

CHROM. 12,449

## ALUMINA AND SUPPORT-COATED OPEN-TUBULAR COLUMNS IN OPEN-TUBULAR MICRO-CAPILLARY LIQUID CHROMATOGRAPHY\*

K. HIBI\*\* and D. ISHII

*Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya-shi 464 (Japan)*

and

T. TSUDA

*Laboratory of Analytical Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya-shi 466 (Japan)*

(First received September 4th, 1979; revised manuscript received October 5th, 1979)

---

### SUMMARY

New methods have been developed for preparing columns in open-tubular micro-capillary liquid chromatography. Layers of aluminium oxide powder were formed on the inner walls of capillary tubing, and then the tubing was drawn into a micro-capillary column. Alternatively, alumina or silica (particle diameter 10–40 nm) was dispersed on the walls of a micro-capillary and used as solid adsorbent or support material for further treatment with octadecylsilane, respectively.

---

### INTRODUCTION

Open-tubular micro-capillary liquid chromatography (OMCLC) may be one way of achieving high efficiency in terms of theoretical plates in liquid chromatography<sup>1-5</sup>. Although micro-capillary liquid chromatography is now in the initial stage of development, the recent results are suggestive of its potential applicability. One of the problems in OMCLC is how to get very efficient and stable columns with different selectivities. Although techniques which have been established in open-tubular capillary gas chromatography (GC) are basically also applicable to micro-capillary liquid chromatography, this is not always possible owing to the fact that the liquid mobile phase is more able to carry the large particles or detach the stationary phase than the gaseous mobile phase and that the inner diameter of a micro-capillary is very small, e.g., about one fifth that used in GC.

---

\* Series Part V; for Part IV, see ref. 8.

\*\* Present address: Japan Spectroscopic Co. Ltd., 2967-5, Ishikawa-cho, Hachioji-shi, Tokyo, Japan.

In this study we attempted to make columns of solid adsorbent coated on a support, using methods similar to those employed in GC<sup>9,10</sup>. Thus, when a hydrophobic colloidal silica was coated onto stainless-steel capillary tubing, the resulting column gave excellent results in hydrocarbon separations in GC<sup>9</sup>. Similar work was done by Horning *et al.*<sup>10</sup> who used silanized silica particles (Silanox<sup>®</sup>, diameter 6–10  $\mu\text{m}$ ) for making a support-coated capillary column (SCOT). In liquid chromatography pellicular column materials<sup>11,12</sup> were made by the deposition of silica (particle diameter 20 nm) on glass beads. Special care should be paid to the adhesion process to the surface shell of glass beads. Although this case is not one of treating the open-tubular glass capillary, it is of interest in the preparation of alumina and SCOT columns in OMCLC.

Modifications of the above techniques have been successfully applied in OMCLC.

