Journal of Chromatography, 104 (1975) 319–326

CHROM. 7951

THERMOSTABLE GLASS OPEN TUBULAR CAPILLARY COLUMNS WITH A POLAR LIQUID PHASE FOR GAS CHROMATOGRAPHY

SHEN-NAN LIN, C. D. PFAFFENBERGER and E. C. HORNING
Institute for Lipid Research, Baylor College of Medicine, Houston, Texas 77025 (U.S.A.)
(Received September 20th, 1974)

SUMMARY

A two-stage process involving a thermo-evaporative technique was employed to coat glass capillary columns with polar phases. Silanox 101 and a small amount of liquid phase were introduced in the first stage; additional phase was introduced in the second stage. The theoretical plate efficiency for 5α-cholestane at 250° for a 32 m × 0.25 mm glass column coated with liquid phase PZ-176 was 1800/m. Column properties remained constant when these columns were used between 180° and 320° for the analysis of biologic samples. No indication of deterioration was observed after months of use. Columns made with phase PZ-176 are useful for the separation of long-chain fatty acid methyl esters, polychlorobiphenyls and α-tocopherol as the trimethylsilyl derivative.

INTRODUCTION

The use of thermostable high-resolution glass open tubular capillary columns for the gas chromatographic (GC) separation of complex mixtures of biologic origin is a relatively recent development. This is not because of shortcomings of theory, but rather because there are only a few practical ways to prepare thermostable glass capillary columns. The basic difficulty, as pointed out by Merle d'Aubigne *et al.*[1], is that most liquid films on glass break up and form micro-droplets when heated.

The glass capillary columns described by Grob[2–5] are made by a surface roughening process. The conversion of a smooth glass surface to one with micro-irregularities provides a means of holding a liquid film in place. A number of etching procedures have been described. These include, besides the Grob procedure, the method evaluated by Novotný and Zlatkis[6,7] and a recent process based on etching soft glass so that sodium chloride crystals remain on the surface[8,9]. We have approached this problem in a different way. The combination of very fine particles of silanized silicic acid (Silanox 101) with a liquid phase on the inner wall of a smooth glass capillary column provides a coating that does not break up on repeated heating and cooling. The chief difficulty in preparing such columns lies in the physical and mechanical aspects of coating evenly the interior of a capillary tube. The procedure of German and Horning[10], involving a two-step coating process, provides SE-30

columns with excellent thermal stability and good column efficiency. Since dynamic coating procedures are used, columns 60 m in length (100,000 or more theoretical plate efficiency) are easily prepared. These columns have many applications[11–14]. Unfortunately, the two-step dynamic process is not useful for the preparation of polar columns. It has not been possible to choose solvent combinations that do not result in uneven coatings and correspondingly low column efficiencies. Static evaporation is slow but known to provide an even coating in a capillary tube; the use of this coating method gives excellent polar phase columns[15], but because of the slowness of the evaporative process the practical limit of column length is about 20 m. A solution to the problem was found through the development of a two-step process based upon (a) a dynamic coating step analogous to the first step described by German and Horning[10], and (b) a second step based upon the technique of Ilkova and Mistryukov[16]. The polar phase employed in this work was PZ-176, a phenyl ether–phenyl sulfone polymer developed by Mathews and Schwartz[17,18].

Columns prepared in this way, and with this phase, showed good efficiency (1700–1800/m theoretical plate efficiency) and were stable to about 320–350°. As noted by Grob and Grob[2], the properties exhibited by any column may be determined to some extent by the nature of the hardware design for injection and detection. We employed the injector design of German and Horning[19]. Further improvement in hardware design may be possible; columns prepared in this way can be used in many applications and with different hardware designs.

EXPERIMENTAL

Coating apparatus

The basic concept of the evaporation system is that of Ilkova and Mystryukov[16]. The oven was constructed of Maronite. A 27 × 1.3 cm cartridge heater was horizontally supported in the center of the oven; the temperature was regulated by a variable transformer. The feeding device employed an electric motor with a gear train which was attached to a steel shaft. A drive speed of 0.5–15 rpm was regulated by a variable transformer. Experience indicated that a rotational speed in the range of 0.5–3 rpm was best. The friction necessary for feeding the capillary column through the oven wall was supplied by a rubber roller. The glass capillary column feed was directed by a forked guide.

Coating procedure

A plug (about one third of the length of the column) of a suspension of 1% (w/v) Silanox 101 (Cabot, Boston, U.S.A.) in a solution of 0.25% (w/v) PZ-176 in chloroform was sent through the capillary column at a rate of 5 cm/sec. The film was dried with a stream of nitrogen gas passed through the column at room temperature for 3 h.

