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Preparation and Evaluation of Polar Stabilized Phase Open Tubular (SPOT) Columns

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Summary

A method is described for the routine preparation of high resolution stabilized phase open tubular (SPOT) columns. A finely dispersed siliceous material (Cab-O-Sil) is treated with benzyltriphenylphosphoniumchloride and dynamically coated onto the inside wall of glass capillary columns. Additional dynamic coating with a polar stationary phase yields thermostable columns of high efficiency. The columns show favourable adsorption properties and good long term stability. The mechanical stability of the stationary phase film is explained in terms of the rheological behaviour of concentrated dispersions. The designation PLOT columns appears inapplicable, and these columns will be referred to as SPOT columns. A theoretical model, describing band spreading in SPOT columns, is proposed and compared with the experimental results. Some applications in routine use and in ultra trace analysis are presented.

Introduction

Glass open tubular columns offer the best prospects in the analysis of complex biochemical and environmental mixtures. Much progress has been made in the development of these columns, especially for apolar stationary liquids. One of the main problems met with in the preparation of polar columns is the poor wettability of the glass wall. Polar coatings, deposited uniformly on untreated glass surfaces, usually soon break up and form microdrops when at higher temperatures. The result is a rapid loss of column efficiency and a useful life of hours to days at best. Of the numerous possible methods of overcoming the problem, that of surface roughening has found the widest application. Surface roughening is based either on the deposition of a layer of fine particles, or on chemical attack of the glass wall. Deposition of a layer of fine particles results in PLOT (Porous Layer Open Tubular) columns, the preparation and properties of which were dealt with extensively in a review by *Ettre* and *Purcell* [1]. Particle dimensions are typically in the range 1–10 μm and the phase ratio amounts to 30–150. For the analysis of high boiling compounds (steroids, pesticides, drugs) columns with high phase ratios, in the range of 200–1000, are beneficial, but the equivalent film thickness of 0.05–0.5 μm is small compared with the size of the

particles normally used. To prepare such lightly loaded columns the choice of a support material of colloidal dispersion seems to be expedient. In 1973, *German* and *Horning* [2] described the use of Silanox 101, a commercially available hydrophobic trimethylsilylated silica with a primary particle size of only 7 nm. The authors did not recognize the colloidal nature of the material. In fact, a particle size of 6–10 μm was quoted. *Blumer* [3] was the first to draw attention to the colloidal dispersion of Silanox 101. Silanox containing apolar SE-30 columns were applied successfully in the biomedical field [2, 4]. The preparation of polar phase columns containing Silanox was reported on by *van Hout* et al. [5] and by *Lin* et al. [6].

We reason however, that Silanox is not the appropriate material for polar stationary phases. The hydrophobic surface is not compatible with polar liquids and will show poor wettability. Indeed, experience in our laboratory indicates that the combination of polar phases and Silanox is of limited practical value. The number of rejects is unacceptable and efficiencies do not rank high. *Pellizzari* [7] used Cab-O-Sil, an unsilanized fumed silica, to prepare columns coated with the moderately polar phase OV-17. The Cab-O-Sil particles were deactivated and cross-linked by *in situ* silanization. The reported lifetime was about 1 month. Recently, our laboratory [8] reported on the successful preparation by dynamic coating methods of lightly loaded (phase ratio 300) OV-225 columns, stabilized by Cab-O-Sil. The key was the treatment of Cab-O-Sil with benzyltriphenyl-phosphoniumchloride (BTPPC). The lifetime exceeded 11 months at 210 °C and the favourable adsorption properties enabled the quantitative determination of underivatized anti-convulsant drugs. In this paper we further elaborate on the use of Cab-O-Sil in the routine preparation of polar high-efficiency thermostable open tubular columns

Experimental

Apparatus and materials

A Pye Unicam series 104 gas chromatograph equipped with FID and Ni[63] ECD was used. The standard injection port was removed and replaced by an all-glass solids injection system [9]. Column connections were made with shrinkable PTFE tubing. At the column exit a length of Dilver P metal capillary 0.2 mm i.d., leads the effluent to a point just below the burner tip (FID) or the radioactive foil (ECD).