## EXPERIMENTAL

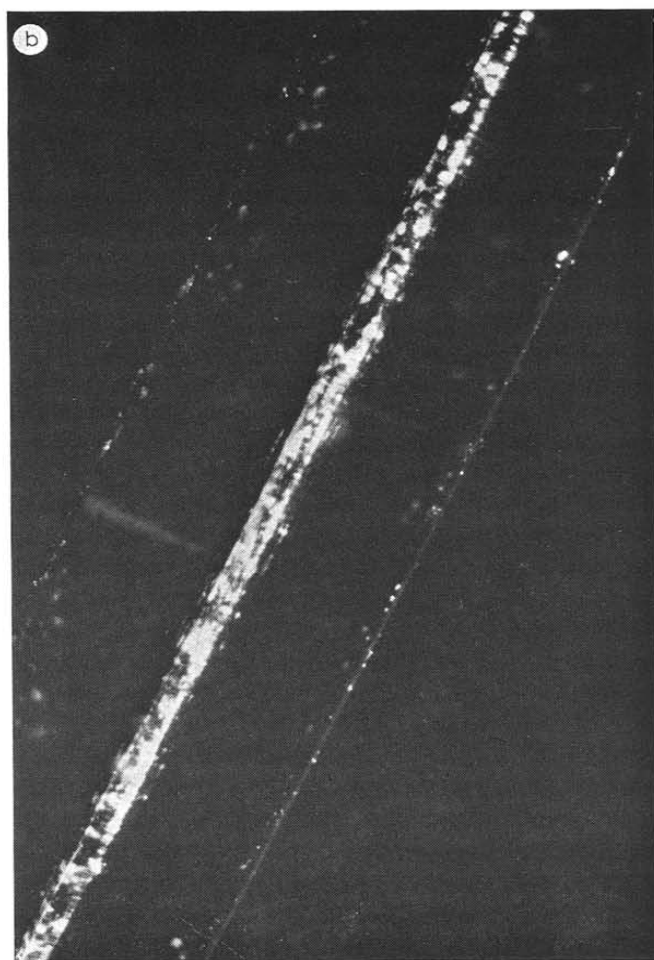
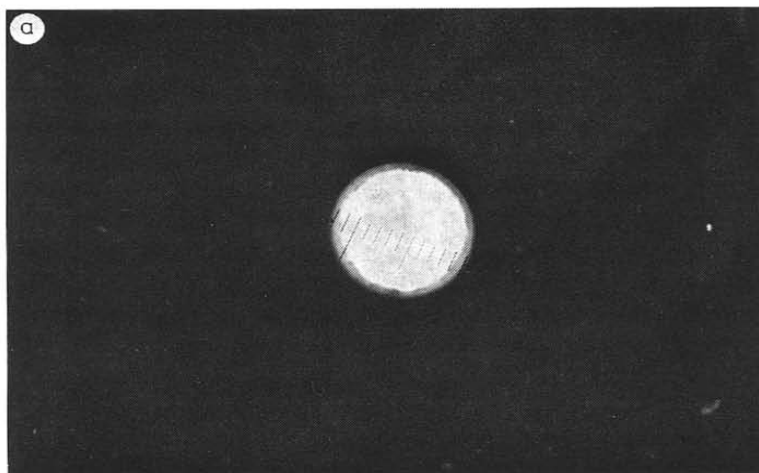
### *Alumina columns*

Columns with activated aluminium oxide were made by two methods. (I) Activated alumina, 0.1–0.2 g, (particle size 10  $\mu\text{m}$ ) (LiChrosorb<sup>®</sup> Alox T; E. Merck, Darmstadt, G.F.R.) ground into a small powder in an agate mortar. Water, 0.3–0.7 ml, was added in the agate mortar, and ground again to give a suspension of activated alumina. This suspension was sucked into a glass tube (*ca.* 80 cm  $\times$  0.3–0.4 mm I.D.  $\times$  6 mm O.D.) and one of the end of the tube was sealed with silicone grease. The tube was then dried in an oven at 400° and *ca.* 2 cm/min with rotation. A thin layer of activated alumina, (thickness *ca.* 10–20  $\mu\text{m}$ ) was produced on the inner wall of the glass tube, shown in Fig. 1a. The capillary was then drawn in the conventional manner using a GDM-1 (Shimadzu, Kyoto, Japan).

(II) A very fine powder of aluminium oxide, Aerosil<sup>®</sup> (Nippon Aerosil, Yokkaichi, Japan) was used as adsorbent. This powder (particle diameter *ca.* 20 nm) was placed in water to give a suspension containing 4% (w/w) of alumina. Sonication of this suspension gave a fully dispersed solution. The glass capillary (30–60  $\mu\text{m}$  I.D.) was filled with this solution, and kept horizontally in an oven the temperature of which was programmed from 35 to 150° at a rate of 1°/min and then set at 150° for 2–12 h. During this process, water evaporated from both ends of the tube and dried aluminium oxide powder was spread on the inner walls, as shown in Fig. 2. The capillary was used for separation without further treatment.

