Journal of Chromatography, 391 (1987) 347–372
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 296

PERFORMANCE AND PREPARATION OF IMMOBILIZED POLYSILOXANE STATIONARY PHASES IN 5–55 μm I.D. OPEN-TUBULAR FUSED-SILICA COLUMNS FOR LIQUID CHROMATOGRAPHY*

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(First received October 7th, 1986; revised manuscript received November 24th, 1986)

SUMMARY

Cross-linked non-polar polysiloxanes were evaluated as stationary phases in open-tubular column reversed-phase liquid chromatography. Coating of 5–55 μm I.D. fused-silica capillaries with stationary phase films of a well defined thickness in the range 0.03–1.96 μm is described for the two polysiloxane gum phases PS-255 (methylvinyl silicone) and SE-54 (methylphenylvinyl silicone). The chromatographic properties of these columns were investigated using split injection and on-column laser-induced fluorescence detection. Gas chromatography was used complementarily in the evaluation of column stability, retention and inertness. A retentive layer thickness to column diameter ratio up to 1:27 could be prepared, and a linear relationship was observed between the retention and the stationary to mobile phase volume ratio. The selectivity was related to the polysiloxane structure and was constant for films thicker than 0.25 μm. The column band-broadening was studied regarding the contribution from stationary phase diffusion, and compared with theory. Depending on film thickness, the stationary phase diffusion coefficient D_s was in the range 10^{-8}–10^{-6} cm^2/s. The highest efficiency, 351 000 plates ($k' = 0.16$), was obtained with a 1.97 m × 11.7 μm I.D. open-tubular-column. An application to the gradient separation of fluorescence labelled amino acids is presented. Preliminary results are also reported on a new type of stationary phase, created by swelling the immobilized polysiloxane with *n*-heptane. A nearly ten-fold increase in retention and a change in selectivity were obtained.

INTRODUCTION

There is a great interest in liquid chromatography (LC) using open-tubular columns (OTC). In theoretical discussions, Knox and Gilbert[1], Knox[2] and Gui-

* Presented in part at the *10th International Symposium on Column Liquid Chromatography, San Francisco, May 18–23, 1986*. The majority of papers presented at this symposium have been published in *J. Chromatogr.*, Vol. 371 (1986) and Vols. 384–386 (1987).

0021-9673/87/$03.50

ochon[3], have shown that OTC LC has inherent advantages compared with packed column LC. One of the main advantages is that OTCs have higher permeability and thus higher efficiencies can be obtained. The prerequisite is that the inner diameter is sufficiently small, *i.e.* in the range of a few micrometres.

In practice, many problems remain before complete success with OTC LC will be attained. Such columns have extremely small physical dimensions, which demand special instrumental arrangements. A lot of promising results have been published covering column preparation and equipment design[4–11]. However, very few applications that demonstrate the real potential of the technique have been made.

The main instrumental difficulty is to design and manage a detection system with such a small volume that the external contribution to peak broadening can be disregarded. On-column detection techniques are therefore of utmost importance. Although soft glass and borosilicate glass capillary columns have been used in OTC LC, fused-silica capillaries possess optical properties better suited to optimal spectroscopic detection conditions. Laser-induced fluorescence detection, where the laser beam can be focused tightly on the small diameter column, is then a practicable solution[12,13]. In addition, fused-silica capillaries have a smooth surface and the flexible material is convenient to handle.

In the literature there are different descriptions of ways to prepare non-polar stationary phases for reversed-phase (RP) OTC LC. An important aspect in OTC LC is to achieve useful capacity factors (k') with mobile phases of realistic compositions. The same degree of retention in an OTC, compared with a packed column, is not easily obtained owing to the high ratio of mobile phase to stationary phase volume[2]. Thus far, capacity factors obtained in OTC LC are only moderate compared with packed column LC.

Generally, the coating procedures used in OTC LC were first developed for gas chromatography (GC). There are three different approaches to applying non-polar stationary phases, based on mechanical deposition of liquids on the wall, chemical bonding of groups derived from monomers, and immobilization of polymers by cross-linking. Thus, the stationary phases are anchored to the wall with different mechanisms with consequently various chromatographic properties.

