Journal of Chromatography, 99 (1974) 63–79

CHROM. 7671

PREPARATION, PERFORMANCE AND SPECIAL APPLICATIONS OF GLASS CAPILLARY COLUMNS

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SUMMARY

A simple procedure for the preparation of glass capillary columns with polar and non-polar stationary liquids, including prior etching and deactivation, is described. A dynamic method of depositing homogeneous solvent films on the capillary walls has been developed. Aspects of the assessment of glass capillary columns are discussed and supplementary proposals for a standardized test procedure with particular regard to temperature stability are made. An improved version of an automated double-column chromatograph for the execution of optimal qualitative and quantitative analyses of aqueous solutions is described.

INTRODUCTION

There is no doubt that the future of analytical gas chromatography (GC) lies in the use of capillary columns with either thin films or thin layers. Different groups of workers have dealt with the problems of the separation and connection of columns and methods for their application in various types of GC analyses[1–7]. The usual requirements for a good GC column must also, of course, be met by capillary columns. Furthermore, characteristic differences between the various types of columns and their operating parameters exist which require the use of different techniques. The criteria for the assessment of capillary columns are treated in detail in this paper with particular regard to our practical experience. Capillary columns have high separation efficiencies at low permeabilities, but very low sample loading capacities. Column lengths of up to 300 m can be used with moderate pressure drops. The application of glass capillaries, however, seems to be unpopular for the following reasons:

(1) the preparation of the columns is difficult and their performance is irreproducible;

(2) glass capillaries are fragile;

(3) connections to commercial gas chromatographs and the sampling techniques used are complicated;

(4) capillary columns are not thermally stable.

On the other hand, glass is a cheap, easily deformable material with a homogeneous and chemically modifiable surface and therefore has been used exclusively in our laboratories and, of course, by many other workers.

METHODS OF PREPARATION OF GLASS CAPILLARIES (USED IN THE LABORATORIES OF THE AUTHORS)

The well-known glass capillary drawing-machine made by Hupe & Busch (now Hewlett-Packard) (Groetzingen, G.F.R.) is used for the production of capillaries with an I.D. of 0.2–0.4 mm and an O.D. of 0.7–0.9 mm from AR-glass, which is a low-melting alkali-type glass from Schott-Ruhrglas (Bayreuth, G.F.R.).

Column lengths of 130 m in one piece can be easily obtained from glass tubes of 1.5 m length, 7.5–8.0 mm O.D. and 3.4–3.6 mm I.D. Different glass types and their influence on surface treating and coating procedures and on the quality of the obtained columns have not yet been investigated. All our capillaries are etched with dry hydrogen chloride gas at elevated temperatures. The etching process requires 2–3 h. No further chemical modification of the surfaces, such as silanization, is carried out. We found that various methods of silanization did not give improved coatings with stationary liquids of different polarities in terms of separation power, tailing behaviour and temperature stability. The coating of etched and silanized surfaces with polar stationary liquids gives poor results, probably because of the hydrophobic character of the silyl groups.

The coating of the etched surface with stationary liquids is carried out by means of a special dynamic method by which concentrated solutions of the stationary liquid (up to 60% in a solvent such as *n*-pentane, methylene chloride or chloroform) may be forced through the capillaries to give homogeneous thin films without the formation of droplets. This effect is accomplished by pushing the solution with a *ca.* 10-cm mercury plug. Thereafter only small amounts of solvent have to be evaporated from the column. The procedure for removal of solvent and transportation through the column usually results in deterioration of the homogeneity of the film. We shall describe this improved dynamic coating procedure in more detail elsewhere[8]. The removal of the solvent is followed by the usual thermal after-treatment, which often improves the separation efficiency of the columns. When high temperatures are applied in this process of artificial ageing of new columns, the polarity of the stationary phase can change considerably, especially when polar stationary liquids are used (see Table I).

TABLE I

KOVÁTS INDICES OF TEST COMPOUNDS OF DIFFERENT POLARITY MEASURED IN THREE DIFFERENT COLUMNS AFTER THERMAL TREATMENT

Stationary liquid	*Treatment*	I_{80}				
		1-Octene	*Toluene*	*Di-n-butyl ether*	*Methyl butyrate*	*n-Butanol*
OV-101	Before heating	788.4	761.7	875.2		
	15 h, 200°	788.4	762.5	875.1		
	Several days, 300°	789.0	763.5	876.9		
LAC-3R-728	15 h, 140°	877.1	1200.0	1039.1		
	40 h, 150°	876.5	1192.3	1031.0		
	Several days, 150°	875.5	1192.4	1026.7		
Carbowax 20M	Before heating	841.0			973.4	1114.9
	15 h, 180°	841.5			975.4	1117.0
	100 h, 180°	842.3			980.7	1127.0

Further investigations of this effect with the careful exclusion of oxygen in instruments fitted with leak-free connections and with oxygen-free carrier gas are being carried out at present. Polar compounds cannot be chromatographed tail-free on non-polar stationary liquids without pre-treatment of the etched surface with a polar deactivating substance. The deactivation of the surface was incomplete when silanizations were performed with the usual reagents. In Fig. 1, chromatograms of a polarity mixture* measured in three different squalane columns are given. Column 1 was simply coated with squalane after the etching procedure, column 2 was silanized before coating and in column 3 the etched walls were previously coated with a dilute solution of a deactivating (preferably strongly polar) substance. The excess of this substance was then removed by flushing the column with the solvent used. The tailing of the polar components of the mixture (cyclopentanone, *n*-butanol, etc.) is stated in the legend of Fig. 1.