The column was then filled with a solution of 3.5% (w/v) PZ-176 and 5% (v/v) pentane in chloroform. One end of the column was sealed with sodium silicate solution. The column was suspended on the shaft outside the oven, and the open end was inserted into the oven.

The oven temperature was set at 87°, and the rotational speed of the shaft was adjusted to 0.75 rpm. After the evaporation process had been started, little supervision

was needed. When the last coil entered the oven, the motor was stopped and the sealed end was broken. A nitrogen stream was then passed through the column at room temperature for 3 h. The column was conditioned by heating in an F & M Model 400 gas chromatograph from room temperature to 300° at 1°/min.

Gas chromatography

This investigation was carried out with an F & M Model 400 gas chromatograph which was modified to accept capillary columns and an inlet system of the kind described by German and Horning[19]. The 1.5 cm of packing in the fore-column was 10% SE-30 on acid-washed and silanized Gas-Chrom P. The inlet gas flow was flow controlled, while the capillary column inlet was under pressure control. The capillary column gas flow-rate was 1–2 ml/min, with a splitting ratio of 10:1 to 20:1. Fore-column helium and flame hydrogen flow-rates were controlled by Brooks Model 8744 flow controllers and measured with Matheson Model 8110-0121 mass flowmeters. A Keithley Model 417 picoammeter and a Texas Instruments recorder were employed. The flame ionization detector was slightly modified to accept a capillary column. Other GC conditions included: sample volume, 5 μl; fore-column inlet splitter, 240°; detector bath, 280°; hydrogen flow-rate, 10 ml/min; air flow-rate, 30 ml/min.

Samples

Theoretical plate efficiencies were measured at 250° with 5α-cholestane and at 200° with methyl linoleate.

Samples of methyl esters of long-chain unsaturated fatty acids were PUFA 1 and PUFA 2 (Supelco, Bellefonte, Pa., U.S.A.).

The sample of human plasma free fatty acids as methyl esters, and containing α-tocopherol and cholesterol as trimethylsilyl ethers, was prepared by a modified Dole[20] procedure. Details will be reported in another paper.

A sample of Aroclor 1254 was obtained from Chromatographic Technology (Houston, Texas, U.S.A.).

RESULTS AND DISCUSSION

The analysis of complex mixtures of biologic origin by GC techniques is best carried out with high-resolution glass open tubular capillary columns, rather than with packed columns. There is general agreement on this point, but much less agreement about the most satisfactory way of preparing such columns. Moreover, since it is not always fully realized that hardware design for injection and detection may materially affect the observed column efficiency, although this was emphasized by Grob and Grob[2], it is difficult to evaluate procedures on the basis of reported theoretical plate efficiency alone. An additional problem is presented by the need for thermal stability in many applications; as Merle d'Aubigne *et al.* have pointed out[1], the chief reason for failure of glass capillary columns is break-up of the liquid film to form micro-droplets when the column is heated.

Several methods of converting a smooth glass surface to one with micro-irregularities have been described. These include the procedure of Grob and co-workers[2–5], the process evaluated by Novotný and Zlatkis[6,7], the soft-glass etching

method of Alexander *et al.*[8] and Alexander and Rutten[9], and a carbonization procedure of Liberti[21]. As indicated by Van Rijswick and Tesarik[22], etching or roughening of the glass surface leads to a liquid film which is of irregular thickness but which is stable and does not readily undergo film break-up when heated.

We have approached the problem of preparing thermostable, high-resolution glass open tubular columns in a different way. When very fine particles of silanized silicic acid (Silanox 101) are combined with a liquid phase, it is possible to obtain a coating that does not undergo film break-up when heated. The function of the glass tube is to provide a physical support for the coating; it should be smooth rather than rough. Etching procedures should not be used in this process. The glass surface should be silanized before coating with SE-30 (for deactivation purposes); when polar phases are used, the glass is not treated (since most polar phases will deactivate glass surfaces). The chief difficulty, when this method is used, is that of achieving a uniform distribution of the coating. The two-step procedure of German and Horning[10] is satisfactory for preparing SE-30 columns, and since a dynamic method is used in each step it is possible to make capillary columns of any desired length (we prefer 60-m columns with 100,000 or more theoretical plate efficiency for use with mixtures of biologic origin). Unfortunately, solvent combinations have not yet been found that permit the preparation of polar-phase columns in this way. One-step dynamic coating, as described by Blumer[23] and by Bertsch *et al.*[24] can be used, but in our experience with glass columns the coating is not as even as is desirable. Static evaporative coating leads to excellent columns[15], but the practical limit of column length is about 20 m; the time required for solvent evaporation from a 60-m column is prohibitive. Further study resulted in the development of a two-step procedure in which the first step was essentially the same as that of German and Horning[10]. Silanized silicic acid does not adhere to a glass surface, but when a small amount of liquid phase is employed as a binder it is possible to obtain a uniform distribution. The second step was based on the forced evaporation method of Ilkova and Mistryukov[16]. The entire column was filled with a solution of the liquid phase. A small amount of pentane was added to provide a very low-boiling constituent, and the coil was driven into a heated oven at a slow rate. The physical state of the coating is determined by the way in which the vaporization of the solvent is carried out. If the vaporization is erratic (due to superheating), the coating will be uneven; if the vaporization occurs at a constant rate and in a smooth way, the coating will be uniform or nearly so. Columns prepared in this way show 1700–1800/m theoretical plate efficiency. They do not deteriorate, and may improve, during use. We prefer 30- to 40-m columns for the separations described here.

