Journal of Chromatography, 147 (1978) 388–394

CHRGM. 10,432

Note

Some observations on the preparation of support-coated open-tubular capillary columns using Silanox*

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(Received June 17th, 1977)

The preparation of support-coated open-tubular (SCOT) capillary columns has been reported from many different laboratories[1–3]. The support has consisted of a wide variety of materials including fumed silica[4,5], graphite[6], sodium chloride[7], and finely sized diatomaceous earth[8] and the resultant columns have often been of excellent quality, having good resolving power and high temperature stability. By and large the dynamic method of coating the columns has been favoured. Nevertheless, there are reports of difficulties encountered in attempting to repeat published work[8], and the preparation of porous-layer open-tubular (PLOT) columns and, in particular of the support layer, still contains an element of art. The inability to reproduce high quality columns, either inter-laboratory or intra-laboratory, usually results from inadequately defined experimental variables. For instance, in several procedures the suspension of the support requires to be sonicated but the time, temperature and method of sonication have not always been reported[8,9]. In contrast, Goretti *et al.*[6] reported that "irradiation conditions are not critical and can be widely modified" when colloidal suspensions of graphite are being prepared. The temperature at which the coating procedure is performed has also been frequently omitted, a significant omission considering the importance of the viscosity of the suspension in determining the flow-rate of the support suspension through narrow capillaries.

We also have experienced some difficulties in reproducing methods for the preparation of PLOT capillary columns most specifically in maintaining uniform flow while coating glass capillary columns with Silanox 101 suspensions in chloroform. On occasion we have observed complete blockage of the columns as noted also by Deelder *et al.*[8]. Considerable improvement was achieved by using a mixture of chloroform and acetone (9:1, v/v) to suspend the support but erratic flow still occurred. Sonication also improved the homogeneity of the Silanox suspensions but did not entirely solve the problem. We have explored some of the causes of this difficulty and herein report our findings.

Silanox supported columns have been very successfully used for steroid analysis. We have also evaluated their applicability to the analysis of volatile derivatives of amino acids.

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* Issued as NRCC No. 16286.

EXPERIMENTAL

Chemicals

Silanox 101 was obtained from Alltech (Arlington Heights, Ill., U.S.A.). This product is now marketed as Tullanox SP available from Tulco (North Billerica, Mass., U.S.A.). Chromosorb R 6470-1 was obtained from Applied Science Labs. (State College, Pa., U.S.A.).

Column preparation

All columns were prepared either from Pyrex or Duran 50 glass (Schott-Ruhrglass, Bayreuth, G.F.R.) which was cleaned using the procedure of Jennings *et al.*[10]. Capillaries (0.3–0.4 mm I.D. × 20 m) were constructed using a Hupe & Busch Model 1045A capillary drawing machine (Hewlett-Packard, Palo Alto, Calif., U.S.A.). Prior to coating, the columns were deactivated either by silylation[1] or by benzyltriphenyl phosphonium chloride (BTPPC)[11,12]. Column coating was initially performed as described by German and Horning[4] using either SE-30 or OV-101 as a stationary phase. Later, however, the suspension of Silanox 101 in chloroform was sonicated[8]. All coating operations were performed at 22° in a draft-free enclosure.

Silanox 101 was fractionated using an Allen Bradley sonic sifter and columns were prepared using both the unsieved material and a fraction of particle size smaller than 38 μm. Columns were also prepared using silanized[13] Chromosorb R6470-1 as a support (Applied Science Labs.).

Chromatography

All gas chromatography was performed using a Finnigan Model 9500 gas chromatograph equipped with flame ionisation detectors and a Grob injector. Helium was used as the carrier gas and as a make-up gas.

Sonication

Suspensions of Silanox 101 in chloroform (1 g per 100 ml) were sonicated at 25° either in an ultrasonic cleaning bath (Branson automatic cleaner) or in a rosette cell using the probe of a Branson Model S-125 sonifier (Branson, Danbury, Conn., U.S.A.). Sonication time was varied. Small aliquots of the resultant suspensions were allowed to dry on a microscope slide and were photographed.

Amino acid derivative preparation

The N-heptafluorobutyryl (HFB) isobutyl esters of the protein amino acids were prepared as previously described[14,15].