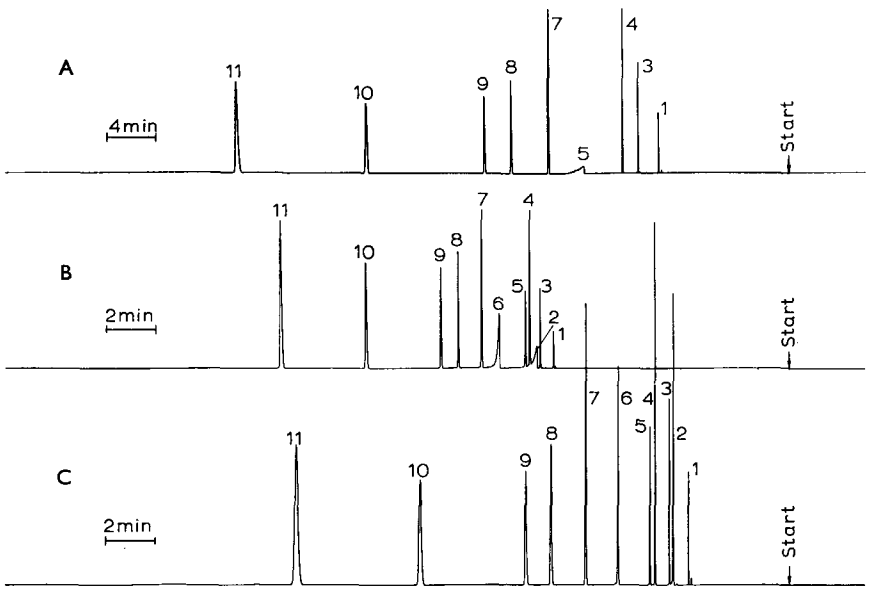

Fig. 1. Chromatograms of polarity mixture in three different Squalane columns. Chromatogram A: column etched, not deactivated; 100 m, 80°, 2.2 bar N_2, 0.1-μl sample. Result: peaks 2 and 6 not eluted because of adsorption; peak 5 tailing. Chromatogram B: column etched, silanized; 100 m, 80°, 1.5 bar N_2, 0.1-μl sample. Result: peaks 2 and 6 not adsorbed, tailing; peak 5 no tailing. Chromatogram C: column etched, Carbowax 20M, deactivated; 60 m, 80°, 1.2 bar N_2, 0.1-μl sample. Result: all peaks without tailing. 1 = *n*-Pentane; 2 = 1-butanol; 3 = *n*-hexane; 4 = benzene; 5 = methyl butyrate; 6 = cyclopentanone; 7 = toluene; 8 = 1-octene; 9 = *n*-octane; 10 = di-*n*-butyl ether; 11 = *n*-nonane.

We therefore assume that the polarity of the stationary liquid squalane is not increased substantially by the deactivation procedure. Similar results with regard to tailing of polar compounds were also obtained with other non-polar stationary liquids such as silicone oils. In Fig. 2, the chromatograms of the McReynolds substances measured with the stationary liquids squalane and OV-225 are shown. The Kováts

* A mixture of compounds of various polarities serving the testing of the polarity of the stationary liquid and the adsorption properties of the support.

Fig. 2. Chromatograms of McReynolds substances in different polar columns.
Chromatogram A: 60 m, squalane, etched, Carbowax 20M, deactivated, 0.1-μl McReynolds test mixture, 120°, 0.7 bar N_2.

Compound	I^S_{120}	*McReynold*
1-Butanol	586.0	590
2-Pentanone	623.1	627
Benzene	652.8	653
Nitropropane		652
2-Methyl-2-pentanol	687.1	690
Pyridine	—	699

Fig. 2. legend (*continued*).

Chromatogram B: 100 m, OV-225, etched, not deactivated, 0.1-μl McReynold test mixture, 120°, 2.0 bar N_2.

Compound	$I_{120}^{OV\text{-}225}$	*McReynold*
Benzene	873.0	653 + 228 = 881
1-Butanol	950.4	590 + 369 = 959
2-Pentanone	957.3	627 + 338 = 965
2-Methyl-2-pentanol	966.2	690 + 282 = 972
Pyridine	1073.5	699 + 386 = 1085
Nitropropane	1132.5	652 + 492 = 1144

Chromatogram C: 140 m, Carbowax 20M, etched, not deactivated, 0.1-μl McReynold test mixture, 120°, 2.5 bar N_2.

Compound	$I_{120}^{CW\ 20M}$	*McReynold*
Benzene	967.0	653 + 322 = 975
2-Pentanone	985.7	627 + 368 = 995
2-Methyl-2-pentanol	1068.3	690 + 387 = 1077
1-Butanol	1118.1	590 + 536 = 1126
Pyridine	1196.5	699 + 510 = 1209
Nitropropane	1214.4	652 + 572 = 1224

retention indices of the components are given and can be compared with those published by McReynolds[9] for packed columns. The comparison shows that much lower Kováts indices for the most polar compounds (especially *n*-butanol) are obtained with our columns. For weakly polar compounds, such as saturated or unsaturated hydrocarbons and even ethers, only a negligible influence of the surface treatment on the retention indices is observed. *n*-Butanol, for example, is not eluted from our standard stainless-steel columns with non-polar stationary liquids. With non-polar stationary liquids, pyridine is not eluted without deactivation with a basic substance of either glass or stainless-steel surfaces. No severe tailing of the different constituents of the polarity mixture, including pyridine in the case of the OV-225 column, is observed. This stationary liquid of the cyanosilicone type can be considered to be polar.

Similar results were found for all common polar stationary phases (see chromatogram C in Fig. 2). No coating with a deactivating agent and no other surface treatment procedure was applied to the polar columns after etching. Typical columns that we have produced using the described method have a separation efficiency of 200,000–300,000 effective theoretical plates at k^* values of >3 with column lengths of 100 m for non-polar stationary liquids. The separation efficiencies of polar columns are lower (30% in terms of theoretical plate numbers)than those of non-polar columns.

Very few reject columns that have a poor separation efficiency or give tailing are produced when this procedure is adopted.

The Kováts indices of the standard components are reproducible only when the same procedure of surface treatment and heating to the proposed temperature limit are applied. The stationary liquids used must be of identical origin. The problems of checking and characterizing the temperature stability of glass capillary columns are discussed in the following section.