Gas chromatographic conditions were:

Sampling system 270 °C, detector 300 °C;

Detector make-up gas 25 $cm^3 min^{-1}$;

Carrier gas N_2 or He (FID) or 95% Ar and 5% CH_4 (ECD).

Scanning electron micrographs were taken using a "Stereoscan", Cambridge Instruments, Cambridge, Great Britain. Glass capillaries 0.4 mm i.d. ± 2.5 % standard deviation were prepared with a home built drawing machine. Pyrex or Duran glass was used throughout, in lengths between 15 and 100 m; the coil diameter was 10 cm. After drawing the columns were flushed with dry acetone and carbon tetrachloride.

Cab-O-Sil[®] is a trade mark of Cabot Corporation, Boston, Mass., U.S.A..Grade M-5, specific surface 190 $m^2 g^{-1}$ [10], particle size 15 nm, was obtained via Packard Instrument Benelux S.A., Brussels-1000, Belgium. Cab-O-Sil is an amorphous silica. The high specific surface is solely due to the high degree of dispersion, the small spheres are non-porous [11].

Benzyltriphenylphosphoniumchloride (BTPPC) was obtained from Aldrich Europe, Beerse-2340, Belgium. OV-225 stationary phase, 25 % cyanopropyl and 25 % phenyl substituted methyl polysiloxane, Ohio Valley Specialty Chemical Corp., Ohio, U.S.A., and AN-600, 25 % 2-cyanoethyl substituted methylpolysiloxane, product of Analabs, Inc., Connecticut, U.S.A., were obtained from Packard Becker B.V., Delft, The Netherlands.
All solvents were A.R. grade.

Preparation of SPOT columns (Cab-O-Sil; OV-225)

Cab-O-Sil stabilized columns are prepared following a modified two-step dynamic coating procedure [2, 8]. An essential step in the preparation of a workable coating dispersion is the treatment of the Cab-O-Sil surface with BTPPC. Therefore 0.3 g Cab-O-Sil is suspended in 4 cm^3 1 % (w/w) solution of BTPPC in methylene chloride. After 10 minutes the Cab-O-Sil is centrifuged off at 1000 g and the supernate is discarded. The residue is washed twice with 4 cm^3 methylene chloride and once with 4 cm^3 carbon tetrachloride. These solvents are separated from the Cab-O-Sil by centrifugation. The gravitational field should not exceed 1000 g. The whole of the so treated Cab-O-Sil is then suspended in a solution of 0.3 g OV-225 in 50 cm^3 carbon tetrachloride. Adequate dispersion is achieved by ultrasonic treatment for 10 minutes. In the first coating step about 30 cm of the column is filled with carbon tetrachloride, immediately followed by a plug of the dispersion of 25 % of the column length.

These plugs are run through the column at 4—5 $cm s^{-1}$. A dummy column prevents a sharp rise of the plug speed at the column end. After the plugs have left the dummy column, the nitrogen pressure is raised to yield a nitrogen flow of 10 $cm^3 min^{-1}$. At this flow rate the column is left drying at room temperature for 4 hours. Then the column is mounted into a gas chromatograph and under

a nitrogen flow of 5 $cm^3 min^{-1}$ programmed at a rate of 5 °C min^{-1} to 250 °C. The column is left at this final temperature for one hour.

In the second coating step additional OV-225 is deposited on the surface layer of Cab-O-Sil. A plug of a coating solution containing 10 % ($^w/_w$) OV-225 in toluene of 25 % of the column length is passed through the column at a speed of 4—5 cms^{-1}, again using the dummy column. When the liquid plug has left the latter column the nitrogen flow rate is raised to approximately 10 $cm^3 min^{-1}$.

At this flow rate the column is left overnight to dry at room temperature. Finally, the column is conditioned using the following temperature program: 2 °C min^{-1} to 240 °C, isothermal at 240 °C during 12 hours, back to working temperature (maximum 230 °C). During conditioning the nitrogen flow rate is 5 $cm^3 min^{-1}$.