Mechanically coated viscous liquid phases, being attractive for the simplicity of column preparation, have been used in OTC LC[14,15], even with fused-silica columns[16]. Owing to a certain solubility of the stationary liquid in the mobile phase, the column stability is lower than that of bonded-phase columns. Furthermore, the exact film thickness is difficult to control.

In the preparation of chemically bonded stationary phases, *e.g.* octadecylsilane (ODS), the glass column material plays an important role. Columns have mainly been prepared from soda-lime glass or borosilicate glass with inner diameters down to 20 μm[17] and 8 μm[18], respectively. These columns have been subjected to various surface treatments, such as etching, in order to increase the inner surface area and the number of silanols available for bonding of the stationary phase via Si–O–Si–C bonds. Assuming that a certain stationary phase surface density can be prepared in different column dimensions, an increased retention can be obtained only with a reduced column inner diameter. Electro-etching of borosilicate columns, as described by Jorgenson and Guthrie[8], resulted in high k' values. Unfortunately, the electro-etching technique is limited to short columns owing to non-uniform etching in longer

capillaries[19]. The highest k' values have been reported on etched soda-lime glass capillaries, although these are difficult to prepare with small diameters owing to a tendency to clog[20]. Fused-silica columns in the range 30–100 μm have also been coated with ODS phases[21]. However, these columns gave low retention owing to insufficient surface coverage.

In principle, two types of siloxane stationary phase can be produced by bonding to surface silanols, *viz.* monomeric and polymeric bonded phases from monofunctional and polyfunctional reagents, respectively. The degree of cross-linking in polymeric bonded phases will depend on the type of silane used. If the trifunctional silanes react effectively they give a more dense network than does a difunctional silane. Extensive cross-linking will result in a phase of resinous character[22]. The rigid nature of such a phase results in restricted diffusion of the solute[23]. Another drawback of these polymeric phases is the difficulty in predicting the exact film thickness.

The third alternative to apply stationary phases in open tubular columns is to perform *in situ* cross-linking of silicone gums (linear high-molecular-weight polysiloxanes). By this technique, it is possible to create stationary phases of variable and well-defined thicknesses obtaining different degrees of retention, even in OTC LC[24]. The cross-linking process, which can be initiated by organic peroxides for example, results in carbon–carbon bonds between methyl and/or vinyl groups attached to silicon atoms (Si–C–C–Si, Si–C–C–C–Si). Only a small degree of cross-linking (0.1–1.0%) is required to change high-molecular-weight siloxane polymers to insoluble rubbers[25].

Silicones possess several advantageous properties[26]. The high flexibility of the Si–O bond explains the liquid-like behaviour of these polymers even at low temperatures[22], which is essential for efficient mass transfer in the stationary phase. It has been shown in GC that the solute diffusivity in gum phases is not altered by this type of cross-linking[27]. However, diffusion rates are lower than in low-molecular-weight solvents. The immobilized silicones are hydrolytically very stable and resistant to various solvents in terms of solubility[28]. Yerrick and Beck[29] reported that methanol and acetonitrile, typically used in reversed-phase LC, caused a minor (*ca.* 3%) swelling of dimethyl siloxane rubbers. The swelling effects on the chromatographic behaviour in RPLC have not been investigated. Recently, swelling of non-polar siloxanes was observed to increase the efficiency in supercritical fluid chromatography (SFC) when carbon dioxide and butane were used as mobile phases[30].

The cross-linking technique was introduced for capillary GC by Grob *et al.*[31,32] and has gained wide popularity. The columns have also been extensively used in SFC[33,34], where the stationary phases must resist strains under supercritical conditions that may be considerably more severe than in GC. For use in SFC, Fields *et al.*[35,36] have prepared immobilized phases with film thicknesses from 0.25 to 1 μm on untreated fused-silica capillaries down to 25 μm I.D. Polyorganosiloxane phases have also been applied in packed-column LC[37]. Silica and alumina particles were coated with immobilized organosiloxanes prepared from oligomers. With thin polymer films (0.27–1.52 nm), capacity factors increased with increasing film thickness.