* Capacity ratio $k = t'_R/t_M$, where t'_R = adjusted retention time of test compound, and t_M = gas holdup time.

ASPECTS OF THE ASSESSMENT OF THE PERFORMANCE OF GLASS CAPILLARY COLUMNS

Separation efficiency and resolution

The maximum separation efficiency in terms of the number of theoretical plates for the total length of a column is doubtless attained with thin-film open tubular columns. Thin-layer open tubular columns (SCOT-type) exhibit a decreased separation efficiency although their sample capacity is much higher. Further improvement of the performance of this type of columns may be achieved in the future (see, for example, German *et al.*[7]).

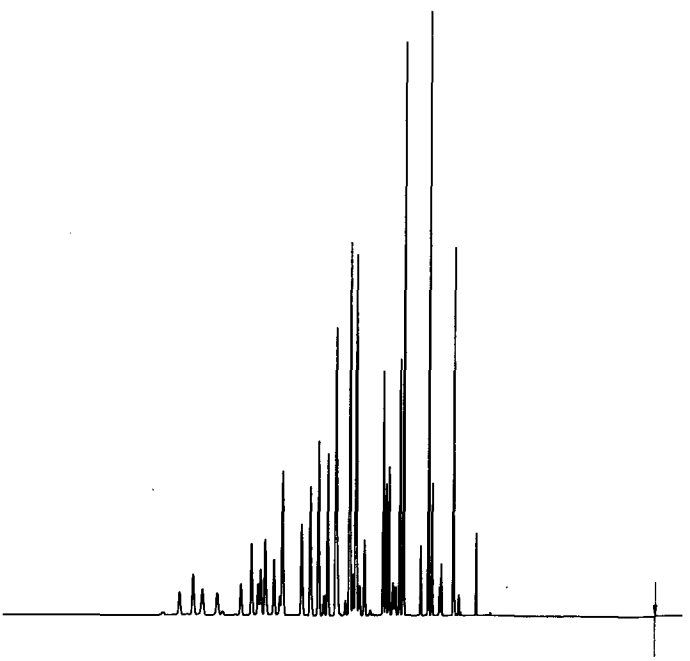

Fig. 3. Separation of isomeric polychlorinated biphenyls (Aroclor 1016). Column: 120 m glass capillary, Dexsil 300, 220°, isothermal.

The chromatogram in Fig. 3 illustrates the resolution that can be achieved by a typical thin-film glass capillary. The separation of isomeric polychlorinated biphenyls (Aroclor 1016) with a 120-m Dexsil glass capillary column in 40 min at 220° is shown. Using a squalane column up to 300 m in length, we were able to separate two cyclic C_8 hydrocarbons, each of which contained six deuterium atoms located at different positions in the molecule. In general, two identical hydrocarbons that differ only by one deuterium atom can be separated using a good 100-m squalane column. The observed difference in Kováts indices for one deuterium atom is 0.65 index unit[10].

Separations of this type are, of course, time consuming. The analysis times for the above separations were between 3 and 12 h. In many practical applications of capillary columns, the analysis time can be reduced by taking advantage of the high

separation efficiency of even short capillary columns. The column temperatures can be decreased in short columns for compounds of low volatility, and degradation of solutes and stationary liquids is thus avoided[11]. The application of temperature programmes to complex mixtures containing many components with a wide range of retention indices is not necessary if the column allows sufficient resolution. Small differences in retention are possibly a consequence of unsuitable operating temperatures for some of the component groups. A sufficient resolution is, however, achieved.

This effect can be seen in the chromatogram shown in Fig. 4, which was obtained from cellobiose radiolysis products after silylation[12]. Sufficient resolution is attained for the deoxyriboses (marked on the chromatogram with an ×) instead of the isothermal mode of measurement.

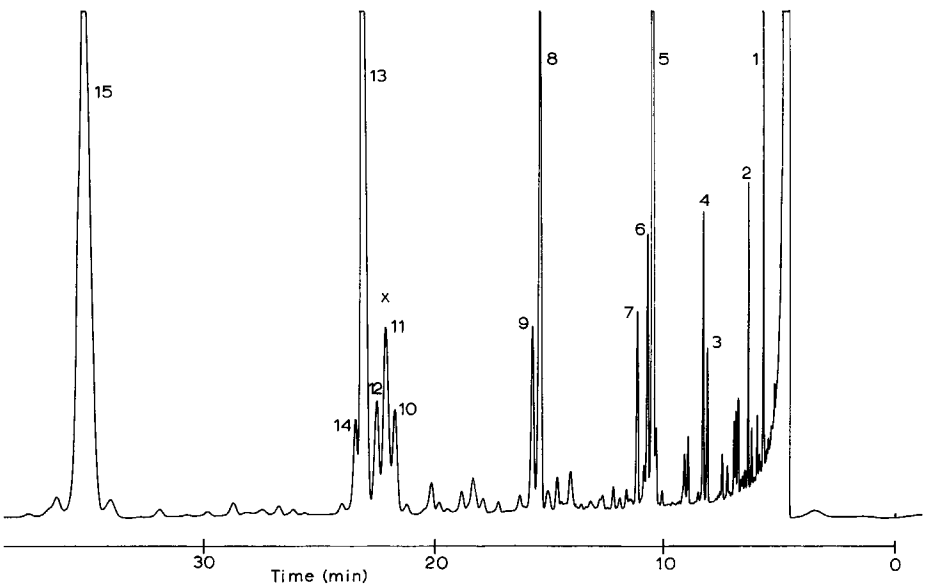

Fig. 4. Radiolysis products of cellobiose, trimethylsilylated after removal of glucose and reduction with $NaBD_4$. Column: 50 m glass capillary, Silicone oil SF-96, 160°, isothermal. 1 = Glycerol; 2 = 1,2,4-butanetriol; 3 = threitol; 4 = erythritol; 5 = 2-deoxyribitol; 6 = 3-deoxypentitol; 7 = 3-deoxypentinol; 8 = arabitol; 9 = ribitol; 10 = 5-deoxysorbitol; 11 = 2-deoxysorbitol; 12 = 3-deoxysorbitol; 13 = 4-deoxysorbitol; 14 = 3-deoxyhexitol; 15 = sorbitol.