Results and Discussion

Discussion of the coating procedure

The treatment of Cab-O-Sil with BTPPC serves a dual purpose. Firstly, BTPPC is an efficient and thermostable deactivating agent. It is used routinely in our laboratory for the preparation of apolar columns [21]. Secondly, it aids in the deposition of the Cab-O-Sil onto the column wall. It was expected that BTPPC, being a surfactant, would act as a deflocculant and thus would decrease the amount of Cab-O-Sil deposited. In fact, however, BTPPC substantially increased the amount deposited. Apparently, the salting-out effect of the ionic BTPPC is stronger than is its deflocculating action. It is for this reason that the Cab-O-Sil should not be left in contact with the BTPPC solution for more then 5—10 minutes, otherwise clogging of the column with large flocculates occurs in the first coating step. The addition of OV-225 stabilizes the dispersion as it counteracts to some extend the effect of BTPPC. It prevents clogging and reduces the amount of Cab-O-Sil deposited. A 1:1 weight ratio of Cab-O-Sil and OV-225 is found to be optimal.

Adequate dispersion is essential and is achieved by ultrasonic treatment for 10 minutes. The final dispersion should not contain any visible flocculates. If this step is omitted, large agglomerates, up to 10 μm, remain in the coating dispersion and column performance is adversely affected.

Summarizing, stable and workable coating dispersions are obtained, only if

— Cab-O-Sil is treated with BTPPC,
— OV-225 is added to the dispersion,
— and a sufficient degree of dispersion is affected by ultrasonic treatment.

Gas chromatography

Plate height (H) versus carrier gas velocity (u) curves and capacity ratios (k) were measured for a series of homologous fatty acid methyl esters $n-C_n H_{2n+1} COOCH_3$, n = 17 — 23. Peak widths were determined by interpolation according to Kaiser [12]. Partition coefficients
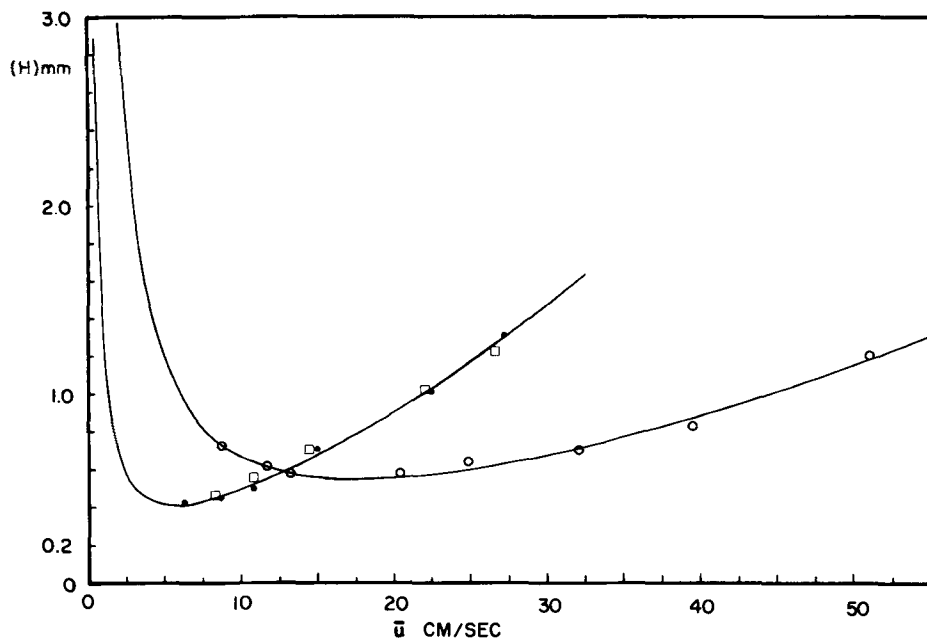
Fig. 1

● H vs ū plot obtained for
  C_{22}-Me-ester at 225 °C

□ Carrier gas nitrogen column 4
● Carrier gas nitrogen column 6
○ Carrier gas helium   column 4

(K) for these compounds were determined in separate
experiments on columns of known phase ratio. From
these values the phase ratios (V_G/V_L) of the columns under
study were calculated. The resistance to mass transfer in
the liquid phase (C_L) was calculated from the combined
H-u measurements in He and N_2 carrier gases [13]. See
also Fig. 1.

