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STUDY ON THE EFFICIENCY OF SUPPORT-COATED OPEN-TUBULAR COLUMNS FOR STEROID ANALYSIS

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SUMMARY

Glass support-coated open-tubular columns for the analysis of steroids in biological samples were prepared with various support materials. The reproducibility of the procedure of preparing the column was investigated and some fundamental chromatographic properties were studied.

INTRODUCTION

A large number of successful applications of glass open-tubular columns for high-resolution separations in biochemical analysis have been reported¹. However, the large-scale use of wall-coated open-tubular (WCOT) columns seemed to be impeded by the tendency of the liquid film on the column wall to break up and form minute droplets². Columns are often found to deteriorate rapidly during use, especially at higher temperatures, owing to the increasing resistance to liquid-phase mass transfer.

This problem was recognized in the early days of capillary gas chromatography, and several methods were proposed for improving the spreading and stability of the liquid film. These methods can be roughly divided into two different groups. In the first approach, the wettability of the smooth column wall is improved either by adding surfactants to the stationary liquid³ or by chemically modifying the glass surface in such a way as to achieve both a zero contact angle and sufficient compatibility for the stationary liquid⁴. The second solution to this problem consists in coating the column wall with a finely powdered support material that is capable of retaining the stationary phase⁵. The rough surface may also increase the volume of the liquid phase. These support-coated open-tubular (SCOT) columns have been commercially available since 1966. Until recently, almost all SCOT columns were made from stainless-steel tubing by a static coating method that required special equipment and techniques⁶. In 1968, Kaiser⁷ described a simple dynamic coating procedure for glass columns using a slurry of the solid support material in a solution of the liquid phase

in a volatile solvent. Probably this easy coating procedure, which can be used in the ordinary gas chromatography laboratory, has contributed to the popularity of SCOT columns in the last few years.

Recently, Horning and co-workers developed SCOT columns for the separation of complex mixtures of biological origin⁸⁻¹⁰. Silanox, a commercially available silica, was used as a support material for both apolar and polar stationary liquids. Although presenting a number of interesting applications, the authors gave only a limited number of data on column characteristics.

The main objective of this study was to evaluate the chromatographic properties in a more quantitative way. Therefore, we prepared a series of SCOT columns with both Silanox and the more conventional diatomaceous earths as support materials and determined their chromatographic properties. Data on the reproducibility of the dynamic coating procedure⁸ are presented.

EXPERIMENTAL

Preparation of capillary tubing

Glass capillaries of I.D. 0.3 and 0.4 mm and length 30-60 m were made from Pyrex tubing on the well-known Hupe and Busch (now Hewlett-Packard) drawing machine. The capillaries were washed with acetone and chloroform. Both untreated and silanized capillaries were used. Silanization was effected by passing a 10% solution of dimethylchlorosilane in dry toluene through the column, the columns subsequently being washed with dry toluene and methanol.

Coating of the columns

The support materials used were Silanox 101, a silanized fumed silica gel⁸⁻¹⁰, and two diatomaceous earth materials, Hyflo Super Cel and Chromosorb R-6740-1 (Johns-Manville, Denver, Colo., U.S.A.), which are almost identical in structure. A 5-10 μm fraction of the Hyflo was used¹¹. Coarse particles were removed from Chromosorb R by sedimentation and the fraction with a particle size below 5 μm was used. A portion of the diatomaceous earth materials was silanized¹². Columns of different length were coated by the two-step dynamic coating procedure described by German *et al.*⁸. A suspension of the support material was prepared by dissolving 0.5 g of the stationary phase in 100 ml of chloroform; 2 g of the support material were added to this solution. The suspension was homogenized by treating it in an ultrasonic bath. A plug, about 20% of the column length, was forced through the column, which had been previously wetted with chloroform, at a constant velocity of about 5 $\text{cm}\cdot\text{sec}^{-1}$ under nitrogen pressure. The column was connected to a short dummy column in order to prevent a sharp increase in the speed of the plug when leaving the column. During the first coating step, the column was placed in an ultrasonic bath.