### *SCOT column*

Silica was dispersed on the inner walls of a capillary by the following procedure. Silica powder (Aerosil<sup>®</sup> OX-50, particle diameter 40 nm; Nippon Aerosil) or silica sol (Cataloid<sup>®</sup> S-50H, particle diameter 10–20 nm; Shokubai Kasei Kogyo, Chiyoda-ku, Japan) as suspended in water and sonicated. The concentration of silica in solution was 1–5% (w/w). These silicas are hydrophilic and suspend well in aqueous solution. The colloidal solution of Cataloid<sup>®</sup> was adjusted to pH 9–10 with NaOH. A borosilicate glass capillary, pre-treated with 1 N NaOH for 2 days, was then filled with the solution. The capillary was placed horizontally in an oven, the temperature of which was programmed from 20 to 130° at a rate of 1°/min and



**Fig. 1.** (a) Cross section of glass tubing coated with activated alumina by method I. The circle in the centre is the inner wall. One division is equal to  $25\ \mu\text{m}$ . (b) Glass capillary column (ca.  $50\ \mu\text{m}$  I.D.) of activated alumina.

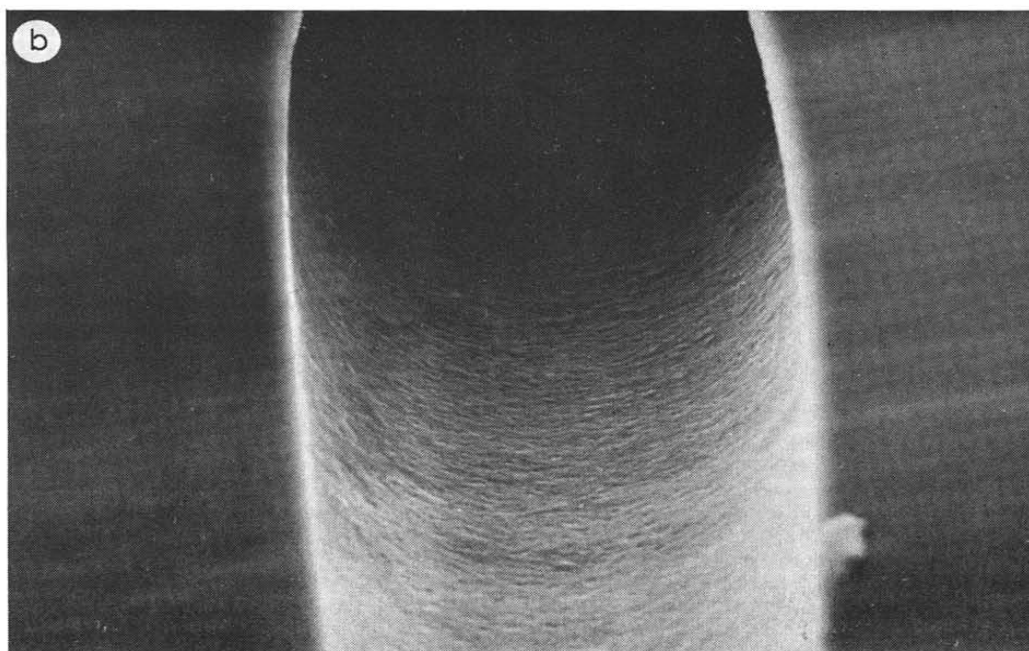
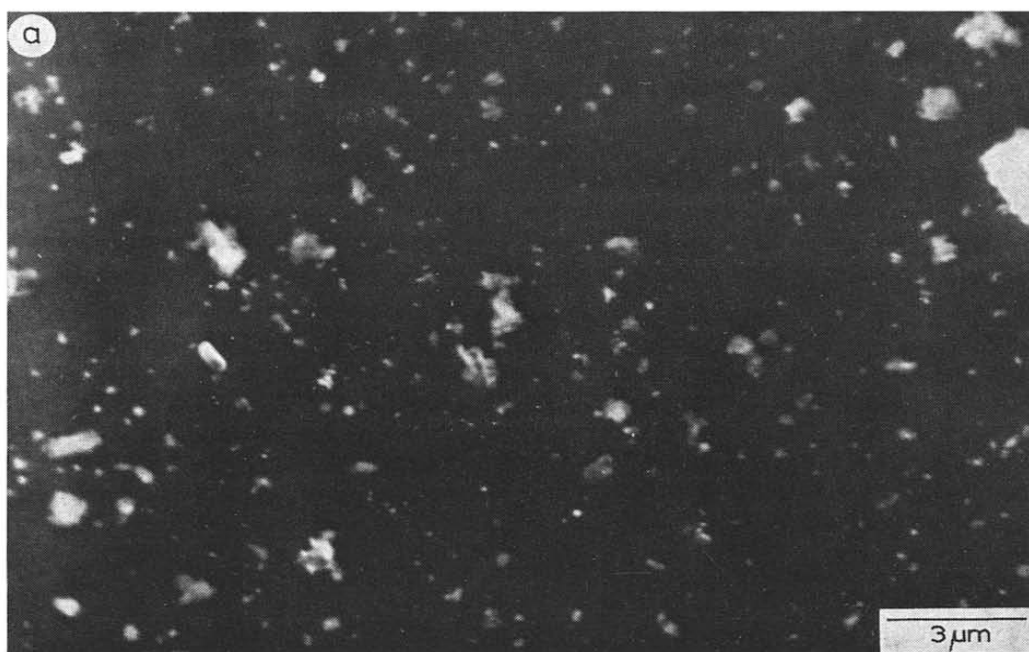