We have already reported some of our results concerning the tailing behaviour of glass capillary columns above, and only a few additional comments on the deactivation procedure are made here.

By coating the surfaces with a thin film of deactivating agent (as described for the first time 12 years ago for stainless-steel capillaries by Averill[13]), the tailing of polar compounds is reduced. Unfortunately, most of these agents, which are preferably strongly polar compounds, are unstable at temperatures above 200°. When, for example, a good OV-101 column that has been deactivated with Carbowax 20M is heated to 200°, the tailing of cyclopentanone and *n*-butanol, which was removed by the deactivation, reappears. The separation efficiency for less polar compounds is

maintained, however, with good peak symmetry. Several types of deactivating agents have been tested, of which Carbowaxes are the best. Further work on the deactivation (possibly by chemically bonded substrates) will be carried out in the near future.

Polarity of columns

For identification by means of retention parameters, columns with defined and reproducible polarities are essential. The polarities of the columns used should always be characterized by McReynolds or Rohrschneider constants. The greatest problem that exists in this field is the lack of stationary liquids with defined polarities. Even products, especially of polymeric structure, that are sold under the same trade-name may have completely different polarities, as can be seen from the Kováts indices measured with cyclopentanone and *n*-butanol with various polypropylene glycols (see Table II).

TABLE II

REPRODUCIBILITY OF POLARITY OF GLASS CAPILLARY COLUMNS

Kováts indices (I_{80}) of three test compounds with three polypropylene glycols of various origins measured in seven columns.

Column	*Phase*	*1-Octene*	*Benzene*	*Cyclopentanone*
1	PPG 1025 (WGA)	811.8	773.7	949.8
2	PPG 1025 (WGA)	811.6	773.5	949.2
3	PPG 1025 (WGA)	811.6	773.5	949.2
4	PPG (Applied Science Labs.)	810.3	769.3	934.4
5	PPG 2025 (WGA)	810.6	769.7	935.3
6	PPG 2025 (WGA)	810.4	769.5	934.7
7	PPG 2025 (WGA)	810.5	769.6	934.8

Using polar stationary liquids at temperatures above 200°, a change in polarity is observed, which is probably accelerated by penetrating oxygen. It is, of course, desirable that the polarity of stationary liquids should not be influenced by the surface deactivation.

Sample capacity of capillary columns

If symmetrical peak shapes have to be obtained for mixtures that contain trace and major components, *e.g.*, for precise determinations of the Kováts indices of all peaks, the dynamic range of sample load[14] is, of course, low, but it depends on the solubility of the components in the stationary liquid. The dynamic range of the sample load is much higher when referring only to the trace or minor components and, moreover, when the major components appear in sections of the chromatogram that are not of interest and therefore do not have to be evaluated. For these components, the column can be overloaded, provided that no overlapping of the major peaks with those of the minor components takes place. For simple quantitative analyses, the requirements of peak symmetry are not as critical. By the use of special sampling techniques, a selectively high sample load only for the minor or trace components can be achieved. The major components may be removed in a pre-separation from the sample, whereas all of the trace components reach the main separation stage. By applying sampling techniques such as the splitless injection of Grob and Grob[15], one of the strongest

arguments against the usefulness of capillary columns (that of poor sample capacity) becomes meaningless. Nevertheless, for samples without extreme ratios of component concentrations, the necessity for using splitting devices (which may cause discrimination of component concentrations with certain types of samples) remains. A connection unit including a splitter and an injection port was tested with samples with a carbon number range from 10 to 20. No severe discrimination of concentrations (*ca.* 1%) was obtained.

A sampling technique for isothermal capillary column chromatography involving the use of a double-column system with intermediate trapping and reinjection is described later.

Temperature stability of glass capillaries

Assessment of the temperature stability of columns is difficult and depends on the type of analytical application involved and on the required standard of performance and reliability of the analyses to be carried out. With isothermal operation, a column is considered to be temperature stable over a defined and not too short period (*e.g.*, longer than 100 h) when:

(a) the separation efficiency and the tailing behaviour do not deteriorate and the film homogeneity remains unchanged;

(b) the background current remains constant and does not exceed a defined limit (this is extremely important for combined gas chromatography–mass spectrometry (GC–MS) work, in which the bleeding products always contribute to the mass spectra of the peaks that are of interest);

(c) the capacity ratio (k) does not decrease rapidly as a substantial part of the stationary liquid contained in the column evaporates.

The temperature at which these requirements are met can be considered to be the limit of the practical application of the column. Sometimes the type of chemical compounds in the injected sample or the oxygen content of the carrier gas may accelerate the deterioration of a column. For the glass capillaries prepared and used in our laboratories, the temperature limits given in Table III together with those for the more

TABLE III

TEMPERATURE LIMITS FOR USE OF GLASS CAPILLARY COLUMNS IN NORMAL GC ANALYSIS AND GC–MS WORK IN COMPARISON WITH THE LIMITS PUBLISHED FOR PACKED COLUMNS BY SUPELCO

Intensity of MS background normalized to 5 V total ionization and 2 ml/min flow-rate of carrier gas (helium) (by courtesy of D. Henneberg, Max Planck Institut, Mülheim-Ruhr, G.F.R.).