Takeuchi *et al.*[38] introduced the cross-linking technique for OTC LC using etched soda-lime glass capillaries in the range 30–40 μm I.D. They cross-linked *in situ* different apolar polysiloxane phases typical for GC. The film thickness was not calculated since the roughened surface area of the capillary was not defined. Jorgen-

son *et al.*[39] applied the cross-linking technique to immobilize a vinyl-modified OV-17 polysiloxane in an etched 16 μm I.D. borosilicate capillary column. They stated that polymer-coated columns offer better stability than ODS bonded columns for LC. Recently Farbrot *et al.*[24] cross-linked non-polar siloxane gums of well-defined film thickness for use in reversed-phase OTC LC. Columns in the range 12–50 μm I.D. were prepared from untreated fused silica. The film thickness was varied from 25 nm to 625 nm, giving larger k' values with thicker films. The reported k' values were at least 10 times larger than values reported for ODS-modified fused silica.

The aim of the work reported here was to prepare non-extractable organosiloxane phases of various film thicknesses in 5–50 μm open tubular columns and to characterize in detail the chromatographic properties. The column development was made to establish practical OTC LC reversed-phase systems with high separation efficiencies. GC was used complementarily to evaluate the stationary phases, *e.g.* regarding retention and stability. The potential of these columns for use in routine LC work was preliminarily investigated.

THEORETICAL CONSIDERATIONS

The distribution of a solute between the mobile and the stationary phase can be described by the capacity factor k', which in partition chromatography is related to the distribution constant K and the phase volume ratio according to

$$k' = K\, V_s/V_m = K\, 1/\beta \tag{1}$$

where s and m denote the stationary and the mobile phase, respectively. An increased retention in OTCs can thus be achieved by decreasing the phase ratio β, *i.e.* by increasing the film thickness for a given column inner diameter. However, the influence of different film thicknesses on column performance must also be considered.

Band-broadening in OTC chromatography is generally described by the mathematical model derived by Golay[40], where the plate height H is expressed as a function of the mobile phase linear velocity u by

$$\begin{aligned} H &= \frac{B}{u} + C_m u + C_s u \\ &= \frac{2\, D_m}{u} + \frac{1 + 6k' + 11\, k'^2}{96\,(1 + k')^2} \frac{d_c^2}{D_m} u + \frac{2k'}{3\,(1 + k')^2} \frac{d_f^2}{D_s} u \end{aligned} \tag{2}$$

where B, C_m and C_s are constants, depending on axial molecular diffusion and on resistance to mass transfer in the mobile phase and in the stationary phase, respectively. By this equation, the column performance is related to the tube inner diameter d_c, the stationary phase film thickness d_f, the solute diffusion in the mobile phase D_m and the solute diffusion in the stationary phase D_s. It is assumed that the stationary phase is a uniform film, coated on the inner wall of the tube.

In order to optimize the column dimensions, *i.e.* the phase ratio and the inner diameter, knowledge of the stationary phase diffusion rate is vital. Assuming a stationary phase diffusion coefficient of 10^{-5}–10^{-6} cm^2/s, Knox[2] and Poppe[41] have theo-

retically shown that a film thickness of 1/25 or 1/14 of the column internal diameter can be allowed for without sacrificing efficiency. Unfortunately, the literature lacks experimental data on stationary phase diffusion rates in OTC LC, especially regarding different immobilized polysiloxane phases. However, a batch measurement of the diffusion coefficient for anthracene in cross-linked OV-101 gave a D_s value of $5 \cdot 10^{-8}$ cm^2/s[42], indicating a relatively slow stationary phase mass transfer.

Fig. 1 shows a plot of plate heights, calculated from eqn. 2, for a 10-μm I.D. column. The chromatographic conditions resemble those used in the experiments of this paper (column no. 14, Table I). These curves demonstrate the effect that different stationary phase diffusion rates and film thicknesses will have on the column performance. With stationary phases where the diffusion rate is low, the maximum permissible film thickness will be smaller than that for films with higher diffusion rates. Considering a stationary phase diffusion coefficient of 10^{-8} cm^2/s, a ratio of film thickness to column inner diameter of *ca.* 1/70 will be more optimal, with respect to efficiency. However, with higher diffusion rates ($D_s = 10^{-6}$ cm^2/s), a very thick film will simultaneously yield a high efficiency as well as an adequate retention and sample capacity. For the thickest film in Fig. 1, a minimum plate height value by a factor of 3.5 is obtained if D_s has a value of 10^{-6} cm^2/s instead of 10^{-8} cm^2/s. Furthermore, the optimal flow-rate is affected. If eqn. 2 is an accurate model for band-broadening in 5–10 μm I.D. columns with immobilized polysiloxanes as stationary phases, then