Fig. 2. Scanning electron micrographs of capillary columns: a, activated alumina column obtained by method I shown at an angle of 30°; b, SCOT column. Stationary phase: ODS; support, silica gel. Column inner diameter: 53  $\mu\text{m}$ .

kept at 130° for 12 h. The inner walls of the capillary were wetted with water, both ends were closed and kept at 130° for overnight. The capillary was dried by passing dry nitrogen through it at 130° for 1 or 2 days.

Chemical bonding of octadecylsilane (ODS) on silica powders was done as follows. About 100  $\mu\text{l}$  toluene solution containing 5% (v/v) octadecyltrichlorosilane were passed through the capillary at a steady flow-rate of *ca.* 0.52  $\mu\text{l}/\text{min}$  for 2–4 h at 100°. We call this reaction procedure the "semi-static method". Then the capillary was washed with 100  $\mu\text{l}$  toluene. To make a thick layer of ODS, the capillary tubing was treated further with 15–20% (v/v) ODS–toluene solution by the dynamic coating method<sup>4</sup> and then kept at 130° for 2 h. The capillary was treated with wet nitrogen gas at 100° to promote the polymerization of ODS<sup>4,13</sup>.

## RESULTS AND DISCUSSION

The aluminium oxide does not lose its activity during the passage through the oven of the glass drawing machine, which was set at 700–800°<sup>14</sup>. The distribution pattern of alumina powder on the inner walls of capillary tubing is shown in Figs. 1b and 2a. There are two groups of scattered powders. One of them is *ca.* 1–5  $\mu\text{m}$  in length and the another is only *ca.* 0.2  $\mu\text{m}$  in length. Both would work as adsorbents. As this column (made by procedure I) did not show any variations of retention times of sample components during the chromatographic operations. This would indicate that these powders stick well on the glass walls. Even though the powders cover only small parts of the walls and are scattered unevenly on the surface, this column shows a good separability. In other words, the solute may travel well between the solids, and this travelling would not cause any unfavourable effect to chromatographic separation under experimental conditions. A typical example of a chromatogram is shown in Fig. 3.

The height equivalent to a theoretical plate ( $H$ ) of  $\alpha$ -naphthylamine and N-phenyl- $\beta$ -naphthylamine are not effected by the sample amount in the region examined, 0.5–15 ng.  $H$  increases linearly with increase of linear velocity ( $u$ ) in the region of 0.4–3 cm/sec. This relation is expressed by  $H \text{ mm} = (0.83 \times u \text{ cm/sec}) + 0.03$  under the following experimental conditions: column, activated alumina, 450 cm  $\times$  52  $\mu\text{m}$ ; mobile phase, acetonitrile–water (0.2:99.8); sample, 0.048  $\mu\text{l}$  iso-octane solution containing 2 ng N-phenyl- $\beta$ -naphthylamine (capacity factor,  $k' = 0.3$ ).