Table I shows the results obtained from 6 different
Cab-O-Sil/OV-225 columns. These data were measured
3 weeks after column preparation. After this period the
column properties remain constant. Immediately after
coating slightly higher coat-efficiencies and capacity ratios
are found.

Structure of the retentive film

From scanning electron microscopic investigation of
columns after the first coating step, it appeared that the
porous layer of Cab-O-Sil on an average was 0.3 μm thick.
After the second coating step the stationary phase fills
all holes between the solid particles. The observed film
thickness of 0.4—1.2 μm is greater than the thickness of
the porous layer. Hence the solid material is dispersed in
the liquid phase, rather than the liquid phase being
dispersed in a porous layer as is the case in PLOT columns.
Under these conditions the solid material cannot adhere
to the wall through surface tension forces, nor can the
stability of the film-surface relationship be explained in
terms of surface roughness. A satisfactory explanation of
the mechanical stability of the film can be found in the
rheological behaviour of structured dispersions. A short
description of structured dispersions, based on a recent
review by *Mewis* and *Spaull* [14], is given below. The follow-
ing concepts are defined explicitly.

1. Dispersion: a two phase solid in liquid system the
   particles of which are colloidal, i.e. between 1 μm
   and 1 nm.
2. Aggregates: tight clusters of particles, strongly held
   together.
3. Flocculates: weak, thermodynamically metastable,
   structures from colloidal particles.
   Flocculates, in contrast to aggregates, may be
   destroyed by the application of shear stresses and may
   reform when these stresses are removed.
4. Agglomerates: weak, but dense flocculates.

In very dilute dispersions the particles do not interact and
the system behaves essentially as a Newtonian liquid.
Above a certain lower concentration limit the particle
interactions cause the dispersion to become structured.
In more concentrated dispersions the particle interactions
fully predominate the resistance to flow and deformation.
The dispersion then shows pronounced non-Newtonian
flow behaviour. The interaction between particles in
structured dispersions consists of an attractive and a re-
pulsive part. The sum of attraction and repulsion energy
equals the total potential energy. The curve, representing
the total interaction energy between particles as a func-
tion of their distance, shows a deep primary minimum
and a less pronounced secondary one, separated by an
energy barrier, cf. Fig. 2.

The position of the potential energy wells corresponds to
preferential interparticle distances. Particles which sur-
mount the energy barrier to the deep primary minimum
become irreversibly bound together: aggregates. The
presence of a secondary minimum leads to flocculates.
Flocculates may be spheroidally-shaped clusters or chain-
like structures. At higher concentrations, chains develop
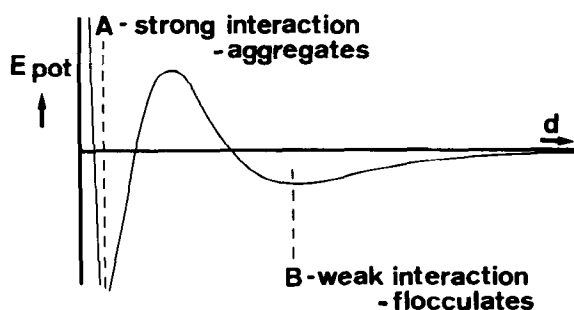into a network, which ultimately extends throughout the

Fig. 2

● Potential interaction energy between particles as function of their distance. Potential energy wells correspond to preferential distances.

Fig. 4

● Structural model of Cab-O-Sil stabilized stationary phase film

whole of the available space. These concepts will be used to describe the structure of dispersions of silica particles.

The size of the primary Cab-O-Sil particles is approximately 15 nm. Scanning electron micrographs of Cab-O-Sil layers revealed that the structural units of the layer were virtually spherical particles 80–150 nm diameter, cf. Fig. 3. Apparently this is the Cab-O-Sil aggregate size. *Bondi* and *Penther* [15] studied dispersions of fumed silica and found that the aggregates formed chain-like agglomerates when the dispersion was not sheared. The structural model is depicted in Fig. 4. *Michaels* and *Bolger* [16] derived theoretically that such structured dispersions should show a finite value of the yield stress, i.e. at low stresses and strains *the dispersion behaves like solid.* A large body of experimental work supports this conclusion [14]. In this
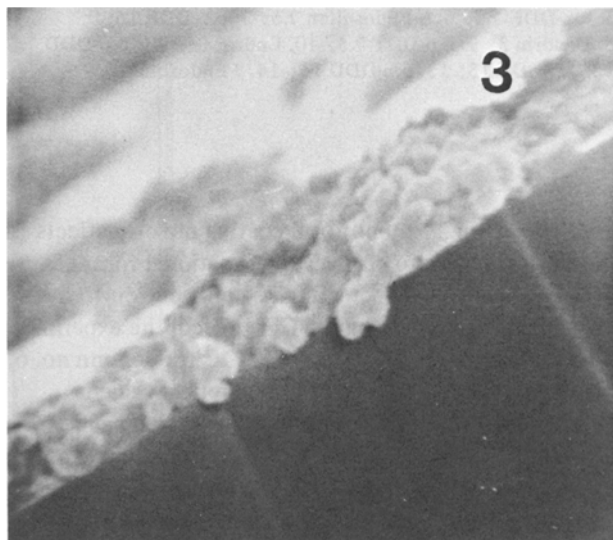
Table I. Column properties for Cab-O-Sil/OV-225 SPOT columns (temp.: 230 °C; obtained from n-fatty acid methyl esters)

Column	1	2	3	4	5	6
Length m	45	62	56	68	48	69
Id mm	0.4	0.4	0.4	0.4	0.4	0.4
Plates m^{-1} (n-C_{23} Me-ester)	2290	2240	2390	2240	2420	2240
H_{min} mm	0.43	0.45	0.42	0.44	0.41	0.44
k (n-C_{23}Me-ester)	4.5	4.2	4.1	5.5	4.4	5.5
V_G/V_L a)	320	340	350	260	325	260
TZC_{19}–C_{23} Me-ester	60	59	56	66	56	65
coating eff.	77	75	78	75	80	76
$\bar{u}_{lin}$ cm/s b)	8.4	8.9	8.1	9.2	9.2	9.1
C_L for C_{22}-Me-ester μs c)	–	–	–	–	–	520

a) at room temperature

b) near optimum velocity of 6–8 cm/s

c) at 225 °C, k = 5.1

Fig. 3

● Scanning electron micrograph (5 × 5 μm) of cross-section of Cab-O-Sil layer after first coating step. Diameter of structural units 0.1–0.15 μm.

Bottom right: cross-section of glass column
Centre: cross-section of Cab-O-Sil layer
Top left: inner surface of column, viewed at an angle of only a few degrees.
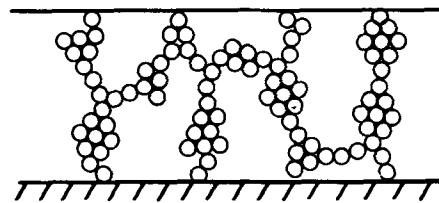
study we observed yield stresses of 100 N/m² and over at volumetric Cab-O-Sil concentrations of only 5 %, albeit at room temperature. The occurrence of such high yield stresses readily explains the remarkable stability of Cab-O-Sil columns, the stress induced by carrier gas flow and surface tension forces being 1–3 orders of magnitude smaller. The designation PLOT column no longer being applicable, we will further refer to this type of column as *stabilized phase open tubular SPOT* columns.

Although the Cab-O-Sil network causes the stationary phase to behave like a solid, the network itself is not static. The Brownian movement constantly breaks down and reforms interparticulate bonds. Thus, during conditioning the stabilizing network may grow through the whole of the stationary phase volume.