In a second step, the columns were coated with additional liquid phase. Two silicone phases were used, the apolar phase SE-30 (a dimethylpolysiloxane) and the polar phase OV-225 (a 25% phenyl- and 25% cyanopropyl-substituted methylpolysiloxane). Solutions containing 2 g of the stationary phase in 100 ml of 2,2,4-trimethylpentane (SE-30) or chloroform (OV-225) were used. The plug velocity was about 2 $\text{cm}\cdot\text{sec}^{-1}$. The remaining solvent was removed by flushing with nitrogen at about 5 $\text{ml}\cdot\text{min}^{-1}$ for 1 h. The columns were mounted in the chromatograph and con-

ditioned under carrier gas by heating them at a rate of 1°min^{-1} to 300° for SE-30 and to 250° for OV-225. The columns were held at the final temperature for 1–2 h.

Gas chromatography

The gas chromatograph, equipped with an all-glass solid injector, has been described elsewhere¹³. Nitrogen and helium were used as the carrier gases. Plate height curves and capacity factors were measured for pure C_{24} and C_{28} *n*-alkanes with nitrogen as the carrier gas. Distribution coefficients for these compounds were determined in separate experiments on packed columns. From these values, the phase ratio in the SCOT columns was calculated.

For a number of columns, the plate height curves were also determined with helium as the carrier gas. The resistance to mass transfer in the liquid phase was calculated from the combined measurements in helium and nitrogen¹⁴.

RESULTS AND DISCUSSION

Although only limited data are available from the literature, the separation efficiency of dynamically coated SCOT columns seems to be inferior to that attained in WCOT columns^{15–17}. Recently, however, German *et al.*⁸ reported on dynamically coated SCOT columns, which combine a high efficiency (about 1600 theoretical plates per metre for $k' = 1$ –1.5) with an excellent temperature stability, when prepared with SE-30 as the stationary phase and Silanox as the support material.

The first objective of this study was to determine some fundamental chromatographic properties of these columns. Therefore, a number of columns were prepared with Silanox as the support for the liquid phase. The preparation of 0.30 mm I.D. columns turned out to be very difficult. In spite of numerous precautions, there were a great many failures, and only a few columns gave acceptable efficiencies. The Silanox suspensions being rather viscous, the Silanox plug showed an irregular displacement during most of the first coating steps and sometimes clogging occurred. Therefore, it was decided to use 0.4 mm I.D. columns, and a series of columns about 35 m long were prepared without difficulties. Some data for these columns are presented in Table I.

TABLE I
CHARACTERISTICS OF SILANOX-SE-30 SCOT COLUMNS

Property	Column No.				
	1	2	3	4	5
Length (m)	30	30	35	50	50
Internal diameter (mm)	0.40	0.40	0.40	0.40	0.40
Temperature ($^{\circ}\text{C}$)	220	220	220	220	220
Capacity factor for <i>n</i> - C_{24}	2.9	3.5	3.4	3.1	3.6
Phase ratio	350	290	300	330	270
Linear gas velocity (cm/sec)	7.6	7.5	6.0	6.5	7.5
Plate height (mm)	0.48	0.40	0.50	0.47	0.51
Theoretical plates per metre for <i>n</i> - C_{24}	2100	2500	2000	2100	1900
$C_L \cdot 10^4$ (sec)	—	2.3	—	4.9	6.6
Separation number (C_{24} - C_{28})	40	44	45	42	43
Coating efficiency (%)	66	84	61	68	58

Capacity factors and plate heights were determined at 220°. Phase ratios, *i.e.*, the ratio of gas volume (V_G) to volume of stationary phase (V_L), were calculated from the capacity factor, k' , for *n*-tetracosane, with the aid of partition coefficients determined on packed columns. A phase ratio (V_G/V_L) of 300–350 corresponds to approximately 0.5 mg of stationary phase per metre of column length. For both the 0.30 mm I.D. dynamically coated SCOT columns described by German *et al.*⁸ and the 0.25 mm I.D. WCOT columns prepared by Luyten¹³, equivalent amounts of stationary liquid (SE-30) are found; the differences in k' values published for *n*-tetracosane are due merely to differences in column temperature and column diameter. This value is much lower than in conventional SCOT columns⁶. However, this low liquid phase volume is particularly useful for the analysis of high-boiling and/or thermally unstable compounds.

Fig. 1 shows the scanning electron microscope (SEM) photograph of the column wall after the second coating step. The small Silanox particles do not form a porous layer on the column wall, but are more or less regularly distributed over the glass surface. Therefore, this column type seems to be related to the hydrochloric acid-etched WCOT columns rather than to the conventional SCOT columns¹⁸.