RESULTS AND DISCUSSION

Difficulties in maintaining consistent flow while coating 0.3 mm I.D. glass capillary columns with suspensions of Silanox 101, a difficulty also observed by Deelder *et al.*[8], prompted us to examine the particle size distribution of the Silanox. A visual examination of the dry material using a Nikon Model 6C microcomparator revealed the presence of particles or aggregates greater than 300 μm (Fig. 1a) although it has been reported[4] that Silanox does not self aggregate. This observation also prompt-

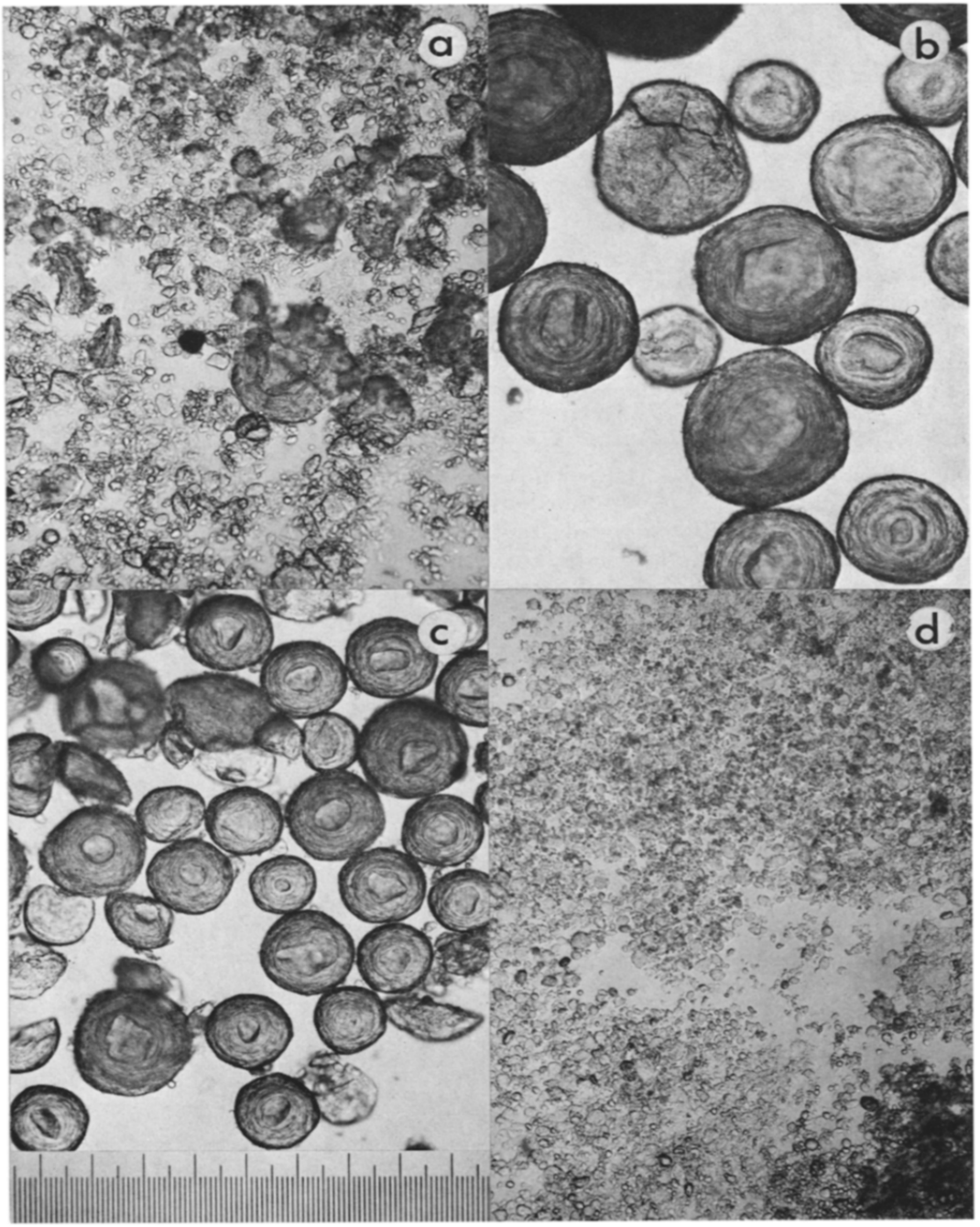

Fig. 1. Photomicrograph of Silanox 101 and sieved fractions (see Table I). (a) Total Silanox 101; (b) fractions IV + V; (c) fraction III; (d) fraction I. Magnification indicated by scale in which each division represents 10 μm.

ed us to sieve the Silanox with the results shown in Table I and Figs. 1b–d. Clearly, in the dry Silanox at least, there are particles or aggregates sufficiently large to cause flow problems in a capillary of only 300 μm I.D. and identical properties were observed for a batch of Tullanox SP.

