

CHROMSYMP. 1635

PREPARATION OF SILICONE-COATED 5–25- μm I.D. FUSED-SILICA CAPILLARY COLUMNS FOR OPEN-TUBULAR LIQUID CHROMATOGRAPHY

O. VAN BERKEL-GELDOF, J. C. KRAAK and H. POPPE*

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam (The Netherlands)

SUMMARY

Fused-silica capillaries of I.D. of 5, 10 and 25 μm were coated with PS-255 or PS-264 in order to achieve highly efficient columns for reversed-phase open-tubular liquid chromatography. Various coating solvents were tested, of which Freon-114 showed the most promising features with respect to speed of coating and ease of handling. The highest efficiency was obtained with a 1 m \times 5.0 μm I.D. column, showing 250 000 and 58 800 plates for solutes with capacity factors 0.2 and 2.6, respectively, at approximately six times the optimum linear velocity.

INTRODUCTION

Open-tubular liquid chromatography (OTLC) will only be competitive with packed-column LC with respect to speed of separation and efficiency provided that capillaries with inner diameters of 5–10 μm can be coated with a uniform, non-extractable layer 0.1–1 μm thick^{1–4}. The requirements put on the magnitude of the external peak broadening, which should be kept at about 1 nl (ref. 2), can be met by applying the techniques of on-column laser-induced fluorescence detection^{5–9} and split injection¹⁰.

For on-column optical detection and for practical reasons, fused-silica capillaries are to be preferred to glass capillaries. However, deposition of a retentive layer by chemical bonding on the fused-silica surface is difficult because of the inertness of this material. Recently, the techniques used for coating and immobilization of silicone phases on fused-silica capillaries to prepare columns for gas chromatography (GC) and supercritical fluid chromatography (SFC) have been applied successfully to the preparation of 5–50- μm I.D. columns for LC^{11–14}.

An important factor in the coating of thick films is the viscosity of the coating solution. On the one hand too high a viscosity of the stationary phase may prevent the coating of thick films because of poor solubility in the coating solvent, and on the other too low a viscosity of the stationary phase appeared to cause droplet formation during evaporation and cross-linking¹⁴. In addition, the physical properties of the coating solvent may play a crucial role in the successful preparation of fused-silica open-tubular columns. The application of different coating solvents is discussed below.

It is known that the methylvinylsilicone gum PS-255 is to be preferred in the preparation of thick, immobilized layers^{1,5}. In this study, PS-255 and PS-264, a methylphenylvinylsilicone gum, were selected for coating small-diameter capillaries.

EXPERIMENTAL

Materials

Fused-silica capillaries of I.D. 10 and 25 μm were obtained from SGE (Ringwood, Australia) and 5- μm I.D. fused-silica capillary from Polymicro Technologies (Phoenix, AZ, U.S.A.).

The stationary phases were polymethylsilicone fluid OV-101 purchased from Chrompack (Middelburg, The Netherlands), PS-255 (dimethyl- + 1–3% methylvinyl-silicone gum) and PS-264 (dimethyl- + 3–7% diphenyl- + 0.5–1% methylvinyl-silicone gum) from Fluka (Buchs, Switzerland) and polymethylhydrosilane and dicumyl peroxide from Merck (Darmstadt, F.R.G.).

The coating solvents were *n*-pentane (J. T. Baker, Deventer, the Netherlands), *n*-butane (Matheson, Oevel, Belgium) and 1,2-dichlorotetrafluoroethane (Freon-114) (Ucar, Nieuw Vennep, The Netherlands).

Polyaromatic hydrocarbons and anthracene derivatives, used as test compounds, were obtained from Janssen (Beerse, Belgium). Stock solutions of these compounds were prepared in acetonitrile–water (40:60, v/v).

All solvents were of spectroscopic quality and were filtered through a 0.2- μm Optex filter (Millipore, Molsheim, France) prior to use.

Apparatus

The chromatographic system is shown schematically in Fig. 1. For 25- μm I.D. columns and low flow-rates, the system was equipped with a helium-pressurized vessel, acting as a constant-pressure pump, which was replaced with an LC pump (Orlita,