Resistance to mass transfer in the liquid phase

From a theoretical point of view the liquid phase mass transfer term, C_L, should increase for PLOT, WCOT and SPOT columns in that order. For SPOT columns, to account for the presence of solid material in the liquid

phase, the C_L-term in the Golay equation must be rewritten as

$$C_L = \frac{2}{3} \frac{k}{(1+k)^2} \frac{d_F^2}{D_L} \frac{T^2}{C\,\epsilon_F^2} \qquad (1)$$

where

d_F is film thickness in absence of solid material

ϵ_F is volume fraction of stationary phase in retentive film

T is tortuosity and

C is constriction of diffusion paths.

Following the procedure of *Knox* and *McLaren* [17] the factor T^2/C for the structural model of Fig. 4 was approximated as a function of ϵ_F. The value of $T^2/C\,\epsilon_F^2$, calculated as a function of ϵ_F, is given in Table II. It appears that the presence of Cab-O-Sil increases the C_L-term substantially, as compared with WCOT columns.

Table II. Correction factors to account for presence of Cab-O-Sil in retentive film, as function of volume fraction of stationary phase in film

ϵ_F	$\dfrac{T^2}{C\,\epsilon_F^2}$
1.00	1.00
0.95	1.27
0.90	1.62
0.85	2.07
0.80	2.65
0.75	3.42
0.70	4.43

Formula (1) predicts a C_L-term for C_{22}-Me-ester at 225 °C for column no. 6, Table I, of 170—230 μs. For D_L the diffusion coefficient of n-C_{25} alkane was used [18] and ϵ_F was estimated from micrographs to be 0.7—0.75 at room temperature. The experimental value of C_L was 520 μs, in reasonable agreement with the predicted value.

The higher C_L-term of SPOT columns as compared to WCOT columns, however, does not affect to an unacceptable extent the column performance. In practice, the efficiency of open tubular columns primarily depends on the gas phase term, Cg. Neglecting the C_L term, *Ettre* [19] defined the coating efficiency CE as

$$CE = \frac{HETP\ min}{HETP\ exp} \cdot 100\ \%$$

where $HETP\ min = r \sqrt{\dfrac{1 + 6k + 11k^2}{3(1+k)^2}}$

and HETP exp is the experimental plate height at optimum carrier gas velocity. The definition of CE is based on the *Golay* equation [20] which is valid for uniformly coated open tubular columns. Deviations of CE from the ideal value of 100 % are caused by C_L being finite and by non-
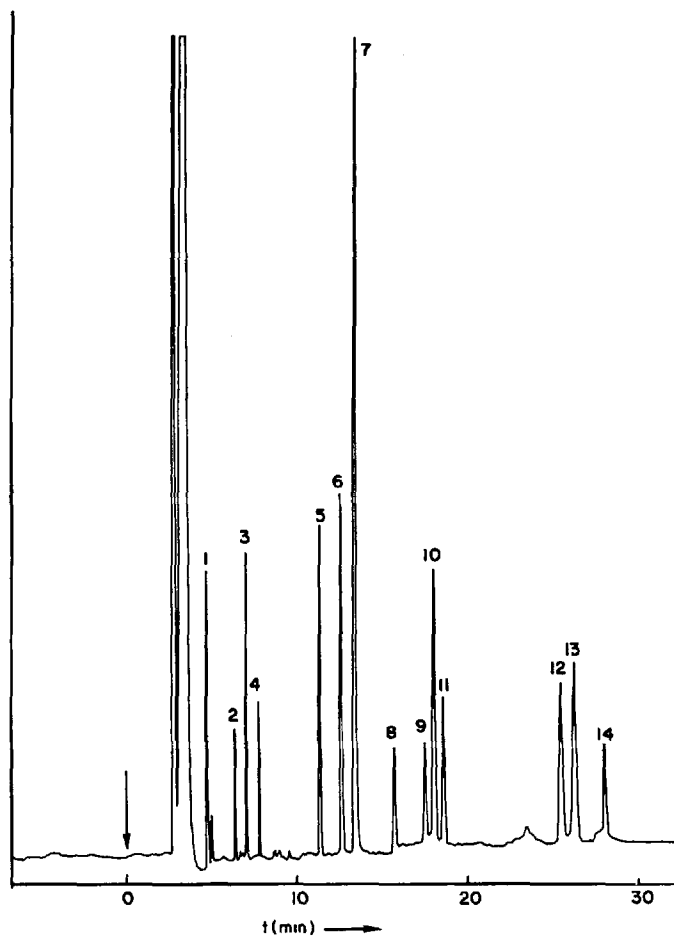