TABLE I

FRACTIONATION OF SILANOX 101

Parameter	*Fraction*				
	I	*II*	*III*	*IV*	*V*
Particle size (μm)	<38	38–106	106–250	250–420	>420
Weights % of total	12.4	19.9	45.9	18.3	3.0

The critical question to be answered is whether or not the large particles observed in the dry state existed in a chloroform suspension but attempts to observe and measure Silanox particles in suspension using a conventional microscope were unsuccessful presumably because of similarity of refractive indices but possibly also because of "disaggregation" of the particles to the nominal primary particle size of 0.7 μm. Therefore, samples of the suspension were allowed to air-dry on a microscope slide with the result illustrated in Fig. 2a. Large particles or aggregates up to 100 μm in diameter are clearly in evidence and the possibility remains that larger particles were present in other areas since the search of the field was not exhaustive. These aggregates could result either from their being present in the original dry material and not dispersing in the chloroform or from the reaggregation of Silanox particles during the drying process. Since flow problems occurred with the suspensions, the former possibility seems more likely.

Following sonication of Silanox suspensions in an ultrasonic cleaning bath for varying times aliquots of the suspension were dried and examined as above. After 10 min sonication, large particles similar to those in the control sample were clearly present (Fig. 2b). Increasing sonication up to 1 h resulted in surfaces which became more uniform (Figs. 2c and 2d). The "crazy paving" effect in Fig. 2d and to a lesser extent in Fig. 2c is an artefact of drying. However, in all cases, and even after 1 h some particles up to 40 μm in diameter and of the type present in the control sample (Fig. 2a) were observed. Direct transmission of the ultrasonic energy using a probe was much more effective in producing a uniform surface and in minimising flow problems while coating (Fig. 2e). Some of the variation in the appearance of the surface is doubtless due to fields of different thickness being observed because it was particularly difficult to apply uniform amounts to the slides and to create uniform drying conditions. However, our primary concern is not with the nature of the surface *per se* but whether the large particles observed in the dry Silanox were readily disintegrated on sonication. Clearly, this is not so and thus we suggest that the presence of aggregates is largely responsible for the difficulties observed in obtaining uniform flow-rates when applying Silanox suspended in chloroform to open-tubular capillary columns.

Capillary columns were also coated using a fraction of the total Silanox (< 38 μm) and, either with or without sonication, uniform flow of the suspension through the column was easily obtained.