Phase	*Temperature limit (°C)*		
	GC–MS	*GC*	*Supelco*
SF-96	175	200	250
OV-101	190	300	350
DC-200	175	200	250
PPG	95	140	150
FFAP	180	200	250
Carbowax 4000	160	160	200
Squalane	85	100	100
Dexsil 300	165	300	500

critical requirements of GC–MS work are realistic. When using temperature programmes, the drift of the baseline depends on the vapour pressure of the stationary liquid, *e.g.*, bleeding of the column at a given flow-rate of carrier gas and a defined detector sensitivity. We have verified that the non-polar stationary liquids OV-101 and Dexsil are particularly temperature stable for programmed analysis. The chromatograms of polyaromatic hydrocarbons shown in Fig. 5 are reproducible over several days, although the background current was high during the measurements at 300°.

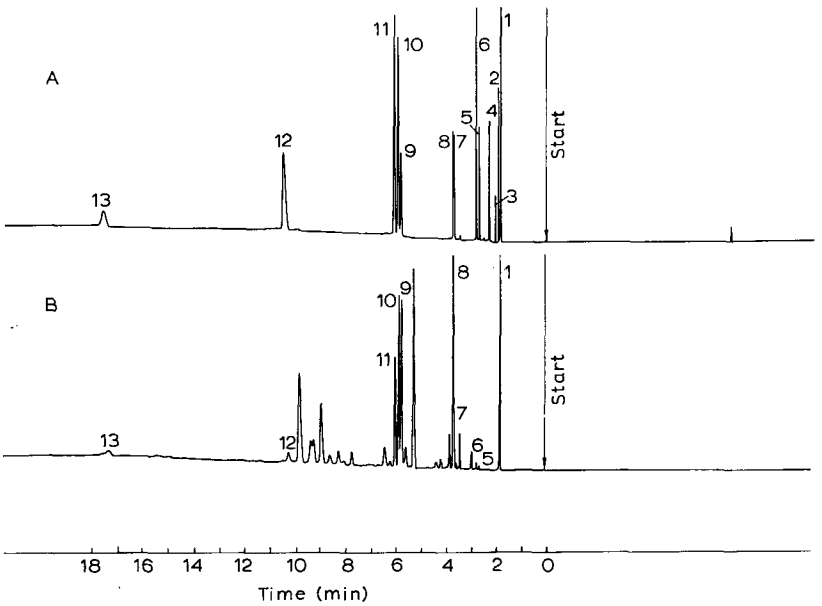

Fig. 5. Polyaromatic hydrocarbons (sample by courtesy of Dr. Hombach, Mülheim-Ruhr). Column: 50 m glass capillary, OV-101, 300°, isothermal, 2.5 bar N_2. Chromatogram A: test mixture. Chromatogram B: polyaromatic hydrocarbons from tar (sample of Dr. Hombach). 1 = Solvent; 2 = naphthalene; 3 = acenaphthene; 4 = anthracene + phenanthrene; 5 = fluoranthene; 6 = pyrene; 7 = benz(*a*)anthracene; 8 = chrysene; 9 = benzo(*e*)pyrene; 10 = benzo(*a*)pyrene; 11 = perylene; 12 = anthanthrene; 13 = coronene.

In a paper by German *et al.*[7], particularly good temperature stability of columns similar to the known SCOT-type columns was claimed. These columns contain a larger amount of stationary liquid than the thin-film type of columns, and the loss of stationary liquid at elevated temperatures is comparatively small in relation to the total amount of stationary liquid in the column. This may be the reason for the reported extended lifetime of these columns. Insufficient information about background current and detector sensitivity can be obtained from the chromatograms and most of the temperature-programmed separations shown were finished at a temperature of 260°. The OV-101 and Dexsil 300 columns used by us are normal thin-film open tubular columns. They have a comparable temperature stability and probably a slightly better separation efficiency. The film homogeneity of the columns remains stable over a longer period at 300°. Of course, the etched surfaces of thin-film capillaries may be considered to have similar properties for the fixation of a homogeneous film of the

stationary liquid as the support of typical SCOT-type columns. The preparation of columns by etching seems, however, to be more convenient. Finally, we would like to repeat that capillary columns with non-polar or slightly polar stationary liquids of the silicone type can be used successfully (*i.e.*, without tailing) at temperatures up to 300° only for compounds that are not too polar. Polar stationary liquids that can be used at relatively high temperatures (above 200°) are not available. The analyst should take into consideration that the use of short capillary columns with a just-adequate resolving power is a good means of lowering the operating temperatures required; degradation of the stationary liquid and decomposable components in the sample are thereby avoided.

TECHNIQUES AND SPECIAL METHODS IN GLASS CAPILLARY CHROMATOGRAPHY

Procedures for the connection of glass capillaries to commercial instruments

For the application of glass capillaries, we use two connections of similar construction, one of which is essentially a splitter, the other a device for connecting the column to the detector, which also allows for the possibility of introducing make-up gas. The ends of the column do not have to be straightened in a flame for the introduction when using silicone rubber sealings. The sample does not come into contact with metal surfaces because both connection parts contain glass inserts. Silicone rubber seals are used up to a temperature of 200°. In the temperature range between 200° and 300°, we use fused glass–platinum joints similar to those described by Ganansia *et al.*[16]. Fig. 6 illustrates how the platinum capillary at the end of the glass capillary is connected with a 1/16-in. Swagelok or a comparable type of fitting. We have previously described[17] column systems into which splitting and make-up gas devices have been integrated. The glass capillaries were shielded against breakage in these units when carrying out connection manipulations. The column was connected to the gas chromatograph by means of four Swagelok fittings without touching the glass capillary. At present, we prefer to use commercial GC instruments equipped with liftable oven lids, which allow easy access to detector and injection port. This set-up is shown in Fig. 7. The connections remain fixed to the instrument and the ends of the column are inserted into the two devices sealed either by silicone rubber or by the described glass–platinum–Swagelok joint. No major problems concerning the breakability of the columns arose with a large number of different instruments during several years experience.