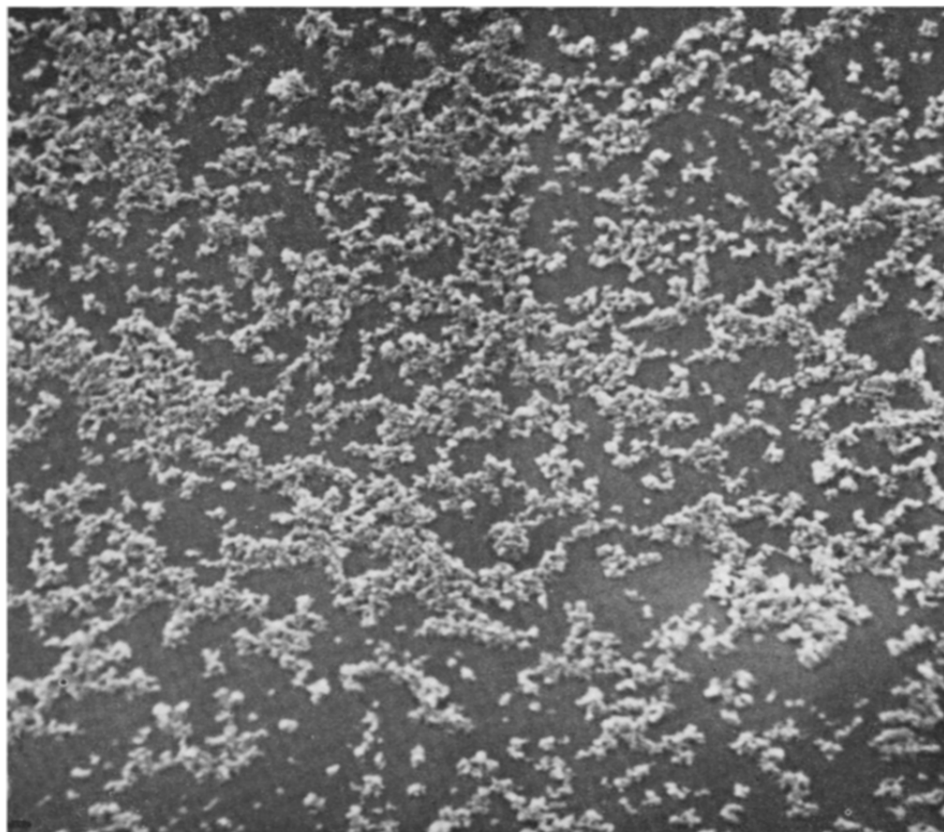


Fig. 1. Scanning electron microscope photograph of a SCOT column coated with Silanox and SE-30. Magnification: 5000 \times .

For a number of columns, the plate height, H , for n -tetracosane was measured at different values of the average linear gas velocity, \bar{u} ; a typical H - \bar{u} curve is shown in Fig. 2. These curves were obtained with helium and nitrogen as the carrier gases. The relationship between plate height and linear gas velocity in open-tubular columns is described by the well-known Golay equation:

$$H = B/\bar{u} + C_G\bar{u} + C_L\bar{u}$$

where B , C_G and C_L are terms that account for the influence of longitudinal diffusion in the gas phase and resistance to mass transfer in the gas and the liquid phase, respectively. In fact, this equation should be modified so as to account for the compressibility of the carrier gas¹⁹. From the differences between the H - \bar{u} curves in helium and nitrogen, C_L can be calculated¹⁴. Values for the diffusion coefficients of gaseous n -tetracosane and nitrogen needed for this calculation were estimated from a general correlation equation²⁰. Values for C_L are presented in Table I; these are comparable in order of magnitude to those found in WCOT columns¹³. Finally, Table I includes data on the separation number²¹ between n -C₂₄ and n -C₂₈ and on the coating efficiency²².