Liquid phase PZ-176 is a phenyl ether–phenyl sulfone polymer developed by Mathews and Schwartz[17]. The useful temperature range is 180–320°, and the upper temperature may be extended to 350° with moderate increase in bleed rate. The selective retention of unsaturated compounds is less marked than is observed for the commonly used polyester phases, but the high thermal stability and the relatively high efficiency attainable with open tubular columns suggested that PZ-176 columns would be useful for analyses of long-chain saturated and unsaturated fatty acids. Ackman[25] pointed out in 1966 that open tubular columns were valuable for this purpose, but even now very few laboratories employ capillary techniques. Figs. 1 and 2 show separations of polyansaturated fatty acid methyl esters with PZ-176. Other

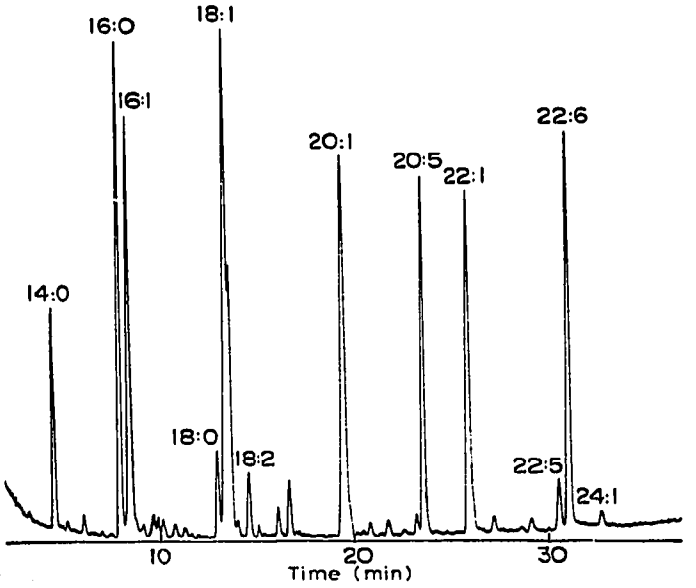

Fig. 1. Separation of polyunsaturated fatty acids (PUFA No. 1, marine source) as methyl esters with a 32 m × 0.25 mm PZ-176 glass capillary column (total theoretical plate number: 58,000 at 250° with 5α-cholestane). The sensitivity of the picoammeter was 3×10^{-11} A for full scale deflection. Temperature programmed at 2°/min from 180°.

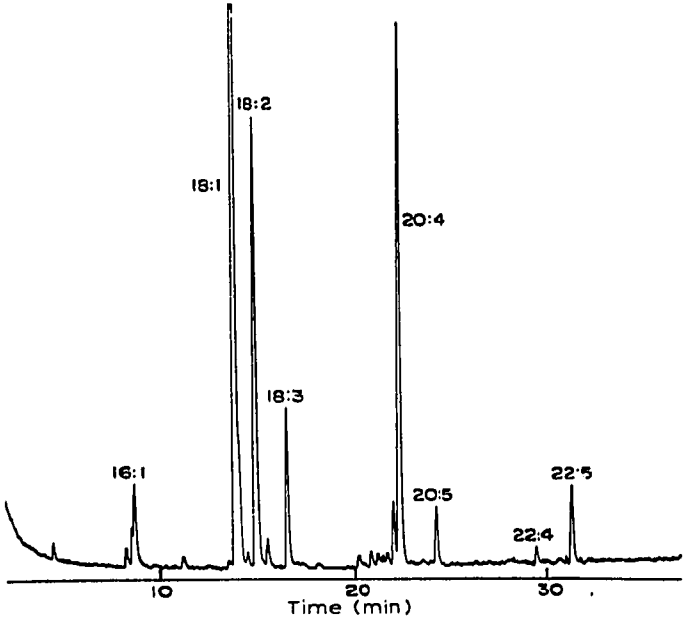

Fig. 2. Separation of polyunsaturated fatty acids (PUFA No. 2, animal source) as methyl esters with a 32 m × 0.25 mm PZ-176 glass capillary column. Temperature programmed as in Fig. 1.