Fig. 5

● Ultra-trace analysis of standard pesticide mixture.
  Column: 26.5 m × 0.4 mm Cab-O-Sil/OV-225   at 217 °C
  Carrier gas: Ar/CH_4 95 : 5
  Detector: EC [63]Ni

1. HCB 1.5 pg; 2. α-BHC 1.5; 3. Aldrin 3; 4. γ-BHC 1.5;
5. o,p-DDE 7.5; 6. α-Endosulfan 7.5; 7. p,p-DDE 15;
8. Dieldrin 3; 9. o,p-DDT 7.5; 10. Endrin 15; 11. o,p-DDD 7.5;
12. p,p-DDT 15; 13. p,p-DDD 15; 14. β-Endosulfan 7.5.

uniform coating. Non-uniform coating directly affects the C_L term but also changes the functional form of Golays Cg term thus increasing its contribution to the plate height and reducing the CE. Indeed, the experimental value of Cg for C_{22}-Me-ester at 225 °C for column no. 6, Table I, was 2480 μs, while the theoretical value is 2180 μs. The theoretical value was calculated using Dg = 0.0652 cm^2/s, as obtained from the Hu-curve at low linear gas velocities. The C_L term appears small compared with the Cg term. The overall effect is a CE of 75—80 %, cf. Table I, which is close to the ideal value. We may conclude that the role of the C_L term is a minor one in SPOT columns as it is in WCOT columns.

Applications

Cab-O-Sil/OV-225 SPOT columns as described in this paper show favourable adsorption properties. This is demonstrated in Figs. 5 and 6, where an ultra trace
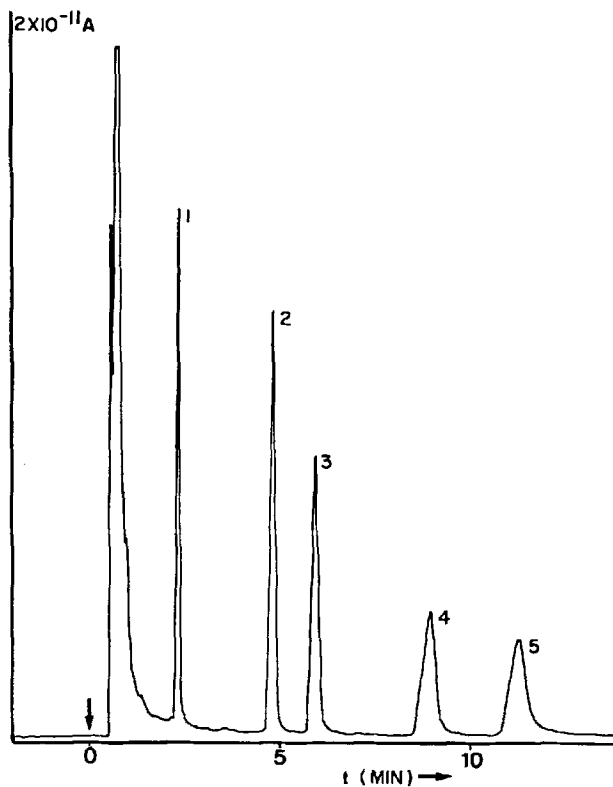
Fig. 6

● Chromatogram of mixture of underivatized antiepileptics.
Column: 4.5 m × 0.4 mm Cab-O-Sil/OV-225 at 210 °C
Carrier gas: N_2

1. Phenobarbital; 2. cyheptamide; 3. carbamezepine;
4. primidone; 5. phenytoin.

analysis of pesticides and a routine analysis of under-
ivatized anticonvulsant drugs are shown respectively. An
example of a slightly more polar stationary phase is given
in Fig. 7 where AN-600 is used instead of OV-225 for the
analysis of TMS-derivatives of steroids.