A characteristic of a SCOT column is that its surface area is larger than that of a bare capillary. This may result in larger  $k'$  values of each sample component and better separabilities. Compared to chromatography on an ODS phase<sup>4</sup>, Fig. 4 shows the improved column efficiency, *i.e.*, by half to one third of  $H$ . The relation between  $H$  and  $u$  is also linear, as in the case of activated alumina. Table I shows the effect on  $k'$  and column efficiency of single or multiple coatings. Double or triple coating gives larger  $k'$  values compared to single coating. As the column efficiency ( $H$ ) depends on both  $k'$  and the diffusion coefficients in the mobile and stationary phases, it is desirable to keep both factors constant when we compare  $H$  values for different columns. Here eluent compositions were arranged so as to yield nearly the same  $k'$  value. Although  $H$  values of singly coated columns are slightly larger than those of multiply coated ones (Table I), the eluent of the former contains a larger percentage

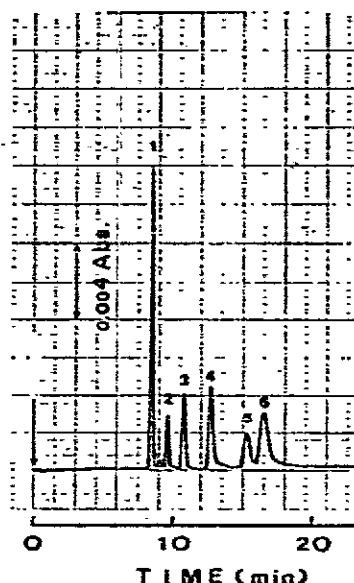


Fig. 3. Separation of aromatic amines on an activated alumina column. Column: 563 cm  $\times$  35  $\mu$ m. Mobile phase: methanol-*n*-hexane (0.01:99.99). Flow-rate: 1.1  $\mu$ l/min. Sample: 0.036  $\mu$ l isooctane solution containing N,N-diethylaniline (20 ng, peak 1), N-phenyl- $\alpha$ -naphthylamine (2.7 ng, 2), N-phenyl- $\beta$ -naphthylamine (3.6 ng, 3), aniline (8 ng, 4),  $\alpha$ -naphthylamine (2 ng, 5) and  $\beta$ -naphthylamine (2 ng, 6). Detection: UV, 235 nm.

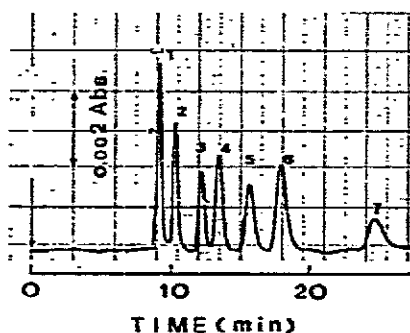


Fig. 4. Separation of fused aromatics on a SCOT column. Column: 563 cm  $\times$  37  $\mu$ m. Stationary phase: ODS on silica gel. Mobile phase: acetonitrile-water (30:70). Flow-rate: 0.8  $\mu$ l/min. Sample: 0.01  $\mu$ l acetonitrile solution containing benzene (50 ng, peak 1), naphthalene (5 ng, 2), biphenyl (0.7 ng, 3), fluorene (1 ng, 4), phenanthrene (0.5 ng, 5), anthracene (0.2 ng, 6) and pyrene (1.3 ng, 7). Detection: UV, 254 nm.

of acetonitrile compared to the latter. So in this case there are no differences between single and multiple coatings.