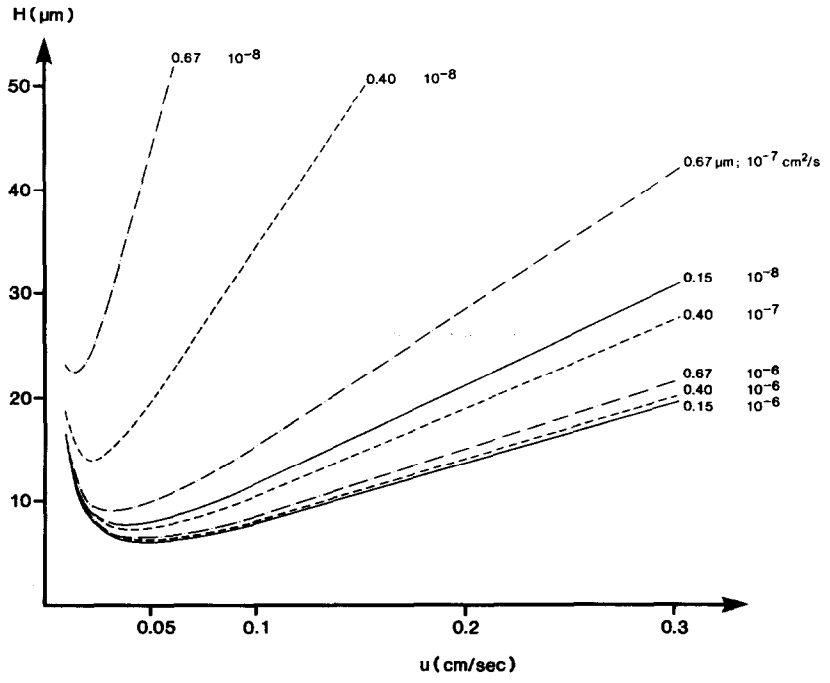

Fig. 1. Dependence of performance of a 10-μm OTC on different stationary diffusion coefficients and film thicknesses. Plate heights calculated from the Golay equation. Stationary phase film thickness and corresponding phase ratio d_f/d_C: (——) 0.15 μm (1/67); (– – –) 0.40 μm (1/25); (— — —) 0.67 μm (1/15). $D_m = 0.75 \cdot 10^{-5}$ cm^2/s; $k' = 0.5$.

TABLE I

DIMENSIONS OF FUSED-SILICA OPEN TUBULAR COLUMNS, COATED WITH IMMOBILIZED POLYSILOXANE LIQUID PHASES

Column	*I.D.* *(μm)**	*Length* *(cm)*	*Stationary phase*	*Concentration, C (% v/v)*	*β* *(V_m/V_s)***	*d_f* *(μm)****
1	55.8	204	PS-255	0.2	499	0.028
2	55.4	204	PS-255	2.0	49	0.28
3	55.2	196	PS-255	2.0	49	0.28
4	54.7	218	PS-255	4.1	24	0.57
5	49.4	203	PS-255	4.8	20	0.62
6	53.1	209	PS-255	7.6	12	1.06
7	52.6	213	PS-255	10.9	8.2	1.56
8	51.6	187	PS-255	13.6	6.3	1.96
9	49.5	200	SE-54	2.0	49	0.25
10	53.8	212	SE-54	3.9	24	0.54
11	54.5	216	SE-54	3.9	24	0.55
12	49.4	225	SE-54	5.8	16	0.74
13	28.4	90	PS-255	2.0	49	0.14
14	11.6	208	PS-255	4.8	20	0.15
15	12.2	43	PS-255	2.0	49	0.062
16	5.3	45	PS-255	4.1	24	0.056

* After coating.
** Before solvent rinsing.

high performance at high separation speeds can only be achieved if the stationary phase diffusion coefficient is sufficiently high.

EXPERIMENTAL

Column preparation

The dimensions of the investigated columns are presented in Table I. The internal diameters of the coated columns were calculated from the column length and the resistance over the mercury-filled capillary, as described by Guthrie *et al.*[43]. A density of 0.98 g/ml for SE-54[44] was used for the calculation of the volumetric concentrations (v/v%), *C*, of the stationary phase solutions in Table I. Since data for PS-255 were not available, the value for a similar methylvinyl silicone gum (SE-33) of 0.98 g/ml was used.

