Journal of Chromatography, 203 (1981) 179–191
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,189

PROPERTIES OF UNTREATED AND TREATED GLASS CAPILLARY SURFACES AND THEIR CONTRIBUTION TO SOLUTE–STATIONARY PHASE INTERACTION AND FILM FIXATION OF THE STATIONARY LIQUID

G. SCHOMBURG*, H. HUSMANN and H. BEHLAU
Max-Planck-Institut für Kohlenforschung und Institut für Strahlenchemie, D-4330 Mülheim a.d. Ruhr (G.F.R.)

SUMMARY

The properties of support surfaces of glass capillary columns pre-treated by various procedures such as etching, leaching, dealkalization and deactivation (silanization, PSD treatment, "baking") determine their successful practical application. A comparative study of the dependence of the performance (tailing behaviour, sample capacity, temperature stability) of polar and non-polar column types on the support surface properties was carried out, using fused silica material which does not require extensive surface pre-treatment. Although the coatability of this material is limited, the availability of fused silica is of great value for the chromatography of highly polar solutes, especially at very low column loads such as arise in trace analysis.

INTRODUCTION

The simple practical application of glass capillary columns in qualitative and quantitative gas chromatographic (GC) analyses is still impeded by various minor as well as major difficulties. Limiting features are the following.

(1) Insufficient deactivation of the support surface for the undisturbed elution of highly polar solutes. Reversible and irreversible adsorption of polar solutes on the surface, which causes tailed peak shapes and incomplete elution from the column, is the most restricting property of improperly manufactured columns. Special deactivation of the support surface has to be carried out especially if coatings with non-polar or weakly polar stationary liquids are to be used. Polar stationary liquids can even be deposited on undeactivated surfaces, however, with less tailing problems with polar solutes because the stationary liquid itself has deactivating properties. Therefore, usually intermediate layers of highly polar stationary liquids which may also be chemically bonded to the surface are generated before coating with non-polar stationary liquids that do not have such strong deactivating properties. For solutes of very high polarity in comparison with the polarity of the stationary liquid or of the intermediate layer, the self-deactivation effect of polar stationary liquids may not be sufficient, however. Reversible adsorptive interaction between the solute and the

0021-9673/81/0000–0000/$02.50

support surface can normally be recognized by carrying out tailing studies with test mixtures containing solutes of varying polarity. The most polar classes of compounds are alcohols, diols, free acids and primary amines and are therefore also used in the above-mentioned test mixtures. In contrast, irreversible adsorption of polar species cannot be excluded reliably from simple tailing studies. Quantitative recovery measurements, especially at extremely low column loads such as arise in trace analysis, have to be carried out relative to non-adsorbed or less adsorbed standards such as alkanes[1].

By the usual procedures for the deactivation of pre-treated and non-pre-treated glass surfaces, intermediate layers between the surface and the stationary liquid may be formed, which contribute to the polarity of the stationary liquid and prevent reproducible manufacture of columns with defined polarity. The selectivity of such columns and the measured retention data may be changed considerably in this way. The same disturbance may arise if excessive amounts of the deactivating reagents (for example, polyethylene glycols) are not removed by solvent rinsing before coating with the stationary liquid. Mixed polarities are then observed.

(2) Imperfect film fixation, *i.e.*, inhomogeneous coating of the support surface. Certain stationary liquids do not adhere to well deactivated surfaces in particular, because the surface energy is too low to prevent droplet formation of stationary phases with a high surface tension. The inhomogeneity of the film of the stationary liquid by which the height equivalent to a theoretical plate (HETP) is considerably increased (the C_L term of the Van Deemter–Golay equation is proportional to the square of d_f), is also increased at higher temperatures because of the decreased adsorption of the stationary liquid on the surface and the decreased viscosity of the stationary liquid. If the surface activity is very high, even homogeneous coatings of the surface do not prevent adsorptive interaction of the solutes through the layer of the stationary liquid.

(3) Insufficient temperature stability of the stationary liquid, depending also on the catalytic properties of the support surfaces and insufficient temperature stability of the intermediate deactivation layer.

Since the availability of glass capillaries made from fused silica[2] of high purity and since the recent progress in the field of surface deactivations[3–5], some of the above limiting factors have become less restrictive in practical capillary GC. The most severe limitation in addition to the problems of quantitative analysis and the sampling techniques therefore required, remains the separation and analysis of very complex mixtures containing many components with very different polarities. For the separation of highly polar solutes from each other and from less polar sample constituents, the chromatographer was considerably restricted in the past in his selection of a column with a stationary phase of adequate polarity for as many solute pairs contained in the mixture as necessary because the highly polar solutes could not be eluted without disturbance by surface adsorption when using non-polar stationary phases. The application of non-polar stationary liquids for the separation of highly polar solutes and conversely the separation of non-polar solutes with polar stationary liquids is hindered mainly by the limited sample capacities of the columns in these instances, however. Overloading problems become even more disturbing when columns with low film thickness are to be used in the GC of compounds of low volatility in order to avoid too high column temperatures. The disadvantage of the above-mentioned approach with regard to selection of phase systems is the limited

sample capacity[6]. This disadvantage is compensated for by the low column temperatures that can then be applied; low column temperatures improve the stationary phase selectivity and the life time of the column.

In analytical practice dealing with mixtures with a wide range of component polarities, stationary phases of medium polarity are preferred in order to achieve adequate selectivity and sample capacity for as many different species as possible.