The scanning electron micrograph of the SCOT column, shown in Fig. 2, shows well-dispersed powders. The powder diameters are 20–40 nm, one hundredth of that of Silanox<sup>®</sup>, which is used for capillary GC. Initially we tried to use Silanox<sup>®</sup>, but its solution could not be passed through the capillary. Filling capillary tubing

TABLE I  
ODS STATIONARY PHASE WITH SINGLE OR MULTIPLE COATINGS  
S = Semi-static coating; D = dynamic coating.

Column			Support	Coating	$k'$ of biphenyl*	$H$ of biphenyl** (mm)
No.	I.D. ( $\mu\text{m}$ )	Length (m)				
A-1	37	5.6	1.5% Cataloid®	5% ODS (S)	0.56	1.8 (1.08; 22)
A-2				20% ODS (D) on A-1	0.84	1.7 (1.16; 24)
B-1	37	4.9	1.5% Cataloid®	5% ODS (S), then 20% ODS (D)	1.22	1.7 (1.03; 25)
B-2				20% ODS (D) on B-1	3.43	1.6 (1.04; 30)
C	53	4.1	1.0% Aerosil®	5% ODS (S)	0.71	2.0 (1.23; 26)

\* Eluent was 40% aqueous methanol.

\*\*  $k'$  values followed percentage compositions of eluent are given in parentheses. The eluent composition was adjusted to yield nearly the same  $k'$  values. Eluent was aqueous acetonitrile.

with a solution of aluminium oxide or colloidal silica is not difficult if the state of suspension is good. The oven drying process, which results in evaporation of solvent, is also very simple. Sometimes, powders aggregated at one place with high density. In this case the column was discarded. It is important to set the capillary in a horizontal position in the oven. If the capillary is vertical an even coating is not obtained. With these precautions, the reproducibility of the procedure was good. Dynamic coating of silica powders on the walls of glass capillaries was also tried, but gave poor results. A dynamic process of deposition of solid onto the surface would be more simple than our process, so it is worth developing such a method.

The most interesting observation in these experiments is that the scattered powders keep their positions during chromatographic operations, possibly owing to static electricity<sup>11</sup>.

The two different approaches to making surface-coated open-tubular capillary columns of activated alumina and silica gel will be helpful in developing high efficiency capillary columns.

## REFERENCES

- 1 G. Nota, G. Marino, V. Buonocore and A. Ballio, *J. Chromatogr.*, 46 (1970) 163.
- 2 D. Ishii, T. Tsuda, K. Hibi, T. Takeuchi and T. Nakanishi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 371.
- 3 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 632.
- 4 T. Tsuda, K. Hibi, T. Nakanishi, T. Takeuchi and D. Ishii, *J. Chromatogr.*, 158 (1978) 227.
- 5 D. Dewaele and M. Verzele, *J. High Resolution Chromatogr. Chromatogr. Commun.*, 1 (1978) 174.
- 6 R. Tijssen, *13th Int. Symp. Advances in Chromatography, St. Louis, Mo., Oct. 16-19, 1978*.
- 7 K. Hibi, T. Tsuda, T. Takeuchi, T. Nakanishi and D. Ishii, *J. Chromatogr.*, 175 (1979) 105.
- 8 D. Ishii, T. Tsuda and T. Takeuchi, *J. Chromatogr.*, 185 (1979) 73.
- 9 R. D. Schwartz, D. J. Brasseaux and R. G. Mathews, *Anal. Chem.*, 38 (1976) 303.
- 10 E. C. Horning, M. G. Horning, J. Szafrank, P. Van Hout, A. L. German, J. P. Thenot and C. D. Pfaffenberger, *J. Chromatogr.*, 91 (1974) 367.
- 11 J. J. Kirkland, *U.S. Pat.*, 3,485,658 (1969).
- 12 C. G. Horvath and S. R. Lipsky, *J. Chromatogr. Sci.*, 7 (1969) 7.
- 13 W. A. Aue and C. R. Hastings, *J. Chromatogr.*, 42 (1969) 319.
- 14 T. Tsuda and M. Novotny, *Anal. Chem.*, 50 (1978) 271.