The OTCs were prepared from 5–50 μm I.D. fused-silica capillaries (Scientific Glass Engineering, North Melbourne, Australia) of 0.5–2 m length by a static coating technique[45]. The coating solutions of polysiloxane phases were made in pentane at least 1 day before use. The concentrations were 2–13.6% (v/v) for PS-255 (methyl, 0.5–1.5% vinyl silicone, Petrarch Systems, Bristol, PA, U.S.A.) and 2–5.8% (v/v) for SE-54 (methyl, 5% phenyl, 1% vinyl silicone from General Electric, Applied Science, State College, PA, U.S.A.). Diisopropyl benzene peroxide (dicumyl peroxide, kindly supplied by H.-B. Larson, Unifos Kemi, Sweden) was added just before filling, and the mixture was then treated ultrasonically for 5 min. The concentration of the peroxide was 1% (w/w) of that of the polymer[46]. In order to remove acidic traces present

on the fused-silica surface[47], the untreated fused-silica capillaries were purged with helium for 30 min. The capillaries were then slowly filled with the stationary phase solutions by applying an appropriate helium pressure, which was gradually increased from 10 to 40 atm for 50 μm columns and to 80 atm for 5–10 μm capillaries. The device for column filling as well as sealing of the column end has been previously described[24]. After filling, the system was slowly depressurized. Static coating was performed with the capillary immersed in a water-bath at ambient temperature by applying vacuum to the column end. After coating, traces of pentane were removed by purging the capillaries with helium gas for 1 h. After flame-sealing, the silicone gum phases were immobilized by heating at 15°C/min to 175°C, then 175°C for 5 min[48]. The columns were then conditioned in a gas chromatograph at 2°C/min to 240°C and maintained at this temperature overnight before GC testing.

After testing, the columns were gently rinsed with acetone followed by dichloromethane and pentane, and subsequently dried in a gas stream before LC use. The columns were prepared for on-column fluorescence detection after the coating procedure. The polyimide outer coating was removed from 1 cm of the capillary by heat from a small butane flame. In order to avoid carbonization of the stationary phase, the column was purged with oxygen during flame-heating.

Gas chromatography

GC separations on 50-μm columns were performed in a Carlo Erba Fractovap 2101 gas chromatograph, modified for carrier gas pressures up to 15 atm and equipped with a flame ionization detector. The carrier gas was hydrogen at an average linear flow-rate of *ca.* 45 cm/s. Split injections (split ratio 1:2000) of 2-μl samples were made with a 10-μl gastight syringe (Hamilton, Bonaduz, Switzerland). The injected amount of each solute was *ca.* 40 pg.

Liquid chromatography

The LC system is presented in Fig. 2. Low volumetric flow-rates were created using a simple split-flow arrangement with an LDC Constametric I pump or a Varian 5000 pump[49]. The volumetric flow-rate was calculated from the measured column dimensions and the retention time of an unretained solute. Injections were made by static splitting or by heartcutting[50], using an electrically actuated Valco valve equipped with an external 20-μl loop. With static splitting, the split-tee was connected directly to the injector outlet (split-tee in position D) and the pump directly to the injector. Split ratios between 1:1000 and 1:25 000 were used to minimize band-broadening from the injection system, as well as to allow the use of pump flow-rates of 0.5–4 ml/min. In particular, the largest ratio was used when determining and comparing the HETP curves for different columns. The relative standard deviation of the retention times was typically 0.3–0.5%. With the heartcutting technique (outlined in Fig. 2), a narrow-bore fused-silica capillary was inserted between the split-tee coupling and the valve injector (between B and C). This was made to lower the volumetric flow-rate during the period of time when the on-off valve (D) was opened. Thereby, the injection times could be varied between 10 and 60 s.

On-column fluorescence detection was performed with a Shimadzu Model RF-530 fluorescence detector, which was modified and equipped with liquid filters to reduce background emission, caused by scattered light from the on-column cell[49].