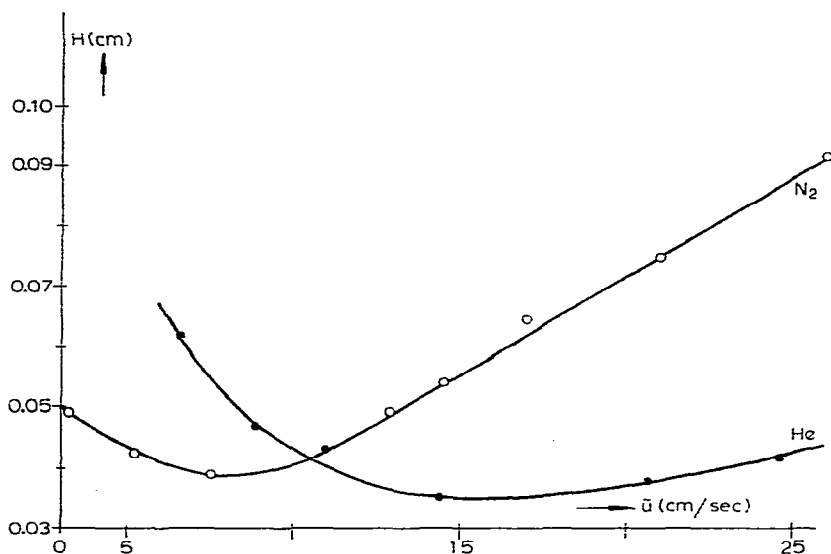


Fig. 2. H - \bar{u} plot for a Silanox-SE-30 SCOT column. Solute: n -tetracosane ($k' = 3.5$). Column temperature: 220°.

The choice of Silanox as a support material seems rather arbitrary. Therefore, we prepared a series of columns using diatomaceous earth materials as the support for the stationary liquid. The use of these materials in dynamically coated SCOT columns was originally proposed by Kaiser⁷. For the separation on packed columns of polyfunctional compounds of biological origin, such as steroids, silanized support materials are required. Therefore, both Hyflo Super Cel (5-10 μ m) and Chromosorb R were silanized¹².

TABLE II
CHARACTERISTICS OF HYFLO AND CHROMOSORB/SE-30 SCOT COLUMNS

Property	Column No.			
	6	7	8	9
Support material	Hyflo	Hyflo	Chromosorb	Chromosorb
Length (m)	35	33	30	50
Internal diameter (mm)	0.4	0.4	0.4	0.4
Temperature (°C)	220	220	220	220
Capacity factor for <i>n</i> -C ₂₄	2.8	3.0	3.3	3.0
Phase ratio	360	340	320	340
Linear gas velocity (cm/sec)	5.3	6.8	6.5	7.5
Plate height (mm)	0.45	0.54	0.43	0.47
Theoretical plates per metre for <i>n</i> -C ₂₄	2200	1800	2300	2100
$C_L \cdot 10^4$ (sec)	—	—	5.8	—
Separation number (C ₂₄ -C ₂₈)	52	44	50	49
Coating efficiency (C ₂₄) (%)	69	57	78	65



Fig. 3. Scanning electron microscope photograph of a SCOT column coated with Hyflo Super Cel and SE-30. Magnification: 500×.

The columns prepared with these materials were as efficient as those made with Silanox (see Table II) and the phase ratios also are almost identical. An SEM photograph of a Hyflo-coated column is given in Fig. 3. Fig. 4 shows the separation of urinary steroids as MO-TMS derivatives with a relatively short column.

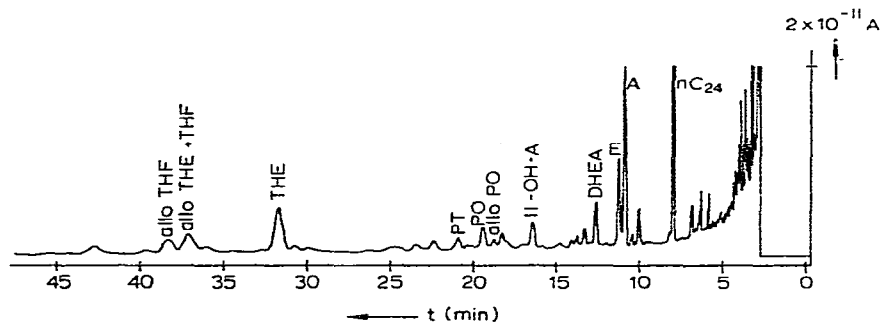


Fig 4. Urinary steroid profile for an adult female (MO-TMS derivatives, sample provided by Dr. W. Leunissen, University of Technology, Eindhoven). Column: 25-m Hyflo Super Cel-SE-30. Temperature: 220°. Carrier gas: nitrogen.