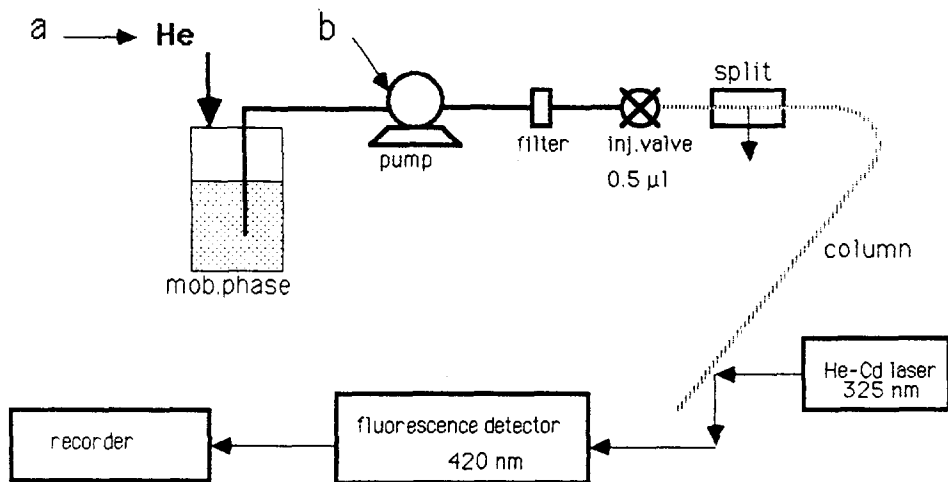


Fig. 1. Schematic representation of the chromatographic OTLC set-up. Supply of the mobile phase by (a) helium pressurized vessel and (b) LC pump.

Giessen, F.R.G.) for 5- and 10- μm I.D. columns. Injections were made by means of a 0.5- μl injection valve (Model 7520, Rheodyne, Berkely, CA, U.S.A.) combined with a laboratory-made splitting device, allowing injection volumes of a few picolitres.

On-column laser induced fluorescence detection was performed with a He-Cd laser as excitation source, excitation wavelength, $\lambda_{\text{ex}} = 325 \text{ nm}$ (Model 4207 NB, Liconix, Sunnyvale, CA, U.S.A., or Model 56X, Omnichrome, Chino, U.S.A.) and a modified fluorimeter (M4Q III, Zeiss, Jena, G.D.R.) as photodetector, with emission wavelength, $\lambda_{\text{em}} = 420 \text{ nm}$.

Column preparation

The capillaries were pretreated and coated as described previously¹⁴. Briefly, after hydrothermal treatment with a 10% (v/v) solution of hydrochloric acid and deactivation of the fused-silica surface by polymethylhydrosilane, the capillaries were coated by the static method. Stationary phase solutions were prepared in *n*-pentane, *n*-butane or Freon-114.

Fig. 2 shows the reservoir for filling the capillaries at ambient temperature with a coating solution, prepared in *n*-pentane at least one day before use. Dicumyl peroxide was added in the ratio stationary phase: dicumyl peroxide = 20:1 (w/w) just before filling.

The filling vessel for coating solutions in either *n*-butane or Freon-114 is shown in Fig. 3. The cooled vessel containing the stationary phase and dicumyl peroxide in the ratio 20:1 (w/w) was filled with the solvent, closed and allowed to stand overnight at room temperature to dissolve the silicone. The capillaries were filled at a temperature of 5°C below the boiling point of the applied solvent (*n*-butane, b.p. = -0.5°C; Freon-114, b.p. = 3.8°C)

After filling, the capillaries were connected to a vacuum pump with both ends when *n*-pentane was used and with one end in the case of *n*-butane or Freon-114. In the latter instances one end of the capillaries was closed by means of a septum. Evacuation was carried out at 10–15°C above the boiling point of the applied solvent.

Next, the columns were purged with helium to remove traces of solvent and oxygen and, after flame-sealing, the silicone phase was immobilized by cross-linking at 260°C. Finally, the column was rinsed with acetonitrile–water (40:60, v/v) and dried at 140°C under a stream of helium.

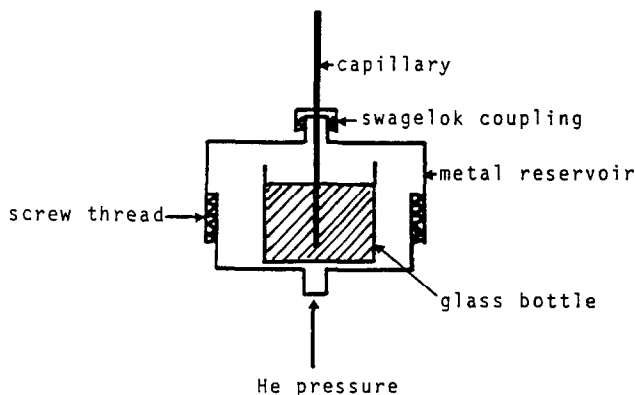


Fig. 2. Schematic representation of the reservoir to rinse and fill the fused-silica capillaries.

TABLE I
DIMENSIONS AND COATING CONDITIONS OF FUSED-SILICA CAPILLARY COLUMNS

Column No.	Length (cm)	I.D. ^a (μm)	Stationary phase	Concentration (mg/ml)	Coating solvent	I.D. ^b (μm)	d_f (μm)	Phase ratio	Coating yield (%)
1	94	25.10	PS-255/OV-101	120	<i>n</i> -Pentane	23.77	0.67	0.126	84
2	98	26.09	PS-255	50	<i>n</i> -Butane	25.51	0.29	0.047	86
3	67	26.09	PS-255	100	<i>n</i> -Butane	24.95	0.57	0.100	84
4	100	28.46	PS-255	60	Freon-114	27.82	0.32	0.048	50 ^c
5	124	10.24	PS-255	40	<i>n</i> -Pentane	10.08	0.08	0.032	34 ^c
6	67	10.18	PS-255	20	<i>n</i> -Butane	10.11	0.04	0.016	79
7	100	5	PS-255	35	<i>n</i> -Pentane	5.00	0.02 ^d	0.017 ^d	93
8a ^e	83	5	PS-255	35	<i>n</i> -Pentane	5.38	0.045 ^d	0.035 ^d	
8b ^e	69	5	PS-255	35	<i>n</i> -Pentane	5.38	0.02 ^d	0.016 ^d	
9	67	25.13	PS-264	35	Freon-114	24.44	0.34	0.059	

^a Before coating.

^b After coating and rinsing.

^c Untreated fused-silica capillary.

^d Calculated from capacity factor *versus* phase ratio plot.

^e 8a and 8b before and after removal of a blocked part, respectively.

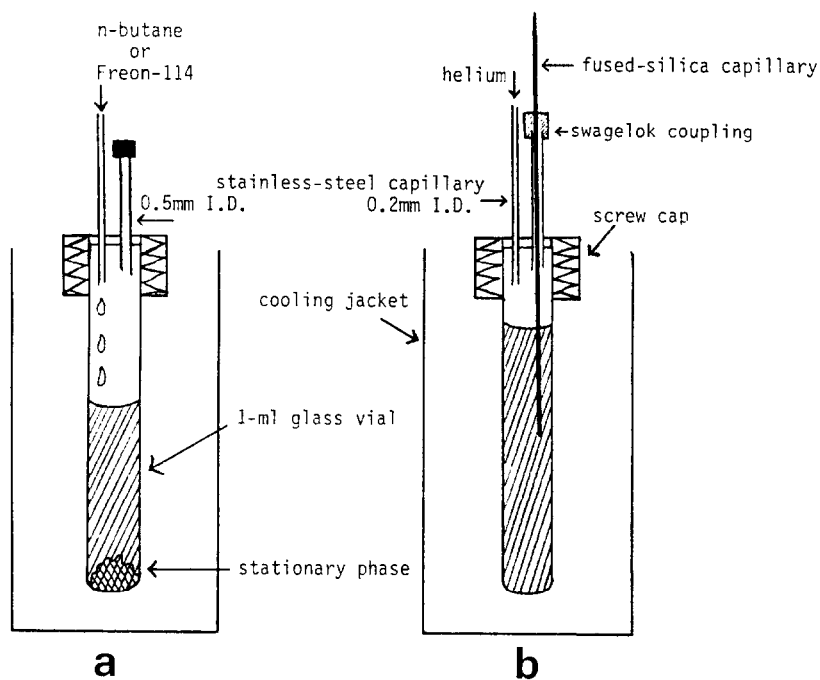


Fig. 3. Schematic representation of the device for (a) dissolving the stationary phase and (b) filling the fused-silica capillaries with the coating solution in *n*-butane or Freon-114.

Film thickness

Before and after coating, the inner diameter of the capillaries was determined by an aerodynamic method, based on the Hagen-Poiseuille relationship, as described previously¹⁶. The dimensions of the fused-silica capillary columns and coating conditions are given in Table I. The expected film thickness, d_f , was calculated from the column diameters before coating and the amount of silicone material applied, according to the equation

$$d_f = \frac{d}{2} \left(1 - \sqrt{1 - \frac{C}{\rho}} \right) \tag{1}$$

where C = concentration of the coating solution in g ml^{-1} , d = capillary diameter before coating and ρ = density of the silicone phase (0.98 g ml^{-1}). For the calculation of the immobilization yield, the film thickness obtained from measurement of the column diameter before and after the treatment was compared with the expected film thickness. The stationary to mobile phase ratio was found from the equation

$$V_s/V_m = d_c d_f / (0.5 d_c - d_f)^2 \tag{2}$$

where V_s = volume of stationary phase, V_m = volume of mobile phase, and d_c = column diameter after coating and rinsing.

It should be noted that for the 5- μm I.D. columns the film thickness and phase ratio were calculated from the capacity factor *versus* phase ratio plots (see Fig. 4).

Retention and efficiency

Capacity factors (k') were calculated from $k' = (t_r/t_0) - 1$, where t_r and t_0 are the retention times of a retained and an unretained solute (anthranilic acid), respectively.

The experimental data for plate heights (H) and linear velocities (v) were fitted to the theoretical values as calculated with the Golay equation:

$$H = \frac{2D_m}{v} + \frac{(1 + 6k' + 11k'^2)d_c^2v}{96D_m(1 + k')^2} + \frac{2k'd_f^2v}{3D_s(1 + k')^2}$$

The diffusion coefficients of the test solutes in the mobile phase, D_m , calculated according to the Wilke–Chang equation¹⁷, are given in Table II.

TABLE II

DIFFUSION COEFFICIENTS OF THE TEST SOLUTES IN ACETONITRILE–WATER (40:60, v/v) CALCULATED ACCORDING TO THE WILKE–CHANG EQUATION

Solute	Diffusion coefficient, D_m ($\text{m}^2 \text{s}^{-1}$)	Solute	Diffusion coefficient, D_m ($\text{m}^2 \text{s}^{-1}$)
Anthranilic acid	$0.70 \cdot 10^{-9}$	9-Methylanthracene	$0.54 \cdot 10^{-9}$
Anthracene	$0.57 \cdot 10^{-9}$	9-Vinylnanthracene	$0.51 \cdot 10^{-9}$
9-Cyanoanthracene	$0.54 \cdot 10^{-9}$	9-Phenylanthracene	$0.46 \cdot 10^{-9}$

Diffusion coefficients in the stationary phase, D_s , of anthracene and pyrene in immobilized polymethylsilicones have been found to be $5 \cdot 10^{-12}$ – $27 \cdot 10^{-12} \text{m}^2 \text{s}^{-1}$ (ref. 14). It was assumed that the diffusion coefficients using a different stationary phase in the present systems are of the same order of magnitude and a value for D_s of $10^{-11} \text{m}^2 \text{s}^{-1}$ was assumed in calculations of the theoretical plate height.

RESULTS AND DISCUSSION

Column preparation

Effect of pretreatment. Although it has been reported that fused-silica capillaries possess sufficient wettability for polymethylsilicones^{18,19}, others have found that it is necessary to pretreat this inert material before coating in order to achieve highly efficient columns^{20–22}. Then the coating procedure should involve four steps: hydrothermal treatment, deactivation, static coating and cross-linking.

The effect of the pretreatment was examined by coating untreated fused-silica capillaries and comparing the coating yield with that for pretreated capillaries. From Table I it can be seen that in the two instances of coating untreated fused-silica capillaries only half of the expected film thickness was obtained. This phenomenon is probably due to a loss of stationary phase by solvent rinsing, which leads to the conclusion that the immobilization of the stationary phase is insufficient. In contrast, hydrothermally treated and deactivated fused-silica columns show a coating and immobilization yield of 84–90%.

Coating solvent. Apart from the structure of the stationary phase and the nature of the capillary surface, the successful coating of capillaries also depends on the choice of the coating solvent and coating temperature²³. The use of dicumyl peroxide as cross-linking agent prevented the application of high coating temperatures. Coating with *n*-pentane as coating solvent at 45°C took several days, leading to an increasing risk of blockage during evaporation, possibly also due to premature decomposition of dicumyl peroxide and cross-linking. A high coating speed at low temperature has been reported by application of liquefied gases as coating solvents^{24,25}. Because of their favourable boiling points and solubility of the stationary phase, *n*-butane (b.p. -0.5°C) and Freon-114 (b.p. 3.8°C) were tested for their applicability as coating solvents. It appeared to be impossible to dissolve over 6% (w/v) of the gum phases in Freon-114 and therefore with this solvent the phase ratio is limited to about 0.05.

In *n*-butane, up to 15% (w/v) of the silicone gums could be dissolved easily. The main drawback of these concentrated coating solutions was the high viscosity, resulting in longer times for filling and evaporation at an increasing risk of blocking and "bubble bursting", especially when coating small-diameter capillaries. Nevertheless, the use of *n*-butane rather than *n*-pentane is to be preferred, because coating is less time consuming (a few hours *versus* a few days).

Probably the long coating times with *n*-pentane at 45°C had an adverse effect on the column stability. During chromatographic testing of the columns, columns 1 and 8a became blocked at high flow-rates and, after removal of the blocked part of the column, a significantly smaller phase ratio was indicated by lower capacity factors. It is assumed that the insufficient immobilization of the stationary phase is due to degradation of the cross-linking agent DCuP under these coating conditions. The coating times with Freon-114 appeared to be similar to those with *n*-butane.

The choice of the coating solvent should be based on the speed of coating at low temperatures, the film thickness required and ease of handling. Considering these aspects, Freon-114 appears to be the preferred choice as a solvent for coating a layer on the inner wall of fused-silica capillaries, unless very thick layers are required, in which case *n*-butane could be used.

Performance of prepared columns

Retention. A linear relationship between the phase ratios of different columns coated with PS-255 and the capacity factor of various test solutes was found, as shown in Fig. 4. The good reproducibility of the coating method is demonstrated by the fact that no significant differences in the retention behaviour of columns with various inner diameters were observed.

As shown in Table III, the capacity factors of the test solutes obtained with the PS-264 column are approximately double those with comparable PS-255 columns. A further increase in capacity factors can be achieved by coating a thicker film of PS-264 with *n*-butane as coating solvent.

Efficiency. A number of columns prepared with the described procedure were tested with respect to efficiency by measuring chromatograms of mixtures of anthracene derivatives at various mobile phase velocities and by the construction of H/v plots from the data obtained. Figs. 5-10 show some results acquired during this study, which will now be discussed briefly in the order 25- μ m I.D. columns (Figs. 5 and 6), 10- μ m I.D. columns (Figs. 7 and 8) and 5- μ m I.D. columns (Figs. 9 and 10).

TABLE III
 COMPARISON OF CAPACITY FACTORS OF ANTHRACENE DERIVATIVES ON COLUMNS COATED WITH PS-255 AND PS-264 WITH ACETONITRILE-WATER (40:60, v/v) AS THE MOBILE PHASE

Stationary phase	Phase ratio	Solute				
		9-Cyanoanthracene	Anthracene	9-Methylanthracene	9-Vinylanthracene	9-Phenylanthracene
PS-255	0.048	0.3	1.3	1.7	2.8	5.1
PS-264	0.059	0.9	3.0	4.0	6.6	13.5

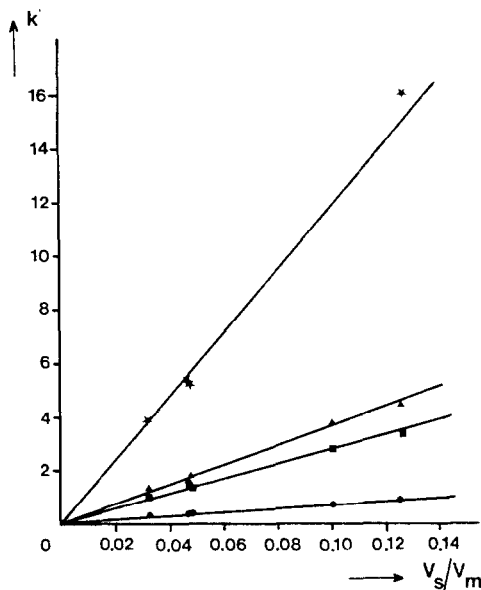


Fig. 4. Capacity factors of test solutes *versus* the phase ratio on different fused-silica columns coated with PS-255. Mobile phase: acetonitrile–water (40:60, v/v). ● = 9-Cyanoanthracene; ■ = anthracene; ▲ = 9-methylanthracene; ★ = 9-phenylanthracene.

The chromatogram in Fig. 5 illustrates the mediocre efficiency that can be obtained with these large-diameter columns. In fact, a packed HPLC column leads to higher resolution in the same or a shorter analysis time. The study of such columns was included, however, in order to obtain a clear idea of the performance as a function of

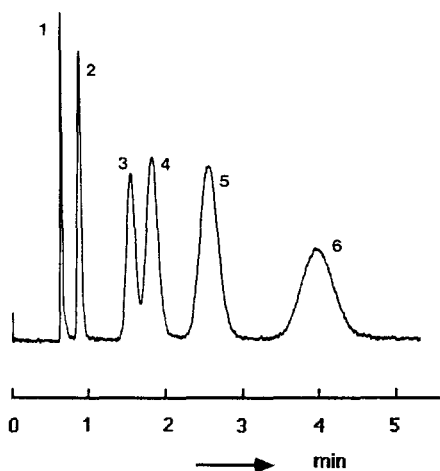


Fig. 5. Chromatogram of a test mixture of anthracene derivatives on column 2, 66.5 cm × 26.1 μm I.D. fused silica coated with PS-255. Film thickness, 0.3 μm; mobile phase, acetonitrile–water (40:60, v/v); pressure, 6 bar; linear velocity, 15.6 mm s⁻¹. Peaks: 1 = anthranilic acid; 2 = 9-cyanoanthracene; 3 = anthracene; 4 = 9-methylanthracene; 5 = 9-vinylanthracene; 6 = 9-phenylanthracene.

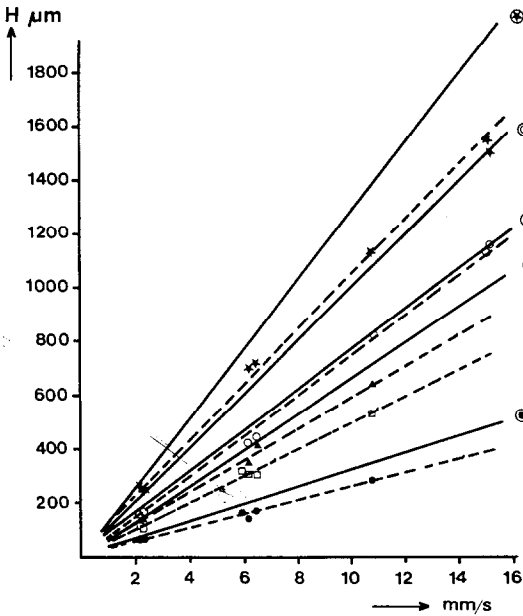


Fig. 6. Theoretical and experimental H versus v plots for anthracene derivatives on column 2. Mobile phase, acetonitrile-water (40:60, v/v). Theoretical curves calculated with D_m values from Table II and D_s values equal to $10^{-11} \text{ m}^2 \text{ s}^{-1}$. Solid lines, theoretical calculated H values; broken lines, experimental H values. ● = 9-Cyanoanthracene, $k' = 0.35$; □ = anthracene, $k' = 1.34$; ▲ = 9-methylanthracene, $k' = 1.75$; ○ = 9-vinylanthracene, $k' = 2.9$; ★ = 9-phenylanthracene, $k' = 5.0$.

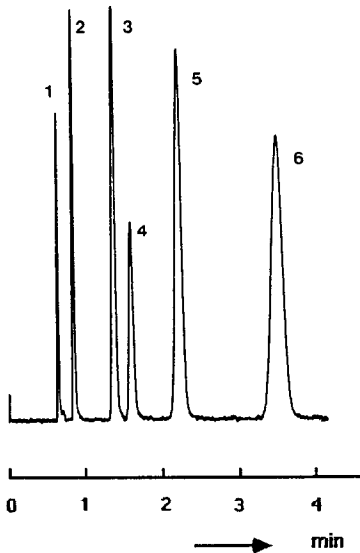


Fig. 7. Chromatogram of a test mixture of anthracene derivatives on column 5, 112 cm \times 10.2 μm I.D. fused silica, coated with PS-255. Film thickness, 0.08 μm ; pressure, 80 bar; linear velocity, 29 mm s^{-1} . Other experimental conditions and peaks as in Fig. 5.

diameter. The experimental H/v plots obtained with these columns, illustrated in Fig. 6, are straight lines that pass through the origin. This is in accordance with theory, as the axial diffusion can be neglected at these large diameters. For comparison, the theoretical lines, calculated with the extended Golay equation, are shown.

The diffusion coefficient in the mobile phase, D_m , was calculated by means of the original form of the Wilke–Chang equation, while the value for the stationary phase was set at $10^{-11} \text{ m}^2 \text{ s}^{-1}$. It should be noted that for all columns the contribution of the stationary phase term, H_{Es} , is less than 10%.

Although the discrepancy between the calculated and experimental curves in Fig. 6 is not dramatic, a systematic deviation occurs, the experimental values for the retained components being 20–40% smaller than the predicted values. This indicates first that uniform stationary layers were probably obtained and second that some inaccuracies were present in the calculation of theoretical H values. Possible causes include inaccurate capacity factors, inaccurate D_m and/or D_s values or secondary flow effects. The first is not likely as the unretained marker (anthranilic acid) elutes together with, or earlier than, compounds such as uranyl acetate, 2-naphthol and salicylic acid.

Inaccuracies in D_m values may occur; *e.g.*, Snyder²⁶ published another calculation scheme for which a higher accuracy is claimed and that generally leads to

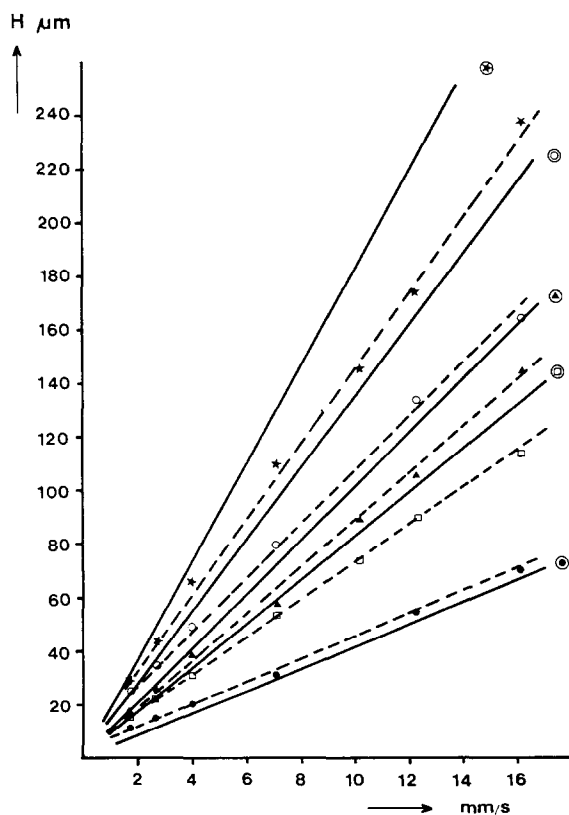


Fig. 8. Theoretical and experimental H versus v plots for anthracene derivatives on column 5. Details as in Fig. 6. ●: $k' = 0.27$; □: $k' = 0.97$; ▲: $k' = 1.28$; ○: $k' = 2.11$; ★: $k' = 3.77$.

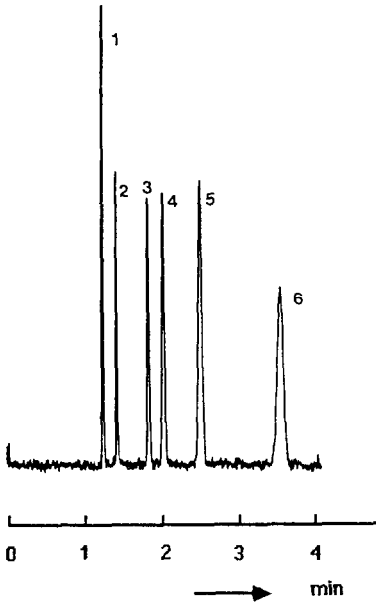


Fig. 9. Chromatogram of a test mixture of anthracene derivatives on column 8b, 67 cm \times 5.4 μ m I.D. fused silica, coated with PS-255. Film thickness, 0.02 μ m; pressure, 50 bar; linear velocity, 9.2 mm s⁻¹. Other experimental conditions and peaks as in Fig. 5.

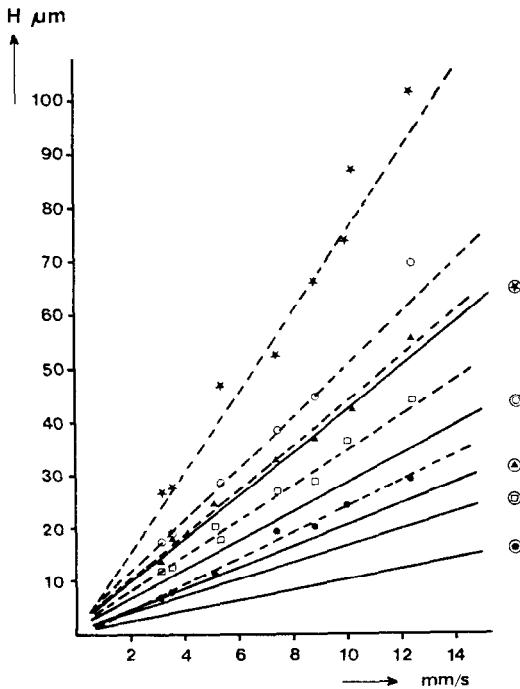


Fig. 10. Theoretical and experimental H versus v plots for anthracene derivatives on column 8b. Details as in Fig. 6. ●: $k' = 0.18$; □: $k' = 0.51$; ▲: $k' = 0.68$; ○: $k' = 1.08$; ★: $k' = 1.96$.

about 30% higher values. The method of choice, of course, would be the experimental evaluation of D_m values, *e.g.*, based on the Aris–Taylor equation as described by Huber and van Vught²⁷. In fact, the H measurements of the unretained solute constitute such a determination in rudimentary form. Unfortunately, the virtual coincidence of the calculated and experimental values for anthranilic acid does not support the assumption of higher values of D_m . As a rough estimate of D_s is inserted for all solutes, inaccuracies in these values are very large. The observed deviation, however, cannot be explained by these inaccuracies as the term containing D_s in the expression for the plate height constitutes less than 10% of the total H value.

As for the secondary flow effect, calculation according to the work of Tijssen²⁸ shows that this effect will not exert any significant influence with the narrow tubes used here. At present, therefore, one has to conclude that the 30% deviation between the theoretical and experimental H values remains unexplained.

The data for the analytically more interesting experiments with 10- μm I.D. columns show a similar deviation, although of smaller magnitude. The experimental curves for the unretained solute are slightly lifted upward, which may indicate the onset of the influence of external volume broadening effects. However, this would hardly affect the H values for more retained solutes.

According to the analysis by Knox and Gilbert¹, 10- μm I.D. columns would be preferable to packed columns when plate numbers, N , in excess of 30 000 are required. Fig. 7 illustrates this. Although the N value is only 4000 for component 5 ($k' = 2.1$), the retention time and pressure are very favourable. Therefore, the operation of a similar column with, *e.g.*, five times this length at the same linear velocity would indeed constitute a very good performance.

Columns of 5- μm I.D. are even more attractive from the point of view of application. In the chromatogram in Fig. 9, peak 5 has 16 800 plates, generated with $t_0 = 73$ s. A few of these short columns in series or one 2-m long, would generate over 50 000 plates in a few minutes. The comparison of the experimental and calculated values for H gives completely different results, the experimental values being 1.5–2.5 times higher than expected. The deviation is more severe for the less retained solutes. It is noteworthy that the peaks are slightly fronting on this column (8b), resulting in peak asymmetries with A values from 0.95 to 0.77 for all solutes. With column 7 (1 m \times 5.0 μm I.D.), the peaks were symmetrical, which leads to a higher efficiency. Plate numbers of 250 000 ($k' = 0.2$) and 58 800 ($k' = 2.6$) were obtained at a linear velocity of 3 mm/s, which is about six times the optimal linear velocity. However, with this column the experimental H values are about 30% higher than expected on the basis of theory.

Although at present we are not in the position to account fully for these deviations, we believe that the following effects play a role:

(i) External peak broadening, especially important for the less retained compounds. This would explain the decrease in the relative deviation at increased retention.

(ii) Stationary phase overload. This, to our knowledge, is the only mechanism that explains the fronting of peaks. For better analytical utility, the layer thickness should be increased by a factor of 4–10, allowing for higher mass loadability.

(iii) Possibly non-uniformity of the stationary layer also contributes to the high H values.

Although these results still leave numerous questions open, we believe that the

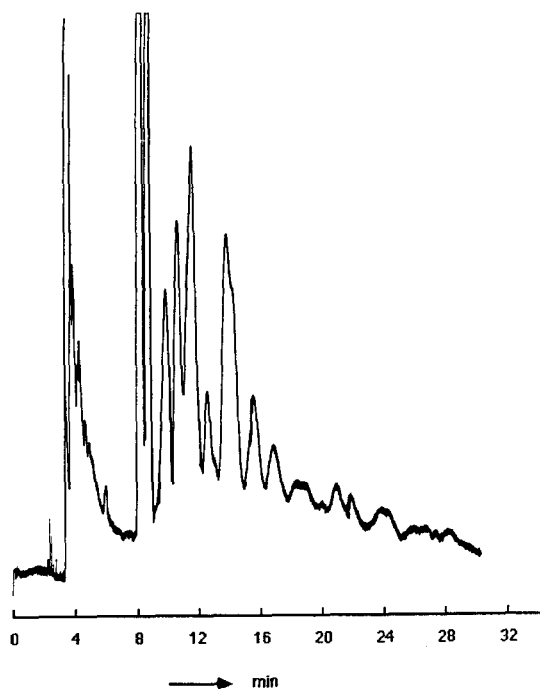


Fig. 11. Chromatogram of a coal tar extract on column 5, 101 cm \times 10.1 μ m I.D. fused silica, coated with 0.08 μ m PS-255. Mobile phase, acetonitrile–water (40:60, v/v); pressure, 15 bar; linear velocity, 5.6 mm s⁻¹; λ_{ex} = 325 nm; λ_{em} \geq 385 nm.

successful demonstration of OTLC performance, close to theoretical predictions (*i.e.*, generally within a factor of 1.5–2), is an important step towards the development of analytically useful OTLC systems. The immobilization of silicones appears to be a viable route towards such systems.

An example of the high separation power of a 10- μ m I.D. column is given in Fig. 11, showing the separation of polyaromatic hydrocarbons in a tar extract.

ACKNOWLEDGEMENT

This work was supported by Pye Unicam Ltd., Cambridge, U.K.

REFERENCES

- 1 J. H. Knox and M. T. Gilbert, *J. Chromatogr.*, 186 (1979) 405.
- 2 J. H. Knox, *J. Chromatogr. Sci.*, 18 (1980) 453.
- 3 H. Poppe, presented at the 9th International Symposium on Column Liquid Chromatography, Edinburgh, July 1–5, 1985.
- 4 J. W. Jorgenson and E. J. Guthrie, *J. Chromatogr.*, 255 (1983) 335.
- 5 H. P. M. van Vliet and H. Poppe, *J. Chromatogr.*, 346 (1985) 149.
- 6 S. Folestad, L. Johnson, B. Josefsson and B. Galle, *Anal. Chem.*, 54 (1982) 925.
- 7 S. Folestad, B. Josefsson and B. Galle, *J. Chromatogr. Sci.*, 23 (1985) 273.
- 8 E. J. Guthrie and J. W. Jorgenson, *Anal. Chem.*, 56 (1984) 483.
- 9 F. J. Yang, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 83.

- 10 V. L. McGuffin and M. Novotny, *Anal. Chem.*, 55 (1983) 580.
- 11 A. Farbrot, S. Folestad and M. Larsson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 117.
- 12 S. Folestad, B. Josefsson and M. Larsson, *J. Chromatogr.*, 391 (1987) 347.
- 13 S. Folestad and M. Larsson, *J. Chromatogr.*, 394 (1987) 455.
- 14 O. van Berkel, H. Poppe and J. C. Kraak, *Chromatographia*, 24 (1987) 739.
- 15 K. Grob and G. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 133.
- 16 P. P. H. Tock, G. Stegeman, R. Peerboom, H. Poppe, J. C. Kraak and K. K. Unger, *Chromatographia*, 24 (1987) 617.
- 17 S. Bretsznajder, *Prediction of Transport and Other Physical Properties of Fluids*, Pergamon Press, Oxford, 1971, pp. 33–39 and 358–383.
- 18 K. D. Bartle, B. W. Wright and M. L. Lee, *Chromatographia*, 14 (1981) 387.
- 19 B. W. Wright, P. A. Peaden, M. L. Lee and T. J. Stark, *J. Chromatogr.*, 248 (1982) 17.
- 20 K. Markides, B. J. Tarbet, C. L. Woolley, C. M. Schregenberger, J. S. Bradshaw and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 378.
- 21 C. L. Woolley, R. C. Kong, B. E. Richter and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1985) 329.
- 22 C. P. M. Schutjes, E. A. Vermeer and C. A. Cramers, *J. Chromatogr.*, 279 (1983) 49.
- 23 R. C. Kong, S. M. Fields, W. P. Jackson and M. L. Lee, *J. Chromatogr.*, 289 (1984) 105.
- 24 T. Wännman, L. Blomberg and S. Schmidt, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 32.
- 25 K. Janák, V. Kahle, K. Tesarik and M. Morká, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 843.
- 26 L. R. Snyder and P. E. Antle, *LC Mag.*, 3 (1985) 98.
- 27 J. F. K. Huber and G. van Vught, *Ber. Bunsenges. Phys. Chem.*, 69 (1965) 821.
- 28 R. Tijssen, *Ph.D. Thesis*, Technical University Delft, Delft, 1979.