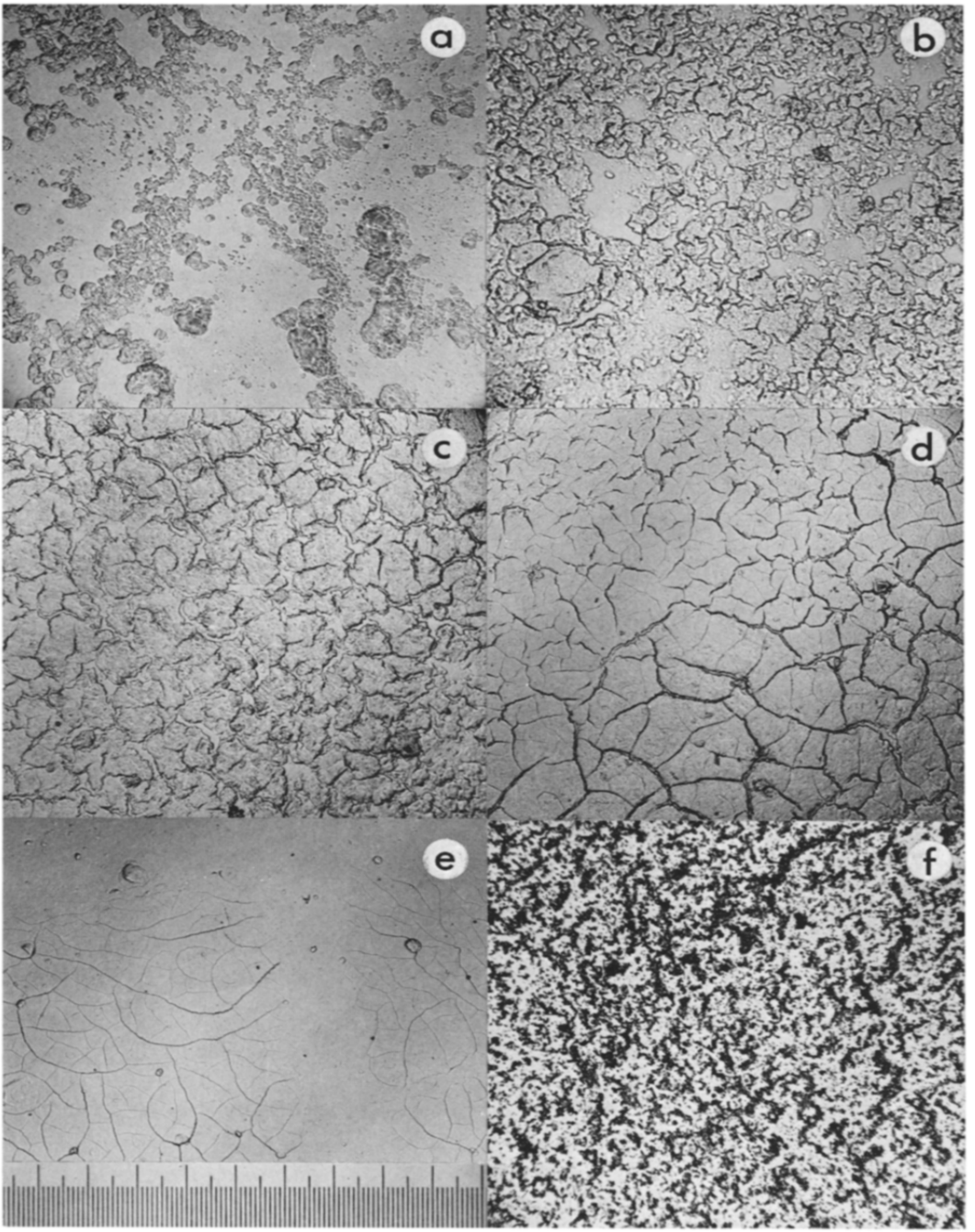

Fig. 2. Photomicrographs of sonicated suspensions of Silanox 101 in chloroform (1%, w/v) after drying (a) control sample, not sonicated, and (b), (c) and (d) suspension sonicated in an ultrasonic bath for respectively 10, 25 and 60 min, (e) suspension sonicated for 5 min using an ultrasonic probe and (f) suspension of Chromosorb R, not sonicated. Magnification indicated by scale in which each division represents 10 μm.

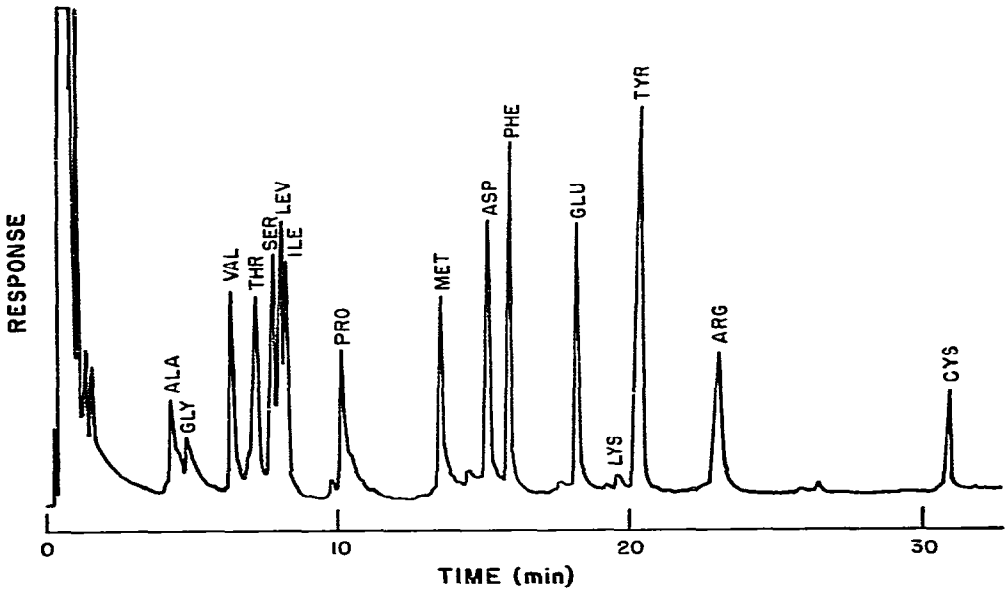

Fig. 3. Chromatograph illustrating separation of N-heptafluorobutyryl isobutyl amino acids using a capillary column (0.3 mm × 20 m) of SE-30 supported on Silanox 101. Starting temperature was 80° and the temperature program rate was 4°/min.

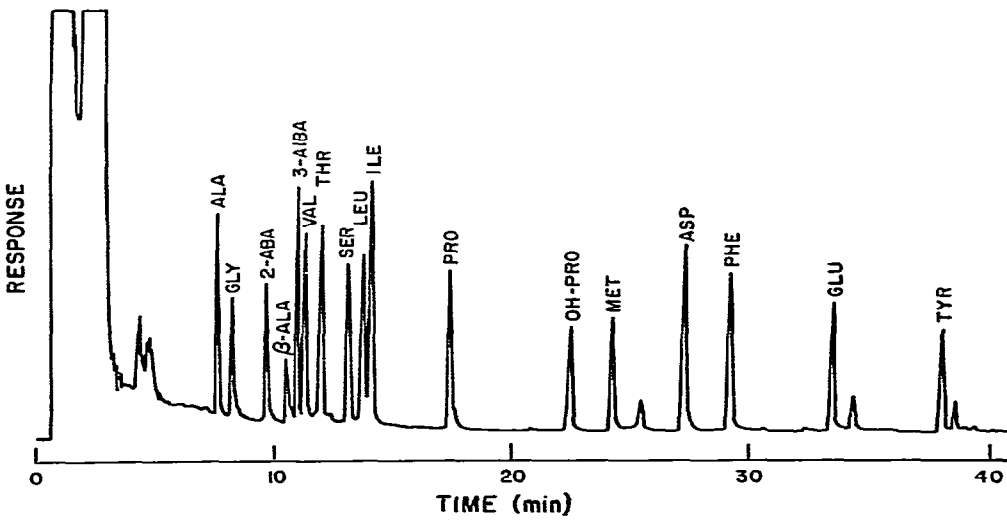

Fig. 4. Chromatograph illustrating separation of N-heptafluorobutyryl isobutyl amino acids using a capillary column (0.3 mm × 20 m) of SE-30 supported on Chromosorb R. Starting temperature was 90° and the temperature program rate was 2°/min. 2-ABA = 2-amino butyric acid; 3-AIBA = 3-amino isobutyric acid.

Treatment of the glass with BTPPC was found to deactivate the glass sufficiently to give acceptably shaped peaks using dodecyl alcohol and tetracosane confirming the results of Rutten and Luyten[12] and, being much more rapid and convenient than silylation and apparently more effective, was used routinely in all column preparations.

Short (20 m) capillaries prepared using a Silanox support layer were evaluated for their suitability for the analysis of protein amino acids as the N-heptafluorobutyryl isobutyl esters[14,15]. The results generally were unsatisfactory because of excessive tailing (Fig. 3) which could not be removed by silylation of the column. Furthermore, there was extensive loss of arginine. The tailing was not the result of excessive dead volume in the column system because in an identical configuration columns prepared using Chromosorb R as a support were much more satisfactory (Fig. 4). Furthermore, the separation, as expected was much superior to that obtained on 10–12-ft. packed columns[14]. For instance, complete baseline separation of alanine and glycine was obtained and the resolution of valine and 3-amino isobutyric acid was excellent. The latter pair of amino acids could not be separated on packed columns.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Dr. R. H. Haskins in taking the photomicrographs and of B. Godwin for technical assistance.

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