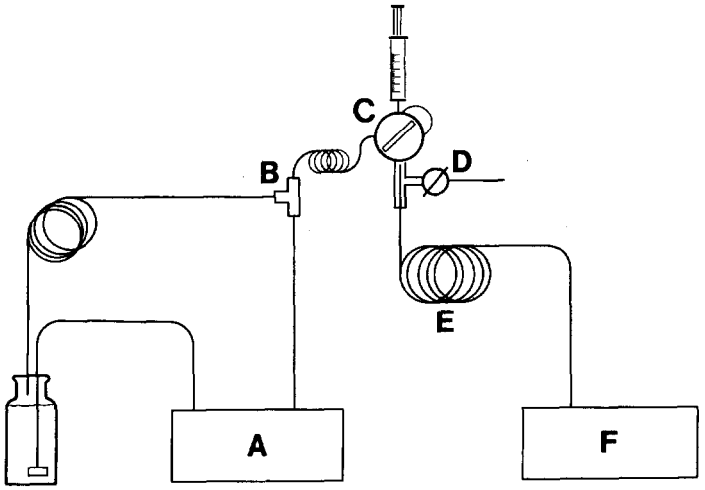

Fig. 2. Instrumental set-up for open tubular column LC. (A) Conventional LC pump; (B) split tee; (C) conventional LC loop injector; (D) on-off valve for "heart-cutting" or restrictor for static splitting; (E) open tubular column; (F) fluorescence detector. For details, see text.

The detection system was appropriate for columns down to 25 μm I.D. For high sensitivity detection, and with columns down to 5 μm I.D., the conventional fluorescence detector was replaced with a laser-induced fluorescence detection system[12,13]. A He–Cd laser, Model 4210NB from Liconix, yielded an output power of 1.5 mW at the UV line 325 nm. The layout of the optical system has been previously described[13,49].

Chemicals

A GC test solution was made in *n*-hexane (p.a.) and the solutes were the *n*-alkanes (reference substances for GC), C_{10}, C_{11}, C_{12} and C_{13}, *n*-octanol and naphthalene, all at *ca.* 40 mg/l. These chemicals as well as *n*-pentane and *n*-heptane (p.a. grade) were from Merck (Darmstadt, F.R.G.).

Different anthracene and fluorene derivatives, suitable for the fluorescence excitation wavelengths available, were selected as model compounds for LC. 9-Fluorenylmethanol was purchased from Fluka (Buchs, Switzerland), 9-phenylfluorene from Sigma (St. Louis, MO, U.S.A.) and 2-aminoanthracene from EGA (F.R.G.). The other anthracene derivatives were synthesized at the Department of Organic Chemistry, University of Göteborg. The solutes were dissolved in the mobile phase prior to injection. For the separation of amino acids, Vaminolac (KabiVitrum), an intraveneous solution containing seventeen of the common protein amino acids was used. The OPA–mercaptoethanol reagent and buffers were prepared according to ref. 51. Acetonitrile and methanol of HPLC grade was from Rathburn Chemicals (Walkerburn, U.K.) and the water was doubly distilled.

Ancillary equipment

Chromatograms were recorded on a Perkin-Elmer 56 strip chart recorder and a Spectra Physics 4270 integrator. Peak widths and retention times were measured manually from the chromatograms as well as calculated from the data recorded by the integrator.

Procedures

Film thickness. For low stationary phase concentrations, the stationary film thickness, d_f, is generally calculated from the column radius r and the stationary phase concentration, C (v/v%):

$$d_f = r \cdot C/200 \tag{3}$$

With increasing concentrations, this expression is no longer valid. For this reason, an exact expression was derived. The film thickness was calculated from the column radius measured after coating, r', and the concentration of the coating, r', solution, according to:

$$d_f = r' \left[1 + \frac{1}{100/C - 1} \right]^{1/2} - r' \tag{4}$$

The mobile to stationary phase volume ratio was obtained from

$$\beta = V_m/V_s = 100/C - 1 \tag{5}$$

Retention. Capacity factors were calculated in LC and GC from $k' = (t_r - t_0)/t_0$, where t_r and t_0 are the retention times of a retained and an unretained peak, respectively. In LC the capacity factors were determined at ambient temperature. With the 50 μm I.D. columns, nitromethane was tested for indicating the column void volume. A weak negative peak was obtained, owing to absorption of the excitation light. The retention time was found to be the same for the fluorene–methanol derivative, with acetonitrile–water (40:60) as mobile phase, except for the thickest films, where fluorene–methanol was slightly retained. With the anthracene derivatives, the 9-anthracenecarboxylic acid was used as an unretained peak.