phases give satisfactory results with C_{18} esters[26]; the advantage of PZ-176 lies in the possibility of extending the range of analysis to materials of much higher molecular weight. Fig. 3 shows a separation of plasma free fatty acids as methyl esters, and α-tocopherol and free cholesterol as trimethylsilyl (TMS) ethers, for an adult human. Details of the extraction method will be published separately. The illustration shows, however, the high capacity of the phase in accepting a large cholesterol component and the thermal stability of the phase in a temperature-programmed separation to 300°.

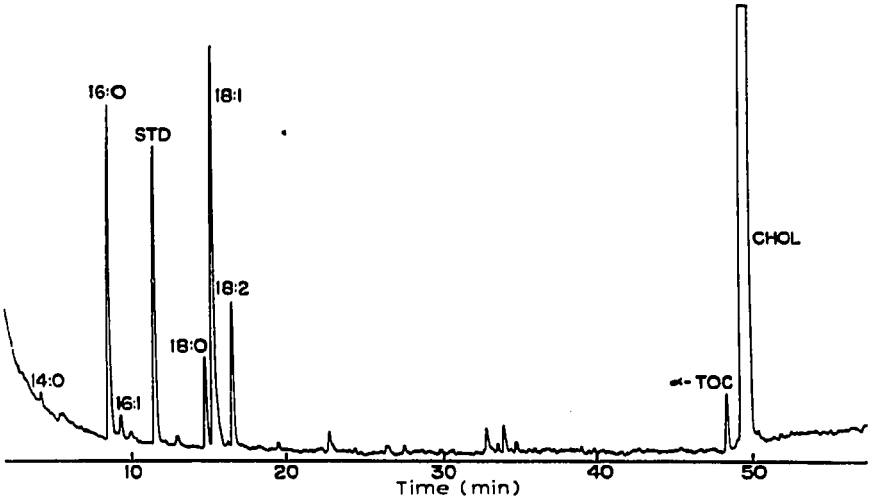

Fig. 3. Separation of plasma free fatty acids as methyl esters, and α-tocopherol and free cholesterol as TMS derivatives, for an adult human. The separation was carried out with a 32 m × 0.25 mm PZ-176 glass capillary column (total theoretical plate number: 58,000 at 250° with 5α-cholestane). The sensitivity of the picoammeter was 3×10^{-11} A for full scale deflection. Temperature programmed at 2°/min from 180°.

These columns may be particularly useful in studies of pesticides and toxic chlorinated aromatic compounds. Fig. 4 shows a temperature-programmed separation of Aroclor 1254. Comparisons with packed column separations[27,28] show the effectiveness of open tubular techniques and phase PZ-176 in separating closely related compounds in the polychlorobiphenyl series.

CONCLUSION

Continued use of glass open tubular capillary columns prepared with Silanox/liquid phase coatings, with either SE-30 or PZ-176 as the liquid phase, indicates that this concept of GC column preparation is entirely satisfactory. Etching of the glass surface is not necessary (and may even be undesirable) when this method is employed. Columns based upon etching or roughening of the interior wall also resist film break-up when heated, and further experience will be required to determine which type of process is most useful for the preparation of thermostable columns. A third type of procedure, involving the use of surface-active additives or chemical alteration of the

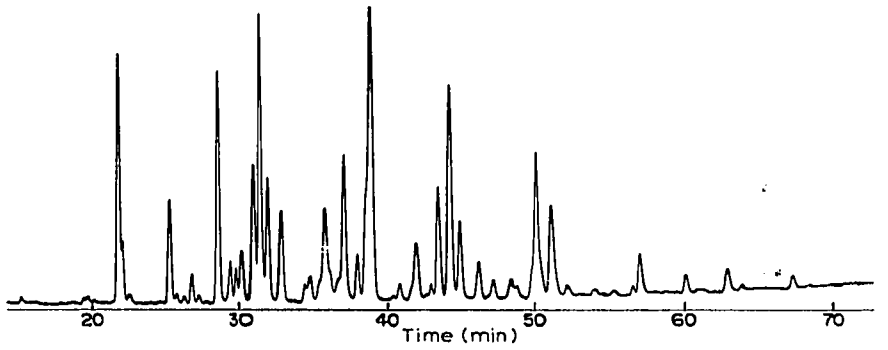

Fig. 4. Separation of Aroclor 1254 (polychlorobiphenyl series) with a 32 m × 0.25 mm PZ-176 glass capillary column temperature programmed at 1°/min from 200°.

glass to provide bonded "chains" which change the wettability of the surface, are not highly relevant at present to the work described here. This approach may be satisfactory for the preparation of columns of low capacity.

Two coating procedures have been developed. These are the two-step procedure of German and Horning[10], for SE-30 columns, and the procedure described here for PZ-176 columns. The high thermal stability of phase PZ-176 makes it possible to extend the range of polar separation capabilities to 320°.

ACKNOWLEDGEMENTS

We are indebted to Mr. A. G. Grill for his skill in design and construction of the oven for the evaporative process. This work was supported by Grant GM-13901 of the National Institute of General Medical Sciences, Grant HL-05435 of the National Heart and Lung Institute, and Grant Q-125 of the Robert A. Welch Foundation.

REFERENCES

1 J. Merle d'Aubigne, C. Landault and G. Guiochon, *Chromatographia*, 4 (1971) 309.
2 K. Grob and G. Grob, *Chromatographia*, 5 (1972) 3.
3 K. Grob and H. J. Jaeggi, *Chromatographia*, 5 (1972) 382.
4 K. Grob, *Helv. Chim. Acta*, 51 (1968) 718.
5 K. Grob, *Helv. Chim. Acta*, 48 (1965) 1362.
6 M. Novotný and A. Zlatkis, *J. Chromatogr.*, 56 (1971) 353.
7 M. Novotný and A. Zlatkis, *J. Chromatogr. Sci.*, 8 (1970) 346.
8 G. Alexander, G. Garzo and G. Polyi, *J. Chromatogr.*, 91 (1974) 25.
9 G. Alexander and G. A. F. M. Rutten, *Chromatographia*, 6 (1973) 231.
10 A. L. German and E. C. Horning, *J. Chromatogr. Sci.*, 11 (1973) 76.
11 A. L. German, C. D. Pfaffenberger, J.-P. Thenot, M. G. Horning and E. C. Horning, *Anal. Chem.*, 45 (1973) 930.
12 E. C. Horning, M. G. Horning, J. Szafranek, P. van Hout, A. L. German, J.-P. Thenot and C. D. Pfaffenberger, *J. Chromatogr.*, 91 (1974) 367.
13 J. Szafranek, C. D. Pfaffenberger and E. C. Horning, *J. Chromatogr.*, 88 (1974) 149.
14 J. Szafranek, C. D. Pfaffenberger and E. C. Horning, *Anal. Lett.*, 6 (1973) 479.
15 P. van Hout, J. Szafranek, C. D. Pfaffenberger and E. C. Horning, *J. Chromatogr.*, 99 (1974) 103.
16 E. L. Ilkova and E. A. Mistryukov, *J. Chromatogr.*, 54 (1971) 422.

17 R. G. Mathews and R. D. Schwartz, Research Engineering and Development Department, Pennzoil Co., Shreveport, La., personal communication.
18 R. G. Mathews, R. D. Schwartz, C. D. Pfaffenberger, S.-N. Lin and E. C. Horning, *J. Chromatogr.*, 99 (1974) 51.
19 A. L. German and E. C. Horning, *Anal. Lett.*, 5 (1972) 619.
20 V. P. Dole, *J. Clin. Invest.*, 35 (1965) 150.
21 A. Liberti, in A. B. Littlewood (Editor), *Gas Chromatography 1966*, Butterworths, London, 1966, p. 97.
22 M. H. J. van Rijswick and K. Tesarik, *Chromatographia*, 7 (1974) 135.
23 M. Blumer, *Anal. Chem.*, 45 (1973) 980.
24 W. Bertsch, F. Shunbo, R. C. Chang and A. Zlatkis, *Chromatographia*, 7 (1974) 128.
25 R. G. Ackman, *J. Amer. Oil Chem. Soc.*, 43 (1966) 483.
26 R. G. Ackman, A. Manzer and J. Joseph, *Chromatographia*, 7 (1974) 107.
27 E. O. Oswald, L. Levy, B. J. Corbett and M. P. Walker, *J. Chromatogr.*, 93 (1974) 63.
28 G. E. Bagley and E. Cromartie, *J. Chromatogr.*, 79 (1973) 219.