Journal of Chromatography, 99 (1974) 103–110

CHROM. 7570

THERMOSTABLE POLAR-PHASE OPEN-TUBULAR GLASS CAPILLARY COLUMNS

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SUMMARY

Borosilicate glass capillary columns coated with polar stationary phases have been successfully prepared by a two-step dynamic/evaporative method. When the liquid film is stabilized by a dispersion of fine particles of silanized silicic acid, the columns are both thermostable and long-lived. Typical column efficiencies are 1700 to 2400 theoretical plates per meter.

INTRODUCTION

Over the past few years there have been both rapid development and expanded utilization of open-tubular glass capillary columns coated with non-polar stationary phases including OV-1, OV-101, SE-30 and SF-96[1–6]. A method for preparing columns coated with SE-30 was recently reported by German and Horning[7]; this method, which involves a dynamic two-step process, creates a liquid film throughout which are dispersed fine particles of silanized silicic acid (Silanox). Columns prepared in this manner maintain film continuity during continual use and have been successfully employed to separate mixtures of urinary steroids[7], urinary acids[8], drug metabolites[9], sugar acids and lactones[10], and urinary polyols and aldoses[11,12].

Glass capillary columns coated with phases more polar than SE-30 are needed in some applications, but use of the dynamic method of coating has not led to satisfactory columns of this type. Alexander and Rutten[13] have pointed out that, although deactivation of the column wall appears less important in preparing polar columns, it is difficult to wet the walls with sufficient stationary phase to provide a continuous film that will maintain its integrity during continual use.

A recent approach to the preparation of polar columns has been to draw the capillaries from soft (soda-lime) glass and to etch the walls with anhydrous hydrogen chloride[13,14]. Such columns, when coated by the method of Bouche and Verzele[15], yield efficiencies of about 2000 theoretical plates per meter for *n*-alkanes.

Current work in the Soviet Union[16–18] involves the use of borosilicate glass which is treated overnight with dilute aqueous hydrofluoric acid, washed to neutrality with water, then washed successively with aqueous potassium hydroxide and methanol. The etched unsilanized column that results is coated by a procedure[16] involving forced evaporation. The capillary is filled with a solution of stationary phase

in a volatile solvent. Then it is slowly introduced into an oven that continuously evaporates the solvent, leaving a thin film of liquid phase on the capillary walls. From the published chromatograms[17], a 40 m × 0.25 mm I.D. column with a phenyl polysiloxane phase shows an efficiency of about 700 theoretical plates per meter for *n*-octane at 85°. Ilkova and Mistryukov[18] reported that treatment of the glass with aqueous hydrofluoric acid alone resulted in lowering the thermal stability of the column. Excessive treatment of the inner surface of the capillary with hot alkali led to rapid deterioration of column performance, especially towards separations of polar and high-molecular-weight compounds.

The approach used here was based on the view that borosilicate glass can be directly coated with polar phases containing a dispersion of Silanox. A two-step dynamic/evaporative method was found to provide thermostable polar-phase-coated open-tubular glass capillary columns exhibiting efficiencies (for methyl linoleate at 175°) of 1700 to 2400 theoretical plates per meter.

EXPERIMENTAL

Reagents

Stationary phase PZ-176 (ref. 19) was generously supplied by Drs. R. D. Schwartz and R. G. Mathews, Pennzoil Co., Shreveport, La., U.S.A. HI-EFF-8BP and SILAR-5CP were from Applied Science Labs., State College, Pa., U.S.A. OV-17 and SP-2401 were from Supelco, Bellefonte, Pa., U.S.A. Silanox Grade 101 was obtained from Cabot, Boston, Mass., U.S.A. Sodium silicate solution (40–42%) was technical grade from Fisher Scientific, Fair Lawn, N.J., U.S.A. Reference esters and sterols were purchased from Supelco.

Drawing the capillary columns

Capillaries were drawn using a Hupe-Busch glass drawing and coiling apparatus (now available from Hewlett-Packard). Novotný and Zlatkis[5] have recently described the general procedure. Pyrex tubes to be drawn were successively rinsed with acetone, methylene chloride, 1% aqueous potassium hydroxide, and methanol, and dried under vacuum. From a 1.25-m Pyrex tube (7.8 mm O.D., 3.8 mm I.D.), a 70-m capillary (1.0 mm O.D., 0.3 mm I.D.) was obtained. The diameter of the coil was 12 cm.

Coating the capillary columns

Twenty-meter lengths of unsilanized borosilicate glass capillaries were coated by a two-step dynamic/evaporative process. First, a plug consisting of Silanox dispersed in a dilute solution of polar phase in chloroform was forced through the column at a rate of 5–8 cm/sec. The dispersion was prepared by dissolving 0.1 g of polar phase in 100 ml of chloroform, then adding 1.0 g of Silanox 101 to the solution and sonicating the mixture at 35° for 15 min. Immediately after sonication a plug of about 1 ml was propelled through the capillary. (Before each subsequent use this mixture was again briefly sonicated.) The solvent was removed by 2 h of nitrogen flow through the column. A thin layer of the polar phase containing Silanox remained on the walls. (At this time the column appeared only slightly opaque.)

In the second step a solution of polar phase in acetone was prepared free of dissolved gases. To accomplish this, 72 mg of phase was dissolved in 40 ml of acetone;

then 10 ml of the solvent were removed under vacuum. The capillary was immediately filled with the solution using reduced pressure. Then one end of the column was warmed to expel a drop of the coating solution. As soon as this had occurred, this end was plunged into concentrated aqueous sodium silicate (water glass). As the capillary cooled, a small amount of sodium silicate solution entered the column. This technique led to a tight seal within an hour. The opposite end was connected to a vacuum pump, and solvent was slowly evaporated at room temperature, the rate being regulated by a variable air leak. A 20-m column required 36–48 h for solvent evacuation. (Too rapid an evaporation rate led to an uneven layer of phase.) Acetone was an excellent solvent for most of the polar phases studied. It exhibited an added advantage in that it extracted some water from the sodium silicate solution, thereby shortening the time required to form a tight seal. If a phase was not sufficiently soluble in acetone (*e.g.* HI-EFF-8BP), a plug of degassed acetone was introduced into the end of the column which was to be sealed. *Caution*: Avoid trapping air inside the capillary coils.

Conditioning the capillary columns

Columns were conditioned under carrier gas (nitrogen) flow by temperature programming 1°/min from 25° to 230° (HI-EFF-8BP), 250° (SILAR-5CP, SP-2401, OV-17) or 300° (PZ-176)[19]. Some columns were held briefly at the upper temperature limit to condition them more completely, but long isothermal periods at the upper temperature limit were avoided when possible.

Gas chromatography

Separations were carried out using a Tracor Model 550 gas chromatograph which had been modified to include a previously described glass inlet system[20] and to accept glass capillary columns (20 m × 0.3 mm I.D.). A Fisher Recordall Series 5000 recorder was employed. Hydrogen was supplied by a Hewlett-Packard Model 18591A hydrogen generator. The flame hydrogen flow-rate was controlled by a Brooks Model 5840 flow controller. All other gas flows were controlled by Brooks Model 8744A flow controllers and measured with Matheson Model 8110-0121 mass flowmeters. The flame ionization detector was modified (see next section) for use with glass capillary columns.

Methylene unit (MU) values were measured through use of *n*-alkanes co-injected with the sample using an initial temperature of 140° and programming at 1°/min. Other gas chromatographic conditions included: sample volume, 0.5 μl; split ratio, 5:1; temperature of precolumn-inlet splitter, 270°; detector bath temperature, 300°; column inlet pressure, 10 p.s.i., resulting in a carrier gas (helium) linear velocity of 18–20 cm/sec (200°); hydrogen flow-rate, 37.5 ml/min; air flow-rate, 200 ml/min; nitrogen make-up gas to the detector, 80 ml/min.

Modified flame ionization detector

The Model 12016 flame ionization detector (standard equipment on the Tracor Model 550 gas chromatograph) is a highly satisfactory detector, but it cannot be expected to respond to small volumes of gases flowing at slow rates. The suggested carrier gas flow-rate through a 6 ft. × 0.25 in. packed column is 60 ml/min, and the recommended burner flow-rates are 40 ml/min hydrogen and 330 ml/min air.

Carrier gas (helium) flow-rate through an open-tubular glass capillary column is rarely more than 2 ml/min. It was therefore necessary to modify the detector as indi-

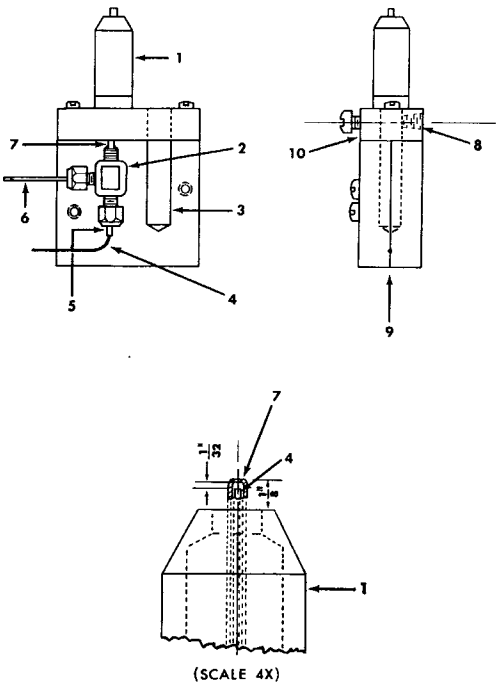

Fig. 1. Modified flame ionization detector: (1) air nozzle through which flame nozzle protrudes 1/8 in., (2) 1/16-in. stainless-steel Swagelok union tee, (3) 100-W cartridge heater, (4) 1/32 in. O.D. (0.020 in. I.D.) stainless-steel tube leading from column in oven to within 1/32 in. of flame nozzle, (5) 1/16-in. stainless-steel aligning tube silver soldered into place on detail "4", (6) fuel gas (hydrogen–nitrogen mixture) inlet, (7) 15-gauge stainless-steel hypodermic needle tubing having a constricted flame nozzle made by rolling the end closed, then drilling out to 0.020 in. I.D. and silver soldering into detail "2", (8) 1/16-in. pipe thread for air inlet, (9) 1 × 2¼ in. split aluminum block, (10) 1/2 × 1 × 2¼ in. aluminum bar.

cated in Fig. 1. The flame housing assembly (not shown) and the original air nozzle (1) were mounted on a heated aluminum split block (9, 10) which housed a union tee (2). This tee permitted the alignment of the two concentric tubes (4, 7) through the air nozzle (1) and made it possible to introduce sufficient make-up gas (nitrogen) into the flame. Through this arrangement, the column effluent (4) was introduced into a mixture of hydrogen and nitrogen (through inlet 6) just above the air nozzle.

RESULTS AND DISCUSSION

The objective of this work was to develop a general method for preparing thermostable and long-lived polar-phase open-tubular glass capillary columns with high theoretical plate efficiency.

Five representative stationary phases employed in this work are listed in Table I. PZ-176, a polyphenyl ether sulfone, is a new phase developed by Drs. R. D. Schwartz and R. G. Mathews. The most outstanding property of this phase is its thermal stability. HI-EFF-8BP is a relatively thermally stable polyester phase. SILAR-

TABLE I

STATIONARY PHASES INCLUDED IN THIS STUDY

Liquid phase	*Chemical composition*	*Supplier*	*$T_{max.}$* (°C)*	*Solvent***
PZ-176	Polyphenyl ether sulfone	not commercial***	300	A
HI-EFF-8BP	Cyclohexanedimethanol succinate	Applied Science Labs.	230	MC
SILAR-5CP	Cyanoalkylphenyl silicone	Applied Science Labs.	250	A
OV-17	Methylphenyl silicone	Supelco	250	A
SP-2401	Trifluoropropylmethyl silicone	Supelco	250	A

* $T_{max.}$ is the recommended upper temperature limit to prolong column lifetime.

** The solvent chosen, either acetone (A) or methylene chloride (MC), was the one most suitable for this method of coating.

*** Inquiries should be addressed to Drs. R. D. Schwartz and R. G. Mathews, Research Engineering and Development Department, Pennzoil Company, Shreveport, La. 71106, U.S.A.

5CP, OV-17 and SP-2401 are known to exhibit different polarities and thermal stabilities depending on the nature of the substituent groups attached to the parent polysiloxane chain[21].

Borosilicate glass capillary columns (not silanized) coated with each of these phases (containing Silanox) were prepared according to the two-step dynamic/evaporative method described here. The maximum temperature of use for each phase is given in Table I. The PZ-176 column was used routinely to 300° for three months without appreciable deterioration. Analyses with this column included both fatty acid methyl esters (FAME) and various sterols as trimethylsilyl (TMS) derivatives. The HI-EFF-8BP and OV-17 columns were used for several weeks to perform FAME analyses with no indication of deterioration. Columns of SILAR-5CP and SP-2401 were used for several months without the development of trailing peaks or loss of efficiency.

Figs. 2–4 are typical FAME analyses on, respectively, PZ-176, HI-EFF-8BP and SILAR-5CP polar phase/Silanox coated glass capillary columns. Theoretical plate efficiencies for these three columns were found to be 1750, 2400 and 2200 theoretical plates per meter, respectively. Columns of OV-17 and SP-2401 exhibited similar values: 1700 and 2000, respectively.

Fig. 5 shows a separation of TMS derivatives of the four isomeric cholestanols (1–4) and stigmasterol (5) plus an internal reference compound (6), cholesteryl butyl ether, on a 20-m column of PZ-176 temperature programmed 1°/min from 200°.

Table II includes MU values from FAME analyses on the columns included in this study. A value ΔMU, defined as the MU value for methyl linolenate minus the MU value for methyl stearate, was used as an indication of column polarity. This led to an order of decreasing polarity: PZ-176 > HI-EFF-8BP $\geqslant$ SILAR-5CP > OV-17 > SP-2401 towards unsaturated fatty acid methyl esters. Only the 20-m column of PZ-176 exhibited a sufficient separation of methyl stearate from methyl oleate to be useful in this application. Current studies in our laboratories involve the preparation and use of 60-m columns of PZ-176/Silanox in fatty acid analyses..

The reproducibility of this two-step dynamic/evaporative method is high provided both steps are carefully performed. The dynamic step deposits a uniform layer

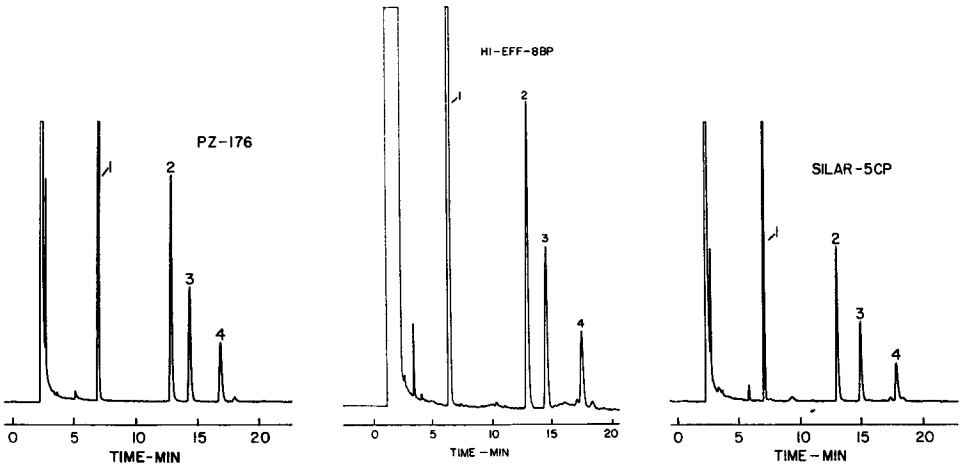

Fig. 2. Isothermal (175°) FAME analysis on a 20-m PZ-176 open-tubular glass capillary column: (1) methyl palmitate ($C_{16:0}$), (2) methyl oleate ($C_{18:1}$), (3) methyl linoleate ($C_{18:2}$) and (4) methyl linolenate ($C_{18:3}$).

Fig. 3. Isothermal (175°) FAME analysis on a 13-m HI-EFF-8BP open-tubular glass capillary column: (1) $C_{16:0}$, (2) $C_{18:1}$, (3) $C_{18:2}$ and (4) $C_{18:3}$.

Fig. 4. Isothermal (175°) FAME analysis on an 18-m SILAR-5CP open-tubular glass capillary column: (1) $C_{16:0}$, (2) $C_{18:1}$, (3) $C_{18:2}$ and (4) $C_{18:3}$.

of Silanox that is firmly held in place by a small amount of stationary phase. Low concentrations of both the dissolved phase and the dispersed Silanox are used. The low viscosity of such a dispersion aids in depositing a layer of Silanox without plugging. The evaporative step is generally straightforward when degassed solutions are drawn into the capillary. In fact, gas trapped in the capillary is the only inherent source of trouble in this coating procedure. Apparently, filling the column with a solution of

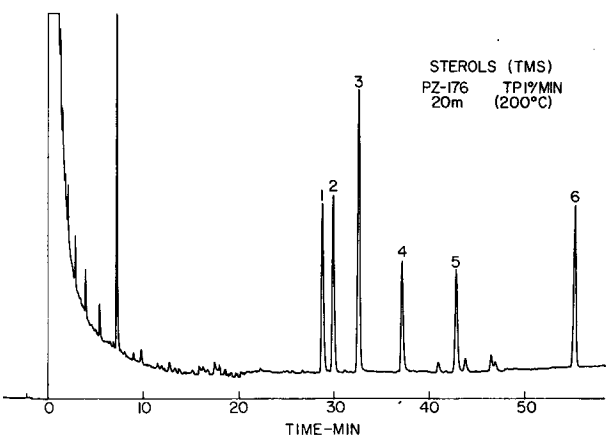

Fig. 5. Separation of the trimethylsilyl (TMS) derivatives of four isomeric cholestanols and stigmasterol plus an internal reference compound on a 20-m PZ-176 open-tubular glass capillary column temperature programmed 1°/min from 200°: (1) 5β-cholestan-3β-ol, (2) 5β-cholestan-3α-ol, (3) 5α-cholestan-3α-ol, (4) 5α-cholestan-3β-ol, (5) stigmasterol and (6) cholesteryl butyl ether.

TABLE II

MU VALUES FROM FAME* ANALYSES ON VARIOUS POLAR-PHASE GLASS CAPILLARY COLUMNS

Liquid phase	*Theor. plates/m***	*MU values****					*ΔMU*§
		$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	
PZ-176	1750	21.25	23.30	23.35	23.69	24.18	0.88
HI-EFF-8BP	2400	21.17	23.27	23.25	23.57	24.09	0.82
SILAR-5CP	2200	21.07	23.01	23.08	23.36	23.81	0.80
OV-17	1700	20.19	22.18	22.07	22.18	22.40	0.22
SP-2401	2000	21.19	22.93	22.93	23.04	23.04	0.11

* Methyl palmitate, methyl stearate, methyl oleate, methyl linoleate and methyl linolenate.

** Determined isothermally at 175° for methyl linoleate except for SP-2401 when methyl linolenate was used.

*** Determined using C_{20}–C_{26} *n*-alkanes and temperature programming at 1°/min from 140°.

§ MU ($C_{18:3}$) − MU ($C_{18:0}$).

liquid phase in an organic solvent does not appreciably disturb the initially deposited Silanox layer. Longer columns require significantly longer periods for solvent evacuation, about one week being required to prepare a 60-m column.

Capillary columns were also prepared from soft (soda-lime) glass which was etched with anhydrous hydrogen chloride prior to the evaporative coating step (no Silanox was used). Columns exhibiting high efficiencies were obtained using several different stationary phases. Fig. 6 illustrates an isothermal (175°) FAME analysis on one such column (20 m of SILAR-5CP exhibiting an efficiency of 2700 theoretical plates per meter for methyl linoleate). Unfortunately, we found that these columns were short-lived, lasting two weeks at most.

ACKNOWLEDGEMENTS

We are indebted to Mr. A. G. Grill for his aid in the modification of the flame ionization detector, and for the drawing used in Fig. 1.

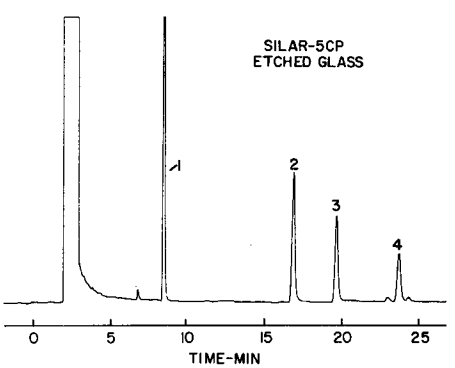

Fig. 6. Isothermal (175°) FAME analysis on a 20-m SILAR-5CP open-tubular HCl-etched soft glass capillary column exhibiting an efficiency of 2700 theoretical plates per meter for methyl linoleate: (1) $C_{16:0}$, (2) $C_{18:1}$, (3) $C_{18:2}$ and (4) $C_{18:3}$.

This work was aided by Grant GM-13901 and Contract NIH-69-2161 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.

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