In the analysis of flavours, aromas and essential oils, for example, stationary liquids such as the medium polarity polypropylene glycols or the more polar polyethylene glycols are therefore preferably applied. Usually not all of the important solute pairs in complex mixtures can be resolved by using a single stationary liquid even with the high separation efficiencies of long capillary columns and by using temperature programming. Coupled columns of different polarities can be used with advantage in multi-dimensional set-ups. For such systems good polar columns and non-polar columns with well deactivated surfaces are needed in order to make the full use of the selectivity change between the two coupled columns. The usefulness of the new types of columns in multi-dimensional GC is illustrated by the chromatograms in Figs. 1 and 2.

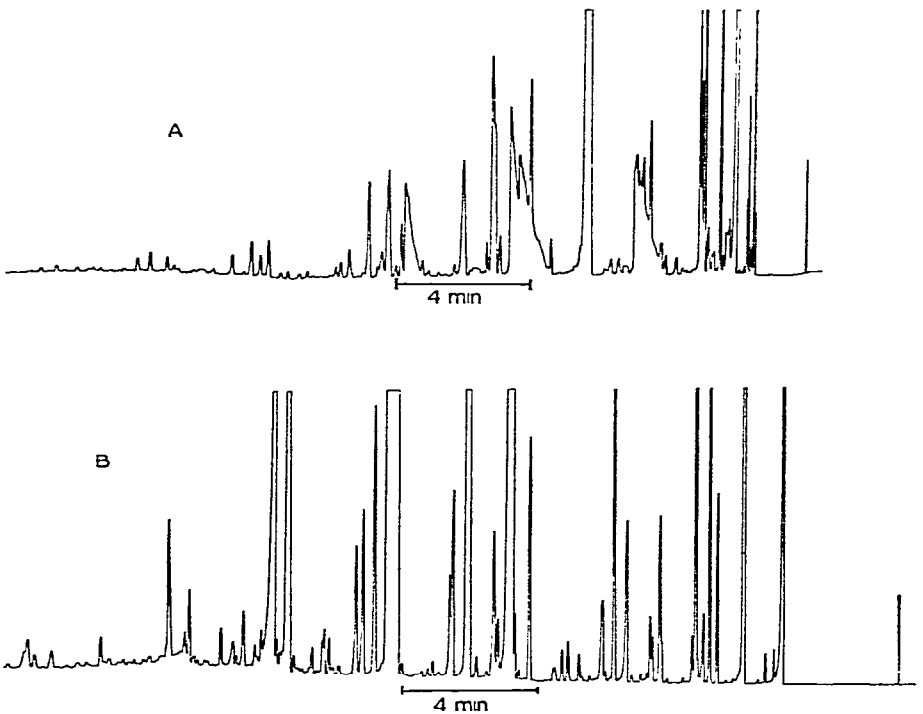


Fig. 1. Multi-dimensional glass capillary GC with an extract of vine aroma using a combination of a well deactivated non-polar methylpolysiloxane (OV-101) fused silica capillary column and a polar polyethylene glycol (Carbowax 20M) leached alkali glass capillary column. Sample: Freon extract of vine aroma and peak identification by courtesy of Prof. A. Rapp, Bundesanstalt für Rebenzüchtung, Siebeldingen, G.F.R.; columns: A, 20-m methylpolysiloxane (OV-101) on fused silica, PSD deactivated; B, 20-m methylpolysiloxane (OV-101) + polyethylene glycol (Carbowax 20M) leached alkali glass; temperature: A and B, 70–220°C, 8°C/min; carrier gas: N_2.

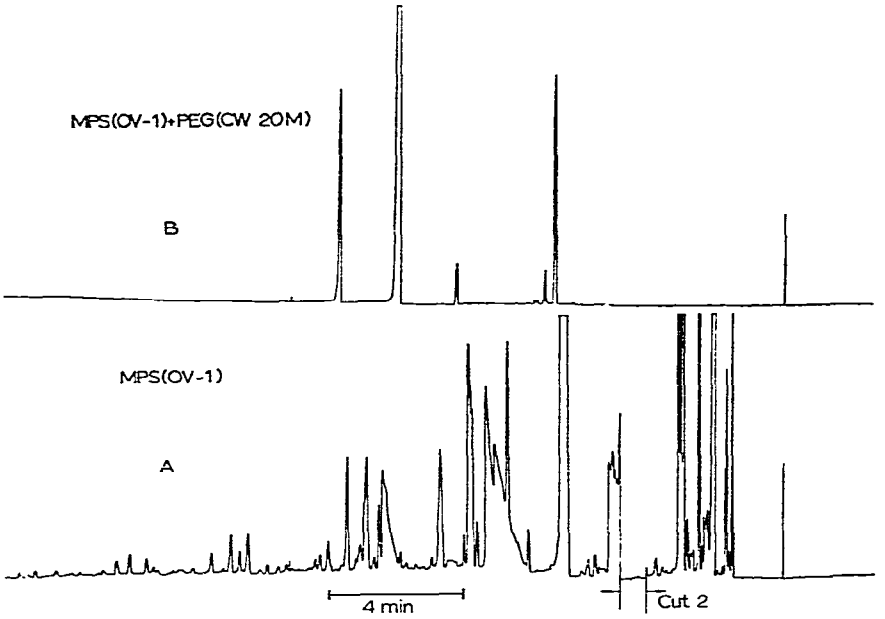


Fig. 2. Multi-dimensional glass capillary GC with an extract of vine aroma. Transfer of selected cuts as marked in chromatogram A: cut 2.