SPOT columns have been used in routine analysis for over
11 months at 210 °C without any reduction in column
performance [8]. At 230 °C, however, OV-225 columns
have a life time of only a few weeks; the recommended
maximum operating temperature is 225 °C. The lifetime
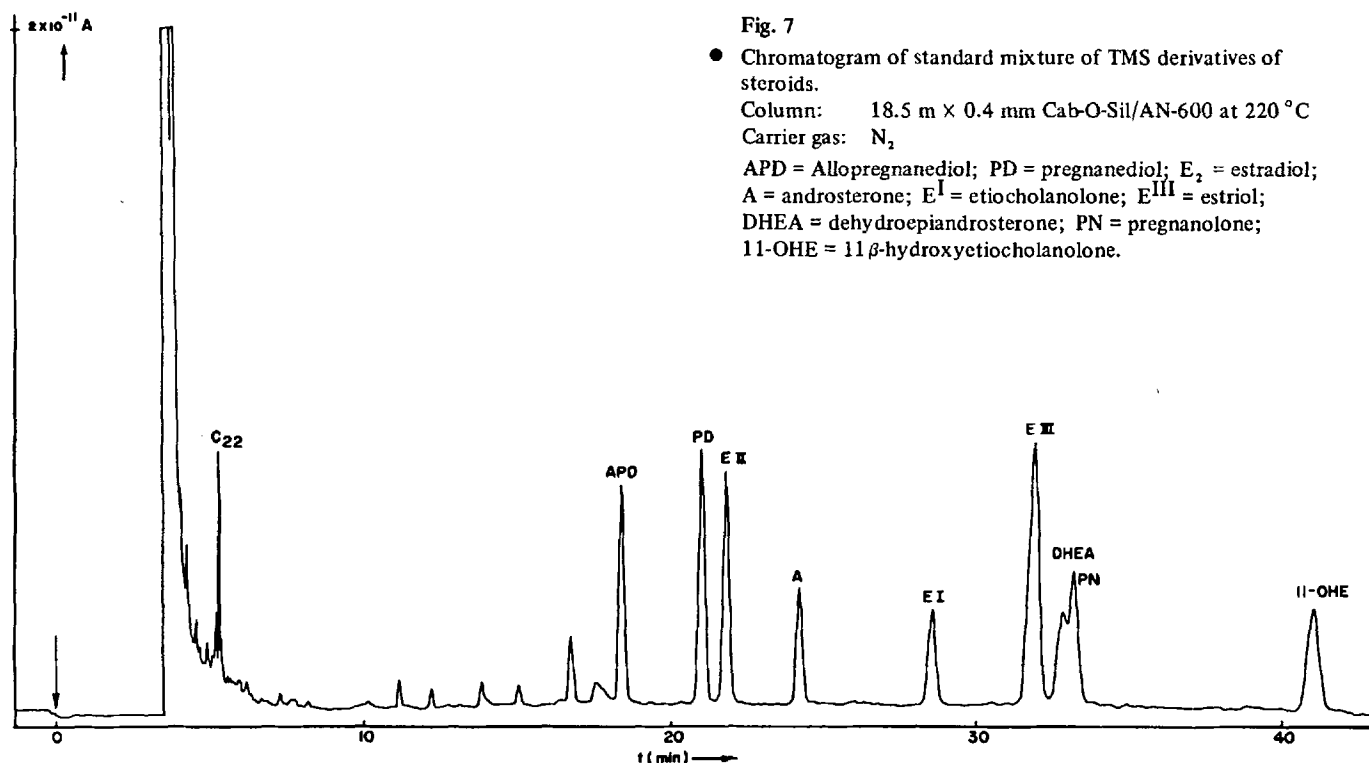is 3–4 months at this temperature.

Fig. 7

● Chromatogram of standard mixture of TMS derivatives of
steroids.
Column: 18.5 m × 0.4 mm Cab-O-Sil/AN-600 at 220 °C
Carrier gas: N_2

APD = Allopregnanediol; PD = pregnanediol; E_2 = estradiol;
A = androsterone; E^I = etiocholanolone; E^{III} = estriol;
DHEA = dehydroepiandrosterone; PN = pregnanolone;
11-OHE = 11 β-hydroxyetiocholanolone.

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