The 50-μm columns were evaluated for GC by measuring capacity factors of *n*-dodecane and *n*-tridecane at 90°C. Owing to the high load of the stationary phase, the gas hold-up time (t_0) marker, butane, was retarded at the separation temperature, 90°C. Thus, t_0 was measured at 220°C and extrapolated from hydrogen viscosity data to the temperature actually used[52], according to:

$$t_0(90) = t_0(220)\ 0.812 \tag{6}$$

The gas hold-up time in a 2-m column is very short and can therefore not be accurately measured by simply injecting a non-retained component. Therefore, an alternative procedure was used, namely recording the time for emptying the column. Butane was injected into a split/splitless injector in the splitless mode (preset split flow 120 ml/min). The injector and column were filled with a dilute gas sample, which was detected by the flame ionization detector. When a signal decrease, due to sample dilution was observed, the split valve was opened. The time from this event to the distinct decrease in the detector signal, which was caused by the replacement of sample in the column by pure carrier gas, determined the gas hold-up time.

Selectivity. The selectivity in LC was calculated from $\alpha = k'_2/k'_1$. In GC,

measurements of Kovats' retention indices (RI)[53] for *n*-octanol and naphthalene were made at 90°C and calculated according to:

$$RI = 100 \cdot \frac{\log t'_{R,x} - \log t'_{R,n}}{\log t'_{R,n+1} - \log t'_{R,n}} + 100n \quad (7)$$

where the compound x is eluted between two adjacent *n*-alkanes with carbon numbers n and $n + 1$, each with the adjusted retention time t'_R.

Efficiency. The theoretical plate height was calculated from:

$$H = \frac{L\, w_{0.5}^2}{5.54 t_R^2} \quad (8)$$

where $w_{0.5}$ is the peak width at half the peak height. For each column the plate heights were determined in LC at five different flow-rates and for at least three injections at each flow-rate. The experimentally obtained plate heights were compared with the plate heights calculated from eqn. 2. The stationary film thickness and the column internal diameter were obtained as described above. The diffusion coefficients for the test solutes in the mobile phase were calculated according to ref. 54: 9-anthracenecarboxylic acid, $D_m = 0.68 \cdot 10^{-5}$ cm²/s; 9-cyanoanthracene, $0.72 \cdot 10^{-5}$ cm²/s; anthracene, $0.78 \cdot 10^{-5}$ cm²/s. The slopes of the experimentally determined HETP curves were obtained from linear regression. This allowed the subsequent calculation of the stationary phase diffusion coefficient D_s by rearranging eqn. 2.

RESULTS AND DISCUSSION

Column preparation

In the present study, all columns were prepared from untreated fused-silica tubing, which is known to possess sufficient wettability for coating with non-polar polysiloxanes[55]. The methylvinyl silicone gum PS-255, suggested for the preparation of thick films in GC by Grob and Grob[46], and the methylphenylvinyl silicone gum SE-54, were selected for comparison of retention, selectivity and efficiency. The general structure of these gum phases is shown in Fig. 3. The vinyl substitution makes these siloxanes easy to cross-link. This is important in the fabrication of thick films, since a decreasing degree of immobilization with increasing film thickness was reported by Grob and Grob[46], who obtained an extractability of more than 50% for a 5-μm SE-54 film, whereas a 0.2-μm film was virtually non-extractable.

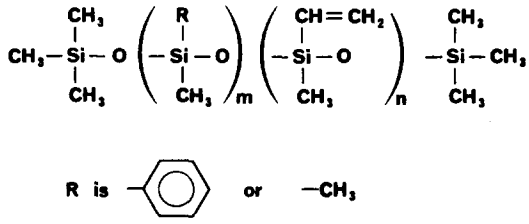

Fig. 3. General structure of the polysiloxane gum phases.