Double-column systems with glass capillaries

By means of the systems described below, we hope to be able to carry out the analysis of mixtures that consist of numerous components with an extremely wide range of volatilities and concentrations. The application of capillary columns to such analyses (which, in our opinion, is to be preferred to the use of classical packed columns in most instances) becomes a necessity when groups of compounds of related or isomeric structures have to be resolved in different sections of the chromatogram (see Fig. 4). As pointed out above, the most important prerequisite for reliable GC analysis lies in the use of mild conditions (especially column temperature). This also means that, in many instances, temperature programmes with high final temperatures may be avoided.

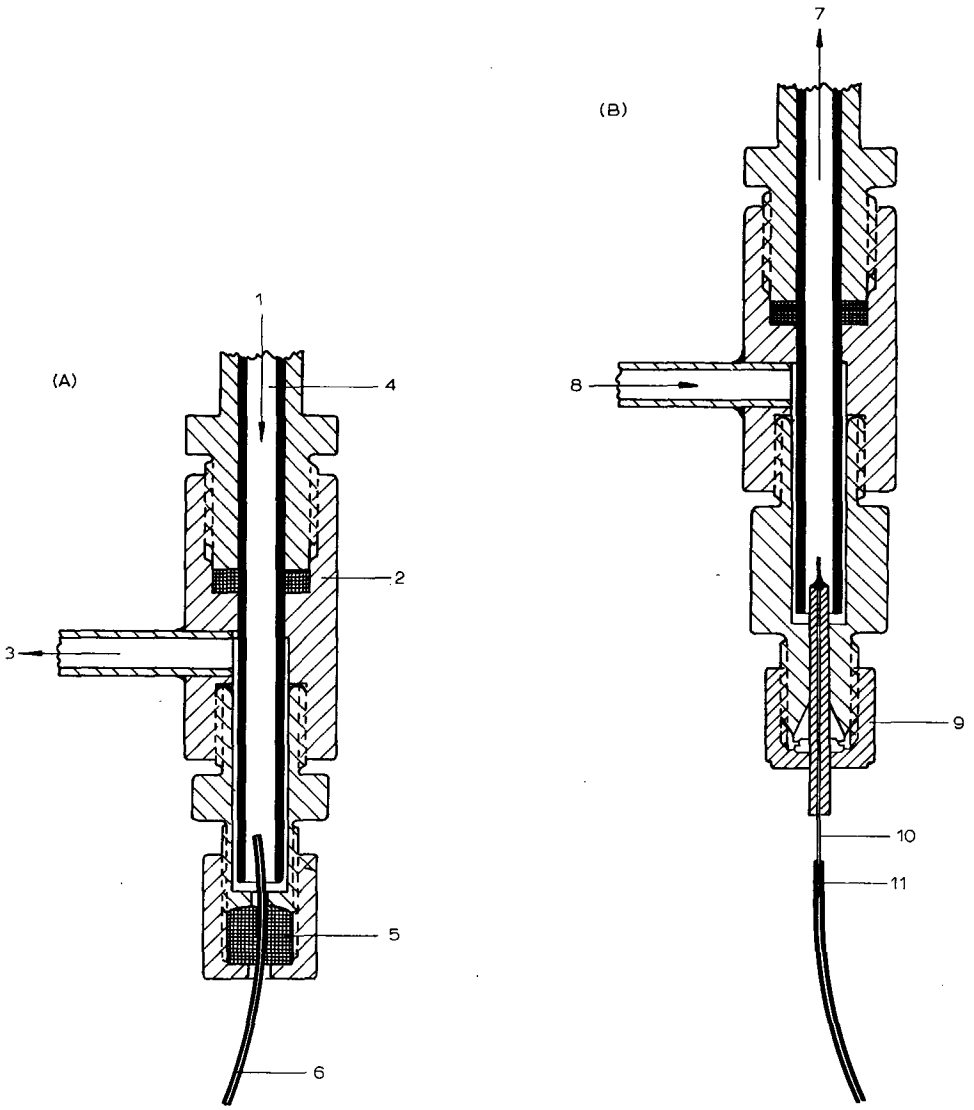

Fig. 6. Connection of glass capillaries. (A) Splitter shown with silicone rubber seal (up to 200°); (B) connection to detector with make-up gas introduction shown with glass–platinum–Swagelok joint (above 200°). 1 = Carrier gas from injection port; 2 = graphite seal; 3 = to splitter throttle; 4 = glass insert; 5 = silicone rubber seal; 6 = glass capillary; 7 = to detector; 8 = make-up gas; 9 = 1/16 in. Swagelok; 10 = Pt–Ir capillary; 11 = fused glass–platinum joint.

In cases of identification by means of retention parameters and of separations in which the maximum resolution possible is required, we prefer to use isothermal GC analysis in capillary columns. With samples with a wide retention range, this type of work has to be carried out in a stepwise manner. In many instances, the resolving power of capillary columns may make temperature programming unnecessary. When using high resolution, long analysis times occur when all of the components, including

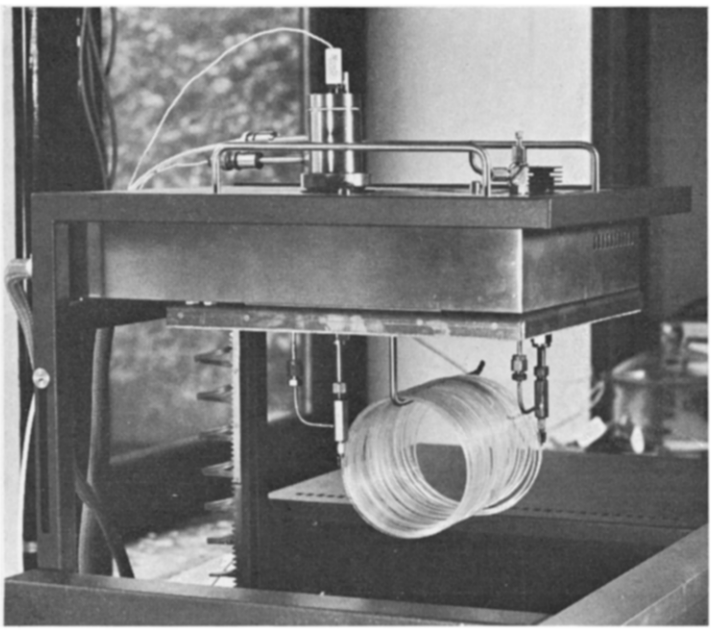

Fig. 7. Connection of glass capillaries to a commercial gas chromatograph with liftable oven lid by means of the devices shown in Fig. 6.