The chromatograms shown of a Freon extract of vine were obtained with a multi-dimensional set-up constructed by Hövermann and Müller[7] (Siemens, Karlsruhe, G.F.R.) using the basic principles developed by Schomburg *et al.*[8]. Two capillary columns with a low volume and zero dead volume are coupled by means of this so-called "live switching unit", which also allows for monitoring the eluate flow from the pre-column using an additional detector. From the pre-column chromatogram obtained with this detector appropriate cuts can be taken as necessary for the effective use of the set-up. The upper chromatogram (A) in Fig. 1 was obtained with the monitor detector after the elution of the sample from the non-polar methylpolysiloxane column (support surface: fused silica). Strong leading and therefore overlapping with many other sample constituents is observed with the free acids contained in the extract. This leading is caused by the poor solubility of such polar components in the stationary liquid and possibly also by intermolecular interactions in the mobile phase. No peaks with leading are observed in the lower chromatogram (B) in Fig. 1, which was obtained with Carbowax 20M as the stationary liquid because of better solute solubility.

In Fig. 2, time interval markings for a "cut" are given in the lower chromatogram (A). By column switching this cut was transferred into the coupled polar PEG (Carbowax 20M) column. Although no intermediate trapping in the inlet of the second column was performed, by which focusing of the solute band could be attained, narrow peak shapes of good symmetry and without appreciably tailing are observed in the chromatogram obtained from the second column. This result is due to the good solubility of the transferred solutes in the polar stationary liquid of the second column and to the excellent flow geometry of the coupling piece used.

Chromatogram B in Fig. 2, obtained with the cut marked in chromatogram A, also illustrates the striking changes in retention sequences caused by the selectivity change. In the analysis of complex mixtures the correct use of such selectivity changes can be made only if properly deactivated support surfaces in columns of high separation efficiency and very different polarities are available, however.

Some aspects of the adsorptivity and tailing behaviour of non-polar and polar capillary columns with specially treated alkali glass and fused silica as the support surface have already been investigated by the authors recently[6]. The major topic was the application of the polysiloxane degradation (PSD) method for surface deactivation to leached or "dealkalized" alkali glass surfaces and to non-pre-treated fused silica material. Non-polar or weakly polar alkylpolysiloxane columns (OV-1, OV-101, SE-30, SE-52, OV-17, Dexsil 400, etc.) with very low adsorptivity for highly polar solutes can be prepared by using the described methods. The performance of such columns is the same as that of the columns obtained by Grob and Grob[3,4] using the silanization of leached surfaces by means of alkyldisilazanes which may contain a varying number of methyl and phenyl groups.

The same surfaces (leached or dealkalized alkali glass and the non-pre-treated fused silica) have also been coated with the polyethylene glycol (PEG) Carbowax 20M. It was surprising that the smooth fused silica surfaces can be easily coated with polar PEG. The fairly good adhesion of this oligomer is probably effected by the presence of silanol groups on such surfaces. With extended use of fused silica columns with Carbowax 20M coatings we observed a limited long-term stability in comparison with columns with leached alkali glass surfaces, however. Surfaces deactivated by Grob and Grob's[3,4] silanization or our[5] PSD method cannot be coated with even weakly polar stationary liquids. In a recent paper, Grob and Grob[4] reported that phenylmethylsilazane-treated, leached surfaces can also be coated with methylphenylsiloxanes (OV-17) and even with the nitrilealkylsiloxane OV-225. Blomberg has presented at this Symposium a paper on the fixation of nitrilealkylsiloxanes to such surfaces by his *in situ* polymerization method. We have found no difficulty in coating alkali glass surfaces with such stationary liquids which have been treated according to our previously published methods[9] (HCl gas, HF gas treatment, etc.). The selection of stationary liquids that adhere well to highly deactivated alkali or borosilicate glasses and especially to the new fused silica surfaces is still limited, as can be deduced from publications, manufacturers' advertisements, etc. Nevertheless, important progress in deactivation has been made, which allows for separations of polar solutes even at very low column loads such as occur in trace analysis.

EXPERIMENTS ON THE PERFORMANCE OF NEW POLAR AND NON-POLAR COLUMN TYPES

Comparative tailing studies with various kinds of support surface

A test method described by Schomburg *et al.*[10] was used to compare the adsorptivity of various glass surfaces before and after the execution of certain pretreatment and deactivation procedures. Test pieces of glass capillaries with defined length are mounted with zero dead volume between the outlet of a column of low adsorptivity, *e.g.*, perfect tailing behaviour, and the detector. The impairment of peak shape of selected highly polar test compounds which are eluted with perfect peak

symmetry from the column to which the test pieces are connected allow conclusions to be drawn about the adsorptivity of the latter.

All types of non-pre-treated, pre-treated, deactivated and alkylpolysiloxane-coated capillary test pieces of length 2 m from alkali, borosilicate and fused silica glass were tested. In Fig. 3 the results obtained with differently treated alkali glass surfaces are given as an example.

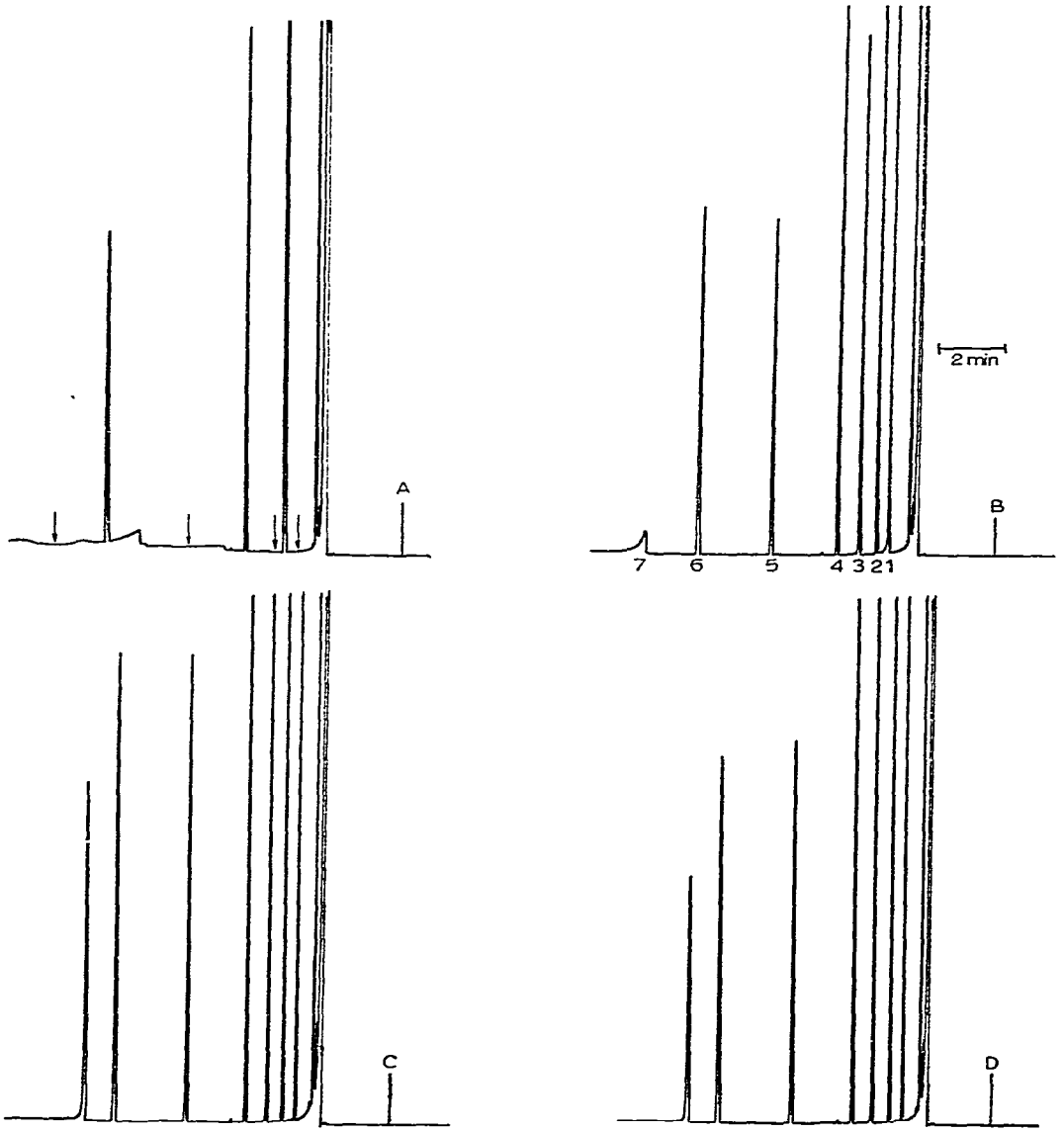


Fig. 3. Surface adsorptivity of alkali glass capillary columns. Test method according to Schomburg *et al.*[10]. Sample: 0.5 μl MPI test mixture, diluted 1:100 with benzene. Peaks: 1 = 1-hexanol, 2 = *n*-nonane, 3 = phenol, 4 = *n*-decane, 5 = methyl caprylate, 6 = *n*-dodecane, 7 = 1-aminodecane; column: 2-m test pieces, coupled with 20-m tailing-free methylpolysiloxane column: (A) untreated, (B) leached, (C) leached PSD (OV-101) treated, (D) leached, Carbowax 20M baked; temperature: 100–160°C, 4°C/min; carrier gas: N_2, 0.7 bar.

The best results regarding tailing and adsorption were achieved by the following, previously published[5,6,9] procedure: 2 h at 450°C with HCl (gas) + H_2O (dealkylation), 2 h at 450°C with HF (gas) + PSD (deactivation) + coating. Such a column was therefore also used as a standard column because even the critical primary amines can be eluted without tailing. If certain steps of the procedure are omitted deterioration of tailing behaviour is observed (see the chromatograms in Fig. 3).

Similar procedures can likewise be successfully applied also to borosilicate surfaces, although the adsorptivity of this material (especially for basic compounds such as primary aliphatic amines) is more difficult to reduce sufficiently. In contrast, fused silica surfaces exhibit the same chromatographic inertness towards the above-mentioned solutes without execution of tedious surface pre-treatment procedures, as was shown by Dandeneau and Zerenner[2], Lipsky *et al.*[11] and Schomburg *et al.*[6]. The fused silica surfaces are generally more suitable for the elution of hydroxyl compounds, compounds such as free acids and acidic compounds in general than for the tailing-free elution of basic compounds such as primary amines, which are also capable of forming hydrogen bonds in addition to their donor properties. For highly polar basic solutes the adsorptivity of the fused silica surface has to be reduced by PSD treatment or any other method of silanization; deactivation by the "classical" PEG (Carbowax 20M) method is likewise applicable, although a change in the polarity of the stationary phase may be observed in the latter instance, especially if an excess of the deactivation reagent is not removed from the column properly by rinsing with a suitable solvent.