Preparation of small-bore columns with the static coating technique is facilitated by the use of coating solutions of relatively low viscosity. In this respect, PS-255 is to be preferred to SE-54 as well as to the methyl silicone gum phase OV-1[46]. The low viscosity methyl silicone oil phases, such as OV-101, produce coating solutions that yield a faster column filling. These phases are, however, more difficult to cross-link and require a considerably higher concentration of organic peroxide to produce the same degree of cross-linking than do the corresponding gum phases[32,56]. Unfortunately, there are indications from previous work in GC of a reduced column inertness from a high peroxide concentration of the radical initiator[31,56,57]. Various initiators for cross-linking non-polar polysiloxanes have been used in capillary GC. Wright *et al.* obtained highly inert columns when cross-linking non-polar siloxanes by repeated treatment with azo-*tert.*-butane[56]. The use of reactive organic peroxides, such as benzoyl peroxide, resulted in increased column activity[31,56,57]. In contrast, the column inertness was not significantly changed when low concentrations of the less reactive dicumyl peroxide (DCUP) were used to cure SE-54[56]. Despite this, a small increase in activity was observed for a polydimethyl siloxane gum, SE-30[57]. A reduced inertness was reported at higher concentrations (more than 1%) of DCUP[58]. Furthermore, residues of DCUP were incorporated in the polymer structure by covalent bonding when it was used to cure a methylvinyl silicone (SE-33)[59]. Thus the amount of peroxide should be minimized, adjusted only to give a sufficient degree of phase insolubility.

A series of 50-μm columns was prepared for evaluation of the maximum amount of stationary phase obtainable (Table I). The static coating technique allows control of the volume ratio of mobile to stationary phase as well as of the film thickness, and columns were successfully prepared from solutions with up to 13.6% PS-255 gum. With the SE-54 gum phase, it was difficult to prepare homogeneous solutions over 6% (v/v). The highest phase concentration (13.6%, column 8, Table I) corresponds to a phase ratio, $\beta = V_m/V_s$, of 6.3. The dimensions of these columns may be compared with the capillary columns generally used in GC, which are typically made from 0.2–0.5% solutions, corresponding to a phase ratio, $\beta = V_m/V_s$, of 200–500. Grob and Grob[46] prepared thick film GC columns with 8-μm PS-255 films in 0.32 mm I.D. capillaries, corresponding to a stationary phase concentration of 9.8% v/v and a β value of 9.3. The even lower phase ratios presented in Table I for columns 7 ($\beta = 8.2$) and 8 ($\beta = 6.3$) show that it is possible to produce capillary columns with a higher load of stationary phase, although the absolute film thickness in the 50-μm columns is smaller.

When very concentrated solutions are handled for coating capillary columns by the static technique, the risk of bubble formation is enhanced, since the coating solution is saturated with gas when it is forced into the capillary by high pressure gas. When nitrogen was used, bubbles were released at the column end during filling. This was prevented by the use of the less soluble helium gas. Occasionally, at low flow-rates of coating solution, the final millimetres of the capillary were not filled unless evaporation of the solvent was hindered by temporarily sealing the end.

The time needed for solvent evaporation in the static coating procedure depends on column dimensions such as length and inner diameter as well as on the viscosity of the coating solution. Accordingly, the 50 μm I.D. columns, all of *ca.* 2 m length, required coating times ranging from 65 min for the less concentrated so-

lutions to 130 min for the most concentrated polysiloxane solutions. In contrast, a column of the same length but with 11.6 μm I.D. (column 14, Table I) required 4 h for coating. The risk of breakthrough (*i.e.* bubble formation in the capillary) was minimized by performing the coating at ambient temperature.

The preparation of 5–10 μm columns, as compared with wider bore columns, means longer times for filling and coating. Consequently, an increased number of failures such as blocking and breakthrough was observed. Despite these difficulties, the presented method allows a successful preparation of OTCs down to 5 μm I.D. However, more work on these small capillaries is needed for coating longer columns with thick stationary phase films.

Using laser-induced fluorescence detection, a considerable increase in fluorescence background was obtained from thick film coatings. The fluorescence emanates from products formed in the capillary during the flame destruction of the polyimide coating at the detection point. This artefact can be avoided by coating the capillary after removal of the polyimide film.

Column stability

The stability in terms of phase immobilization was examined by GC. Capacity factors were measured before and after solvent rinsing. The loss of stationary phase was typically 8–10%, irrespective of film thickness. The effect of a 9% loