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Note

Coating support-coated open tubular capillary columns with Silar 10C or Alltech CS-10 and Silica T40 for separation of isomers of fatty acid methyl esters

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Successful coating of capillary columns, especially with highly polar stationary phases, is a difficult operation for many laboratories[1]. There are experimental variables still not well defined and/or not well understood[2,3]. Grob and Grob[1] have evaluated the existing procedures and have established some simplified general rules as a guide. These rules may require some adaptation to suit individual requirements.

In this laboratory, support-coated open tubular (SCOT) capillary columns were coated with Silar 10C or Alltech CS-10 on solid supports of Chromosorb R-6470-1, Silanox 101, hydrochloric acid washed Silanox 101 or silica T40. Only columns containing Silica T40 as solid support produced satisfactory separation of positional and geometrical isomers of fatty acid methyl esters.

EXPERIMENTAL

Soda-glass (Eigentum Schott-Ruhrglass, Bayreuth, G.F.R.) columns (100 m × 0.45 mm I.D.; coil diameter 130 mm) were used. The columns were washed with methanol, chloroform, diethyl ether and acetone (*ca.* 20 ml of each solvent), thoroughly dried under a stream of nitrogen and coated immediately to avoid exposure to the air. Columns soaked overnight with 50% aqueous hydrochloric acid and then washed with water, methanol and acetone were also used.

A column was placed horizontally in a small container made of foamed polystyrene insulation. The top end of the column was connected with heat-shrinking tubing to the coating reservoir, and the other end to a buffer column (15–20 m) in order to maintain the same coating speed in the last part of the column[4]. A lid was placed on the container to ensure a uniform temperature[5] throughout the column for steady coating speed and uniform solvent evaporation and film spreading. The column was coated at room temperature (approximately 25°C).

Silica T40, a pure silicon dioxide of particle size 5–30 nm (Wacker-Chemie, München, G.F.R.) was used for the solid support. Approximately 0.2 g of support was weighed into a 20-ml vial, 8 ml of acetone was added, and the suspension was sonicated for 1–1.5 h in an ultrasonic bath (Bransonic 52; Branson Cleaning Equipment, Stamford, CT, U.S.A.). During this time, the vial was taken out of the ultrasonic bath four or five times, and the suspension was decanted into a new vial to

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remove the particles that had conglomerated on the wall of the vial above the suspension. Then 0.8 g of Silar 10C (Applied Science Labs., State College, PA, U.S.A.) or Alltech CS-10 (Alltech, Arlington Heights, IL, U.S.A.) was added, and the suspension was further sonicated for *ca.* 30 min. After standing, the suspension was stable for some days. Suspensions of Silanox 101, a hydrophobic silicon dioxide of average particle size 7 nm (Cabot Corp., Boston, MA, U.S.A.) and hydrochloric acid washed Silanox 101 (soaked overnight in 50% aqueous hydrochloric acid and washed with water, methanol and acetone) behaved in the same way as the suspension of silica T40. However, Chromosorb R-6470-1 (Applied Science Labs.) of particle size 1–4 μm did not stay suspended for more than a few minutes.

The column was coated dynamically[6]. A wetting plug of acetone (3 ml), followed by the slurry (8 ml), was introduced into the column under pressure of nitrogen. Both plugs were pushed through the column at a slow coating speed of 1–1.5 cm/sec and a nitrogen pressure of 100–150 kPa. A fast coating speed of 8–10 cm/sec and a pressure of 350–400 kPa were also used for producing a thicker film[7].

The solvent was evaporated slowly from the slow-coated columns at 100–150 kPa. Fast solvent evaporation[8] was applied for fast-coated columns at 350–400 kPa. The evaporation continued for 3–4 h.

The column was conditioned overnight by raising the oven temperature at 0.2–0.3°C/min to 195°C with a carrier-gas flow-rate of 1–2 ml/min.

A Packard Model 7401 gas chromatograph with helium as carrier gas was used for the GLC analyses; it was fitted with a flame-ionisation detector and was adapted for capillary columns with a splitless injection system.

Carbon disulphide was used as the sample solvent. Sample-solution sizes were 0.04–0.06 μl, and the concentration was 10–20 mg/ml.

RESULTS AND DISCUSSION

The object of this work was to produce, for our own use, highly polar SCOT capillary columns capable of separating isomeric fatty acid methyl esters; several solid supports and techniques were tested.

Columns washed with aqueous hydrochloric acid before coating were not better than non-washed columns, but acid-washed Silanox 101 (made hydrophilic) produced more efficient columns than when Silanox 101 (hydrophobic) itself was used; in both instances, the columns had short useful lives[9,10]. Although the hydrophobic properties of Silanox 101 are considered unsuitable[11] for use with polar phases, it has been reported[12] that Silanox 101 and polar phases can be directly coated on borosilicate glass. Chromosorb R-6470-1 produced unevenly coated columns with low efficiency, probably because of its large particle size. Silica T40, hydrophilic and with a small particle size, proved to be the most suitable solid support for even coating.

Acetone was found to be a better solvent for dispersing silica T40 than was chloroform or acetonitrile. Other workers[12] report that acetone is an excellent solvent for polar phases, and this was confirmed by our experiences. Slow coating of silica T40 slurry in acetone and slow evaporation greatly improved the uniformity of the liquid film. Columns produced as described had a useful life of *ca.* 2 months at an oven temperature of 170–180°C (ref. 12).

Compared with the other solid supports tested, Silica T40 gave the best

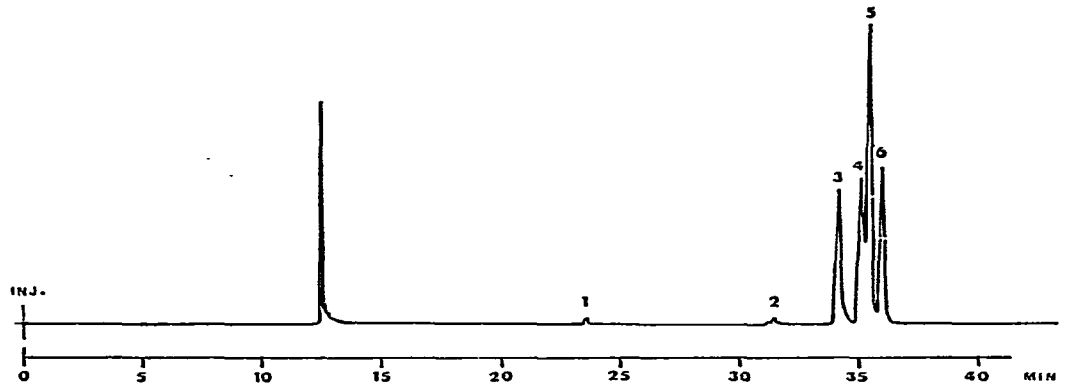

Fig. 1. Gas chromatogram of a mixture of fatty acid methyl esters. Peaks: 1 = palmitate; 2 = stearate; 3 = 9-*trans*-octadecenoate; 4 = 6-*cis*-octadecenoate; 5 = 9-*cis*-octadecenoate; 6 = 11-*cis*-octadecenoate. Column coated with Alltech CS-10 and silica T40. Carrier gas flow-rate, 3 ml/min. Oven, injection and detector temperatures 180, 260 and 280°C, respectively.

performance. An efficiency of 60,000–70,000 effective theoretical plates for methyl oleate was regularly attained and satisfactory separation of the four methyl octadecenoates (9-*trans*, 6-*cis*, 9-*cis* and 11-*cis*) was achieved (Fig. 1). Considering also that it takes only 1 day to produce a column, the procedure can be recommended to those workers who prefer to make their own columns.

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