It is difficult to indicate the temperature stability of a column in an unambiguous way; in fact, the estimation depends on the reliability required by the analytical application concerned. Table III gives some characteristic data for an SE-30 SCOT column upon prolonged use at 220°. A slow decrease of the capacity factor, k' , is observed, which indicates some loss of stationary phase or alterations in its chemical composition, *e.g.*, by oxidation. The plate height, however, remains almost constant over a 3-month period, showing that no changes occur in the distribution of the liquid film over the surface.

TABLE III

CHARACTERISTICS OF A HYFLO/SE-30 SCOT COLUMN UPON PROLONGED USE AT 220°

Property	Week		
	0	6	12
Capacity factor for $n\text{-C}_{24}$	2.8	2.5	2.1
Linear gas velocity (cm/sec)	11.2	11.4	11.3
Theoretical plates per metre for $n\text{-C}_{24}$	1800	1650	1650
Separation number ($C_{24}\text{-}C_{28}$)	44	41	40

In Table IV, some characteristics of the columns described above and of equivalent columns described in the literature are presented for comparison. In fact, the major difference exists in the phase ratios; as stated earlier, this difference is due rather to the variation in column diameter than to the amount of stationary phase on the column wall per unit column length. From the point of column efficiency, expressed in terms of plate height, WCOT columns are slightly superior to SCOT columns. The complex preparation technique of the former type of column seems to be a serious disadvantage.

TABLE IV
CHARACTERISTICS OF SCOT AND WCOT SE-30 COLUMNS

Property	Column type		
	WCOT*		SCOT
Internal diameter (mm)	0.25	0.25	0.40
Length (m)	20-30	30-70	30-50
Coating procedure	Dynamic	Static	Dynamic
Capacity factor for $n\text{-C}_{24}$ (220°)	5-6	4-7	3
Theoretical plates per metre for $n\text{-C}_{24}$	1000-2000	2000-3000	2000
Coating efficiency (%)	30	60	60

* Data from ref. 13.

On theoretical grounds, one would expect the SCOT columns of 0.4 mm I.D. to be less efficient than those of 0.3 mm I.D. In our columns, the plate height is largely determined by gas-phase effects, that is, by the two first terms on the right-hand side of the Golay equation. The contribution of these gas-phase effects can be calculated by subtracting from the measured value of the plate height the contribution due to diffusion in the liquid phase, $C_L \bar{u}$. The predominance of gas-phase effects results from the proportionality between C_G and the square of the column radius. In practice, however, no unfavourable influence on the overall efficiency has been observed.

In some applications, there is a need for columns with phases of polarity higher than that of SE-30. Recently, Van Hout *et al.*⁹ reported on SCOT columns for polar silicone phases, in which Silanox was again used as the support material. These columns are prepared by a two-step dynamic/static procedure. In our opinion, however, Silanox is not the appropriate material for polar stationary phases. The highly hydrophobic surface is not compatible with polar liquids, and will show poor wettability. We prepared SCOT columns for OV-225 as the stationary phase by the two-step dynamic procedure, using non-silanized glass tubing and both Chromosorb R and Hyflo as support materials. Some data are given in Table V. Obviously, these columns

TABLE V
CHARACTERISTICS OF HYFLO AND CHROMOSORB-OV-225 SCOT COLUMNS

Property	Column No.			
	10	11	12	13
Support material	Hyflo	Hyflo	Chromosorb	Chromosorb
Length (m)	35	35	35	30
Internal diameter (mm)	0.4	0.4	0.4	0.4
Temperature (°C)	220	220	220	220
Capacity factor for $n\text{-C}_{25}$	1.2	1.1	1.1	1.3
Linear gas velocity (cm/sec)	9.9	10.4	8.5	8.0
Plate height (mm)	0.88	0.97	0.85	0.80
Theoretical plates per metre for $n\text{-C}_{25}$	1150	1050	1200	1250
$C_L \cdot 10^3$ (sec)			3.2	2.1
Separation number ($C_{25}\text{-C}_{37}$)	24	22	26	31
Coating efficiency (%)	29	27	31	32

are not as efficient as those with an apolar liquid, owing mainly to a rather high C_L contribution, indicating uneven distribution of the liquid film.

Further improvements will be possible if a higher density of the support material over the glass surface can be obtained.

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