those of higher retention, are to be eluted at constant temperature. Sometimes even these compounds with high retentions cannot be removed from the column in a reasonable time, in which case we make use of double-column systems, which have already proved to be very effective with regard to the aspects discussed above. By applying double- or multiple-step chromatography, cuts are made from the eluate obtained by a pre-separation step. Interfering chemical reagents (for example, those originating from silylation procedures), solvents or components that are not of interest can be removed from various regions of the chromatogram. The removed species are excluded from the main separation in the capillary column, thus preserving its performance. Often only a selected cut of the pre-separated eluate is allowed to enter the main column, this cut containing a group of components of special interest for the analyst which must be separated under reproducible conditions for identification purposes. As described in a previous paper[18], double-column systems with capillary columns should be used with an intermediate trapping and re-injection procedure. The whole system is designed for valveless column switching according to the techniques described by Deans[19] in an automatic mode, *e.g.*, a timer- or computer-controlled mode (see Fig. 8). In order to illustrate these techniques, two examples of their practical application are given below.

Splitless injection of selected cuts for isothermal analysis with trapping and re-injection. Isomeric silylated sugars[12] had to be separated isothermally for qualitative analysis via retention parameters and for optimal quantitative evaluation. The separation was combined with the removal of the excess of the silylation reagent and the components with higher retentions that were not of interest. In the arrangement shown

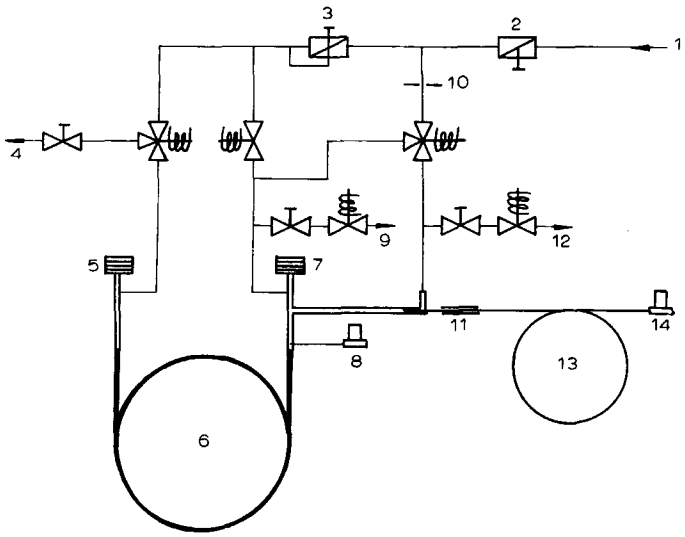

Fig. 8. System for double-column chromatography with intermediate trapping and re-injection, suitable also for direct injection of aqueous solutions. 1 = Carrier gas; 2 = pressure regulator; 3 = flow controller; 4 = vent for back-flushing; 5 = injection port for heart-cut and back-flushing; 6 = pre-column (packed); 7 = injection port for aqueous solutions; 8 = control flame ionization detector for pre-separation; 9 = vent for cutting; 10 = leak for make-up gas; 11 = trap; 12 = outlet of splitter; 13 = glass capillary column; 14 = flame ionization detector for main separation.

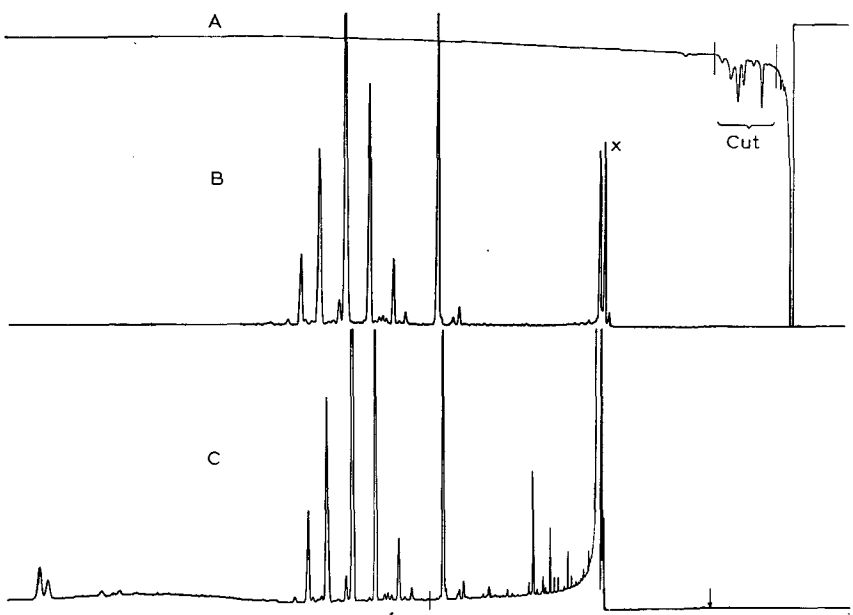

Fig. 9. Removal of solvents, silylating agents (>95% of sample) and components of high retention by cutting, back-flushing, intermediate trapping and re-injection. Chromatogram A: pre-separation on a packed pre-column (the cut period is marked). Chromatogram B: separation of trapped products in main column (× = remainder of solvent and silylating agent). Chromatogram C: original sample measured in main column under conditions identical with those for the trapped products.