Because of the absolute absence of alkali from fused silica, the temperature stability of alkylpolysiloxanes is still slightly better than that of the best carefully prepared alkali and borosilicate surfaces. Moreover, the surface treatment for the common glasses preferably requires the use of acidic chemicals such as HCl and HF, which also catalyse siloxane degradation if the surfaces remain contaminated with them.

Comparative testing of readily coated columns

In the second part of our comparative studies we tested readily coated alkylpolysiloxane and polyethylene glycol columns. The support surfaces were either pre-treated (etched or leached) alkali glass or non-pre-treated fused silica. The alkylpolysiloxane coatings were always generated after previous PSD deactivation. By an analogous thermal "conditioning" of PEG columns after coating, the performance can also be slightly improved. The PSD treatment cannot, of course, be applied if subsequent coating with polar stationary liquids is to be carried out.

We also investigated the quality of commercially available fused silica columns. We used a test mixture which guaranteed a more rigid adsorptivity test than the usually used Grob mixture and selected the following compounds: *n*-hexane, *n*-nonane, phenol, *n*-decane, methyl caprylate, *n*-dodecane, 1-aminodecane (for more basic surfaces) and *n*-butyric acid (for more acidic surfaces).

The adequate evaluation of the chromatograms obtained, *i.e.*, the assessment of peak shapes and the relative peak areas of adsorbed highly polar test solutes relative to that of a non-adsorbed alkane, requires careful consideration and control of various other parameters of the column itself and of the chromatogram generation and recording in the instrument. In addition to the influence of extra-column effects such as poor flow geometry (dead volumes) and adsorption on system surfaces, the

following parameters are important: (a) film thickness of the stationary liquid and homogeneity of the film (no droplets)[6]; (b) column temperature (adsorptivity decreases with increasing temperature); (c) column load per component[1]; (d) sensitivity of detection and recording: a small tail on the peak base can easily be overlooked and may be deleterious for trace analysis if the trace peaks are located close to the tailed major peaks (see Fig. 4) in which two chromatograms of the same separation are given which have been plotted on a display screen with the standard attenuation and also at 250-fold increased sensitivity; the latter chromatogram reveals the tailing at the peak basis of the main peaks; (e) irreversible adsorption which is not correlated with the tailing of the peak of the adsorbed species and can be measured quantitatively in relation to non-adsorbed species only[1].

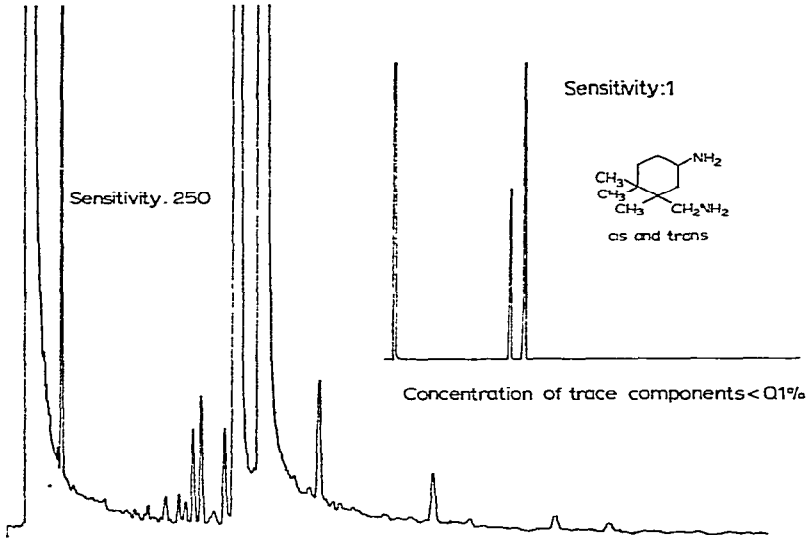


Fig. 4. Peak shape assessment at extremely different sensitivities (1 and 250) of chromatogram recording. Sample: 1 μl isophorondiamine (two isomers) in *n*-pentane (1:50), splitting ratio 1:100; column: 20-m Carbowax 20M, I.D. 0.27 mm, leached alkali glass support surface; temperature: 160°C; carrier gas: H_2, 0.7 bar; computer-reconstructed gas chromatogram, analysis time: *ca.* 12 min.

Especially when testing new well deactivated non-polar alkylpolysiloxane columns, overloading by highly polar solutes such as diols, phenols and free acids, which are naturally also the best test compounds for adsorptivity, can be avoided only by high dilution of the test mixtures[6].

The results of these GC measurements on column performance cannot be fully presented here, and are summarized only:

(1) With all types of columns tested primary aliphatic amines exhibited the strongest tailing (and irreversible adsorption), including columns made with the new inert fused silica. These compounds showed less tailing in non-polar alkylpolysiloxane columns if the prior deactivation was carried out by PSD treatment. In polar Carbowax 20M columns with either leached alkali glass or non-pre-treated fused silica surfaces the tailing was surprisingly stronger. The higher adsorptivity of leached alkali glass surfaces is probably due to contamination of the surface with

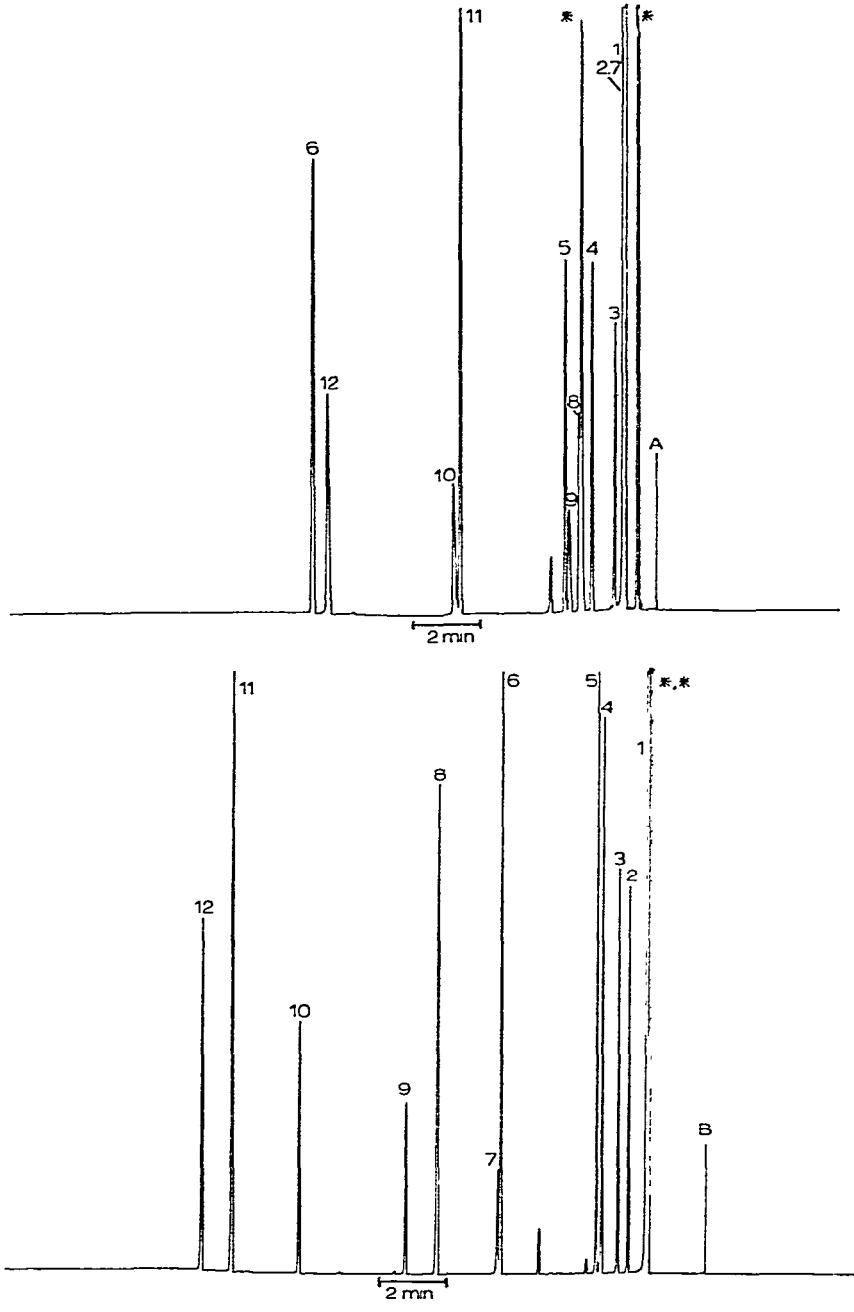


Fig. 5. Peak shapes of typical polar solutes found in vine aroma extracts. A, Non-polar methylpolysiloxane (OV-101), fused silica; B, polar polyethylene glycol (Carbowax 20M), fused silica. Sample: 1 μl; 1 = ethyl acetate (solvent), 2 = 2-methyl-1-propanol, 3 = 1-butanol, 4 = 2-methyl-1-butanol, 5 = 2-hexanol, 6 = ethyl octanoate, 7 = acetic acid, 8 = 2-methylpropanoic acid, 9 = butanoic acid, 10 = hexanoic acid, 11 = phenol, 12 = octanoic acid, * = impurity; columns: A, 20-m methylpolysiloxane (OV-101), 0.25 mm I.D. fused silica, PSD-deactivated; B, 20-m polyethylene glycol (Carbowax 20M), 0.25 mm I.D., fused silica; temperature: A, 35–100°C, 8°C/min; B, 60–200°C, 8°C/min; carrier gas: H_2; A, 0.35 bar; B, 0.40 bar.

HCl, whereas with fused silica the presence of too many silanol groups on the surface may be the reason.

(2) All Carbowax 20M fused silica columns showed a slight but always present tailing for alcohols. Some of the commercially available columns did not show the optimal performance that is achievable today.

(3) On extended use for the separation of highly polar solutes, the lifetime of Carbowax 20M fused silica columns seemed not to be as long as with etched or leached alkali glass as the support surface.

(4) The temperature stability of alkylpolysiloxane fused silica columns was slightly better than that of borosilicate or carefully dealkalized alkali glass columns.

(5) The coatability of fused silica by polar stationary liquids was not as good as those of etched or leached alkali glass surfaces. The choice of stationary liquids to be used with fused silica is still limited.

Finally, we carried out applicational test measurements with the different types of columns, including classes of compounds such as polyaromatic hydrocarbons including heterocyclic types*, unsilylated sterols, polar constituents of vine aroma extracts and essential oils and flavours. For example, an artificial mixture of the most demanding constituents of vine aroma extract with regard to tailing and irreversible adsorption was separated in non-polar methylpolysiloxane OV-101 and polar PEG Carbowax 20M columns both made with fused silica material. Both chromatograms showed only minor tailing even for the highly polar free acids, but leading of the peaks of the same compounds appeared except when low column loads by dilution of the test mixture were applied (Fig. 5).

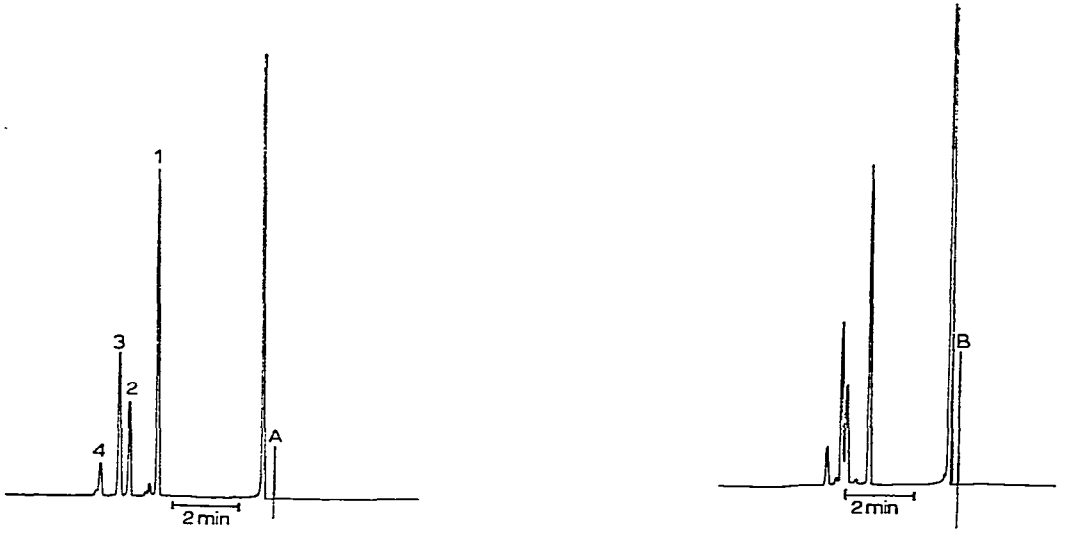


Fig. 6. Separation of underivatized sterols on non-polar and polar fused silica columns. Sample: 0.5 μl sterols, diluted 1:100 with 1-methylnaphthalene, splitting ratio 1:60; 1 = cholesterol, 2 = campesterol, 3 = stigmasterol, 4 = β-sitosterol; column: A, OV-1 on fused silica, 20 m × 0.34 mm I.D.; B, Carbowax 20M on fused silica, 16 m × 0.34 mm I.D.; temperature: A, 270°C; B, 260°C (isothermal); carrier gas: H_2, 0.4 bar.

* No chromatograms are given here.

With the non-polar column A (methylpolysiloxane on fused silica) much lower column temperatures (35–100°C) have to be applied in order to achieve higher capacity factors, k'. Short analysis times for less volatile solutes at higher temperatures are of advantage but longer columns should be used for more volatile solutes in order to achieve good resolution. Generally lower column temperatures may be advantageous for thermolabile compounds.

Also with four unsilylated sterols the same negligible difference in tailing behaviour can be seen for both the non-polar and the polar column (Fig. 6).

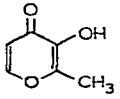
If the selectivity of the non-polar methylpolysiloxane phase is also suitable for the separation of such compounds, methylpolysiloxanes may be superior to the polyethylene glycol Carbowax 20M because of its much higher temperature stability.

Taking these results into consideration, the problems involved in the separation of complex mixtures containing very polar and much less polar constituents seem to be much less disturbing in analytical practice than hitherto because of the availability of well deactivated non-polar columns and the inert fused silica material. Similarly good results in the comparison of non-polar and polar new column types were also obtained with an artificial mixture of the major and significant constituents of certain essential oils (see Fig. 7 and Table I). Comparing the two chromatograms, it can bee seen that lower column temperatures are required for the separation in the non-polar column. Several retention sequences changed dramatically (see, for example, peaks 14, 15, 18, 19 and 20). Only slight "leading" of the "free acid" peak 17 (pentanoic acid) is observed, whereas the highly polar propanediol exhibits moderate tailing in the non-polar methylpolysiloxane column because of the very low initial column temperature of 25°C.

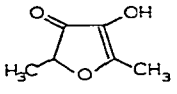
TABLE I

TYPICAL POLAR SOLUTES FOUND IN FLAVOURS AND ESSENTIAL OILS

The compounds are numbered according to their retention sequence in Carbowax 20M (see Fig. 7).



maltol (19)



furaneol(20)

1	Methyl 2-methylbutyrate	13	1-Octanol
2	Ethyl butyrate	14	1,2-Propanediol
3	Ethyl 2-methylbutyrate	15	Octyl butyrate
4	Limonene	16	Benzyl acetate
5	Ethyl hexanoate	17	Pentanoic acid
6	Hexyl acetate	18	β-Ionone
7	*cis*-3-Hexenol acetate	19	Maltol
8	1-Hexanol	20	Furaneol
9	*cis*-3-Hexenol	21	Triacetin
10	Hexyl butyrate	22	γ-Decalactone
11	Octyl acetate	23	Eugenol
12	Linalool	24	Triethyl citrate

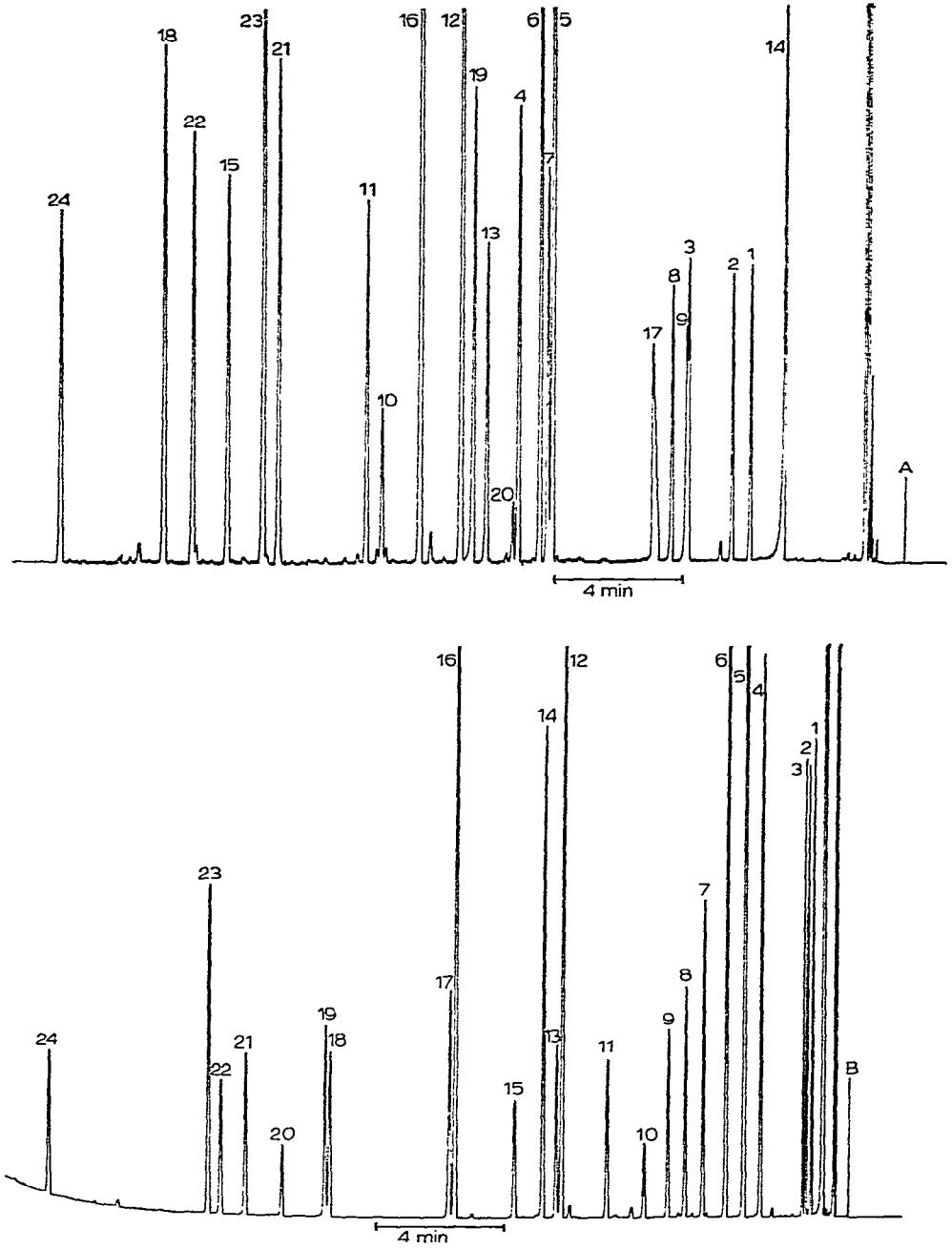


Fig. 7. Peak shapes of some typical polar solutes found in flavours and essential oils. A, with a non-polar methylpolysiloxane (OV-101) column, fused silica; B, with a polar polyethylene glycol (Carbowax 20M) column, fused silica. Sample: for composition see list of compounds in Table I; columns: A, 20-m methylpolysiloxane (OV-101) on fused silica, PSD-treated, 0.25 mm I.D.; B, 20-m polyethylene glycol (Carbowax 20M) on fused silica or leached alkali glass, 0.25 mm I.D.; temperature: A, 25–230°C, 6°C/min; B, 50–200°C, 6°C/min; carrier gas: H_2, 0.4 bar.

REFERENCES

1 G. Schomburg, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 461.
2 R. Dandeneau and E. H. Zerenner, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 351.
3 K. Grob and G. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 31.
4 K. Grob and G. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 197.
5 G. Schomburg, H. Husmann and H. Borwitzky, *Chromatographia*, 12 (1979) 651.
6 G. Schomburg, H. Husmann and H. Behlau, *Chromatographia*, 13 (1980) 321.
7 W. Hövermann and G. Müller, *Technisches Messen*, 1980, Heft 6, 131.
8 G. Schomburg, H. Husmann and F. Weeke, *J. Chromatogr.*, 112 (1975) 205.
9 G. Schomburg, H. Husmann and F. Weeke, *J. Chromatogr.*, 99 (1974) 63.
10 G. Schomburg, H. Husmann and F. Weeke, *Chromatographia*, 10 (1977) 580.
11 S. R. Lipsky, W. J. McMurray, M. Hernandez, J. E. Purcell and K. S. Billeb, *J. Chromatogr. Sci.*, 18 (1980) 1.