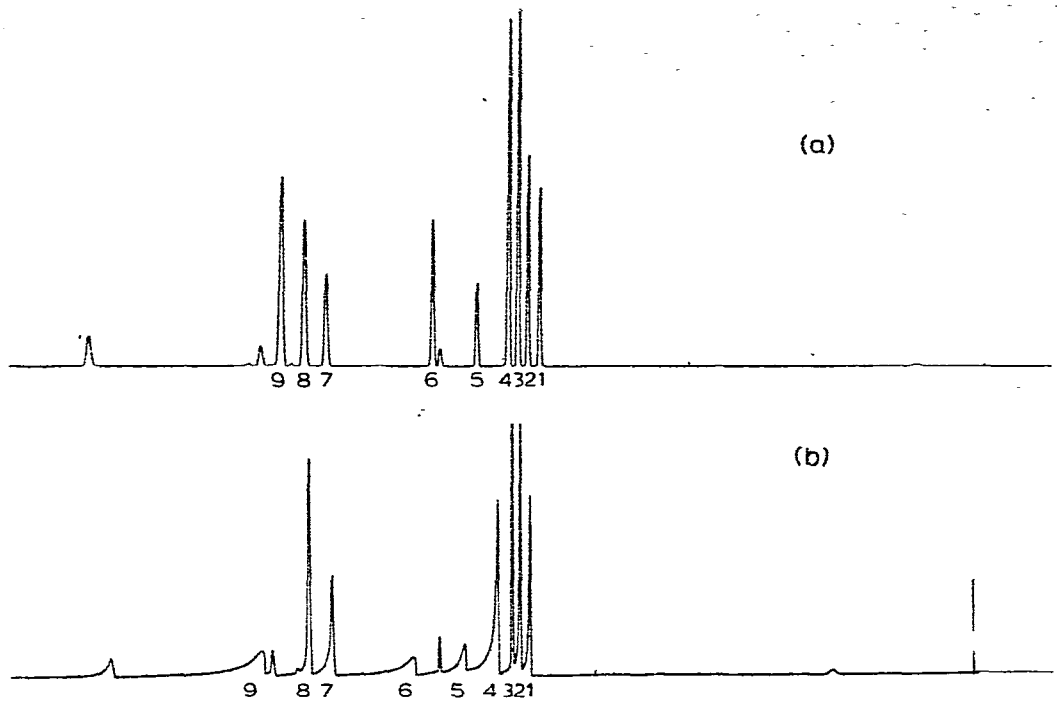

Fig. 7. Separation of amines with Carbowax 20M capillary column. (a) Surface deactivated with NaOH; (b) surface without NaOH deactivation. Peaks: 1 = isopropylamine; 2 = diethylamine; 3 = triethylamine; 4 = *n*-propylamine; 5 = isobutylamine; 6 = *n*-butylamine; 7 = 4-aza-(4)-octene; 8 = tetramethylethylenediamine; 9 = *n*-pentylamine.

of a particular column. Before this treatment, the solvent is removed at temperatures up to 80°.

(5) In recent experiments, columns could also be made without treating the surfaces with hydrochloric acid but by applying the mercury drop method. Columns with a good standard of tailing behaviour, separation efficiency and temperature stability have been obtained with OV-101, polypropylene glycol and Carbowax 20M. The reliability of the coating procedure in terms of percentage of good columns is, however, not as good as after HCL-treatment of the surface.

The separation efficiency, polarity and tailing behaviour of the columns after preparation are checked by measuring chromatograms of standard test mixtures. Optimal and reproducible results are obtained in most instances, the number of poor columns obtained with this coating procedure being negligible. The separation efficiency of columns with polar stationary liquids in terms of theoretical plate numbers at high k' values are, in general, 10–30% lower than that of non-polar or weakly polar columns. No satisfactory explanation has been given for this phenomenon to our knowledge. The thermal stability of columns coated with all types of stationary liquids with a wide range of polarities is excellent and is as good as that of packed columns in our experience with more than 1000 columns. The thermal stability of columns made by our procedure depends only on the stability of the stationary liquid itself. By removal of the volatile constituents of the original stationary liquid at tem-

peratures up to 350° under high vacuum before the coating[27,28] and by thermal conditioning of the coated column itself, temperature limits for the application of the most usual columns have been found to be the following: OV-101, 300°; Dexsil, 300°; Carbowax 20M, 270°; polypropylene glycol, 240°; and Poly-S 179, 400°. These data differ from those which we published previously[8]. For polypropylene glycol, we previously reported[8] a temperature limit of 140°.

The pre-treatment of stationary liquids proposed by various workers changes the original polarity of the phase in terms of the Kováts retention indices of selected test compounds. It is disappointing that the simple procedure of thermal pre-treatment is not applied to each commercial stationary liquid by the manufacturer.

The column bleed at the given maximum temperatures is still low enough for no restricting background in GC–MS measurements to be observed[29]. Figs. 2 and 3 show chromatograms obtained with columns with new stationary phases or with improved standards of tailing behaviour and temperature stability.

AUTOMATED MULTI-DIMENSIONAL GAS CHROMATOGRAPHY WITH CAPILLARY COLUMNS[10]

In capillary column work, long columns with high resolution and limited sample capacity are used. In general, the analysis of complex mixtures cannot be effected by a single chromatographic separation, even when temperature programming is applied. No adequate column temperature for optimal separation in all sections of the chromatogram can be established nor an optimal sample load for all significant constituents. For mixtures with a wide range of volatilities, very long chromatograms may be obtained when high resolution in long capillary columns is needed. Time can be saved by removing the components of high retentions by a pre-separation with a short column, which may also be operated at elevated temperatures. The non-volatile components remain in the pre-column and are removed forwards or backwards (back-flushing) by flow switching operations.

Another important facility of a well designed double-column system is the removal of volatile components, preferably solvents and reagents, by pre-separation. The transfer of the eluate from the pre-column into the main column is started after the solvent has been vented between the pre-column and main column (cutting). If the pre-separation is adequate, the removal of solvent can also be performed when the components of interest have retentions similar to that of the solvent and are located on the tail of the solvent (heart cutting). For quantitative and qualitative analysis of very dilute solutions of species of analytical interest, selective sampling, *i.e.*, without splitting of these trace constituents, has to be carried out (splitless injection, "selective" sampling).

By using an intermediate trap between the two columns, repetitive pre-separation of trace components in the trap (enrichment) is possible. Packed columns can be used as pre-columns because of their greater sample capacity. The main separation is carried out subsequently with the accumulated material on a capillary column with a much greater signal-to-noise ratio. By applying intermediate trapping, isothermal main column separations with optimal reproducibility of retentions and peak heights are achievable. The starting point of the separation is well defined for precise

retention measurements. When using two columns of different polarity, two sets of retention data can be obtained from a single sample introduction into the closed system, with the advantage that the peak correlation for the ΔI^* calculation can be carried out undisturbed by peak overlapping. The flow switching that is required in such pneumatic systems of multi-dimensional chromatographs in order to reverse the flow directions has to be valveless[30]. These techniques can also be applied in capillary column work (Schomburg and Weeke[31]). As described previously, all flow switching manipulations can easily be executed automatically, for example by using solenoid valves that are located outside the heated chromatographic system. The solutes never come into contact with heated valves. The cold trap can also be operated automatically by blowing cold or hot nitrogen on to the section of column that acts as trap. Further improvements in the versatility of the double-column system can be achieved by using separate column ovens for the pre-column and main column. Temperature programming of the pre-column may be of especial advantage for the analysis of mixtures with a very wide range of chromatographic volatilities. Typical examples of double-capillary column separations with different analytical objectives including cutting, back-flushing, trapping and enrichment have been given in our previous publications.

In our opinion, complex mixtures may not, and in many practical cases cannot, be separated perfectly in a one-dimensional system. Very often representative portions of the eluate of the pre-separation could be analyzed and evaluated instead of the total mixture without losing important information. These sections of the chromatogram of the pre-separation should contain, however, internal standards of precisely known concentrations for quantitative analyses, *i.e.*, for the determination of the absolute concentrations of the selected species in the original mixture, whereas the relative peak areas in the selected section can be determined without an internal standard.

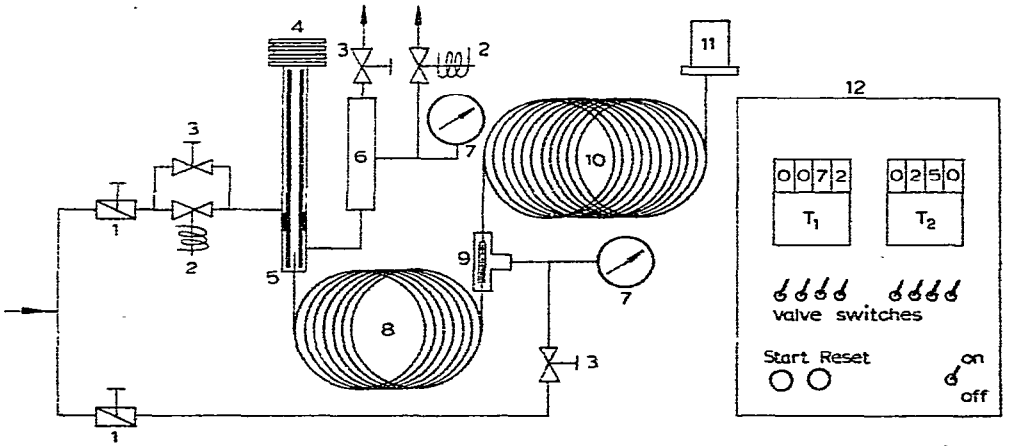

Fig. 8. Simple double-capillary column system for back-flushing and for variable column polarity when using columns of different polarity. 1 = Pressure regulators; 2 = solenoid valves; 3 = needle valves; 4 = injector; 5 = splitter; 6 = adsorption tube; 7 = pressure gauge; 8 = pre-column (short section of main column or column with different polarity); 9 = connection piece; 10 = main column; 11 = FID; 12 = timer and switching unit. T_1, period of normal flow direction in pre-column and main column; T_2, period of reversed flow direction in pre-column.

* ΔI is the difference of the Kováts indices in polar and non-polar stationary liquids ($I_{polar} - I_{non\text{-}polar}$).

Especially in routine analyses, for process and product quality control, many analyses per day have to be executed with high precision and accuracy. The proper application of capillary columns can help to save time and to improve precision. Heart cutting and back-flushing can also be achieved by using simple automated versions, which we have designed for application in routine analysis (Fig. 8).

The figures illustrate the simple set-up, which can be integrated easily into every commercial instrument. A capillary column is divided into a short section that acts as the pre-column and a longer section that is the main column, and a simple T-connector to the main column allows the back-flushing of the involatile components from the pre-column. Of course, these components can be detected for quantitation only by a second detector between the pre-column and main column and not together with the species separated in the main column. Considerable time can be saved because non-volatile species need not be eluted before the next chromatogram is started. In practice, no sample is really free of components with low volatility, and therefore the back-flushing should be applied in every analysis in order to prevent the appearance of peaks with high retention in the subsequent chromatograms. If it is necessary to remove excessive amounts of solvents or reagents, a double-T version of the connecting part between the two capillary columns has to be used. In this still simple system the solvent can be vented between the two columns and selective sampling with dilute solutions of significant species can be carried out.

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