in Fig. 8, which cannot be described in detail in this paper, a pre-separation (see chromatogram A in Fig. 9) and a main separation (see chromatogram B in Fig. 9) were performed. It can be seen that the bulk (>90%) of silylating reagents is removed from the eluate, leaving a minimal remainder. The species with higher retentions no longer appear in the isothermal chromatogram and thus analysis time is saved, as can be seen in chromatogram C in Fig. 9, which was measured under identical conditions without pre-separation for comparison. These species remain in the pre-column and are back-flushed from there. The pre-separation was performed on a 1 m × 1/8 in. column packed with SE-52 with a sample load of about 1 μl. The cut from the eluate was made during an interval which is marked in chromatogram A in Fig. 9. The eluate leaving the packed column during the selected period is led into a small trap consisting of a short length (50 mm) of platinum capillary (*ca.* 0.15 mm I.D.) connected to the glass capillary by a fused joint. The trap is cooled by blowing nitrogen (at a controlled temperature) during a programmed interval. The species of interest contained in the eluate are trapped. The volatile solvents or reagents are removed by venting between the pre-column and main column, the components of high retention are back-flushed from the pre-column and vented between the injection port and the pre-column.

After column switching the trapped part of the sample is evaporated instantly and introduced into the main column by heating the trap with a stream of hot nitrogen. The optimal ratio of the flow-rates of the carrier gas in the pre-column and main column requires adequate splitting between the pre-column and trap, by which a certain but not large loss of material of the species of interest occurs. Taking this aspect into account, the technique described is not perfectly splitless, but nevertheless sufficient sample is available for the main separation in most instances. Because of the removal of the solvent peaks, higher detector sensitivities may be applied, thus compensating for the mentioned loss of sample. Chromatogram B in Fig. 9, showing only the peaks of the species of interest, is obtained in the main separation. No temperature programme was necessary in order to start this chromatogram (*cf.*, Grob and Grob's technique of splitless injection[15]). The technique of Grob and Grob is, in general, restricted to the removal of very volatile solvents or major components from solutions in which components of low volatility are to be chromatographed. The group of peaks in chromatogram B marked with an × is obtained from the solvent peak tail on which the sample components of interest are eluted. The appearance of these peaks can be avoided by using modified pre-columns with improved sample capacity or tailing behaviour. The various consequences and possibilities that arise from the described technique cannot be discussed in detail here.

Trace analysis of aqueous solutions with glass capillaries. Water is a highly polar solvent, and good capillary columns must be protected against large amounts of water, which could change the surfaces and the adsorption of the deactivating agents and stationary liquids. There are two chromatographic methods for the removal of water. In a pre-column with a strongly polar stationary phase, such as Carbowax, the retention of water is increased to such an extent that in many instances the trace components of interest which are less polar are eluted in front of water, although they may have a much higher molecular weight. In this case, the earlier eluted volatile components are led into the main column with, or sometimes without, trapping. The water that remains in the pre-column is back-flushed from there. This method is used frequently in our laboratories and is applicable to other polar solvents such as methanol.

(For example, acetaldehyde could be determined at concentrations lower than 1 ppm in aqueous solutions.)

The alternative method described below is more generally applicable. With a non-polar Porapak pre-column, very short retentions are achieved for water and other low-molecular-weight hydroxyl compounds. All components that contain more carbon atoms are extremely retarded and remain in the very first part of the pre-column. Using the double-column technique described above, the transfer of these species into the trap would require high flow-rates of carrier gas and elevated temperatures when performing the elution in the same direction as the pre-separation was carried out. We have designed an arrangement (Fig. 8) in which, by valveless column switching, the flow of carrier gas is reversed in the pre-column. The trace components of interest are now back-flushed into the trap and introduced in the usual way into the capillary column. There are two injection ports, which can be used for the normal double-column technique (the sample is injected into port 1) and for the separation of aqueous solutions (injection into port 2 between the pre-column and main column).

Chromatograms obtained of dilute solutions of xylenes in methanol and water are given in Fig. 10. Methanol was chosen as a solvent to demonstrate the correct operation of the removal procedure by flame ionization detection. Only negligible amounts of the polar solvents reach the main column. This arrangement has not been fully developed, and we expect to be able to improve the performance and to demonstrate the efficiency of this modified version of a double-column system in various practical problems.

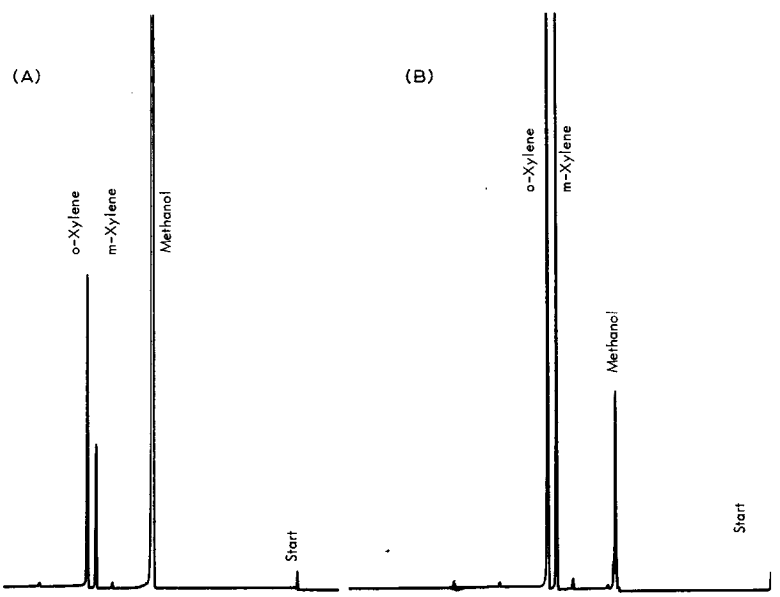

Fig. 10. Separation of a 5% solution of two xylenes in methanol. (A), Original sample; (B), the methanol was removed down to a minimal remainder by a pre-separation on a Porapak column (1 m). Main column: 50 m glass capillary, OV-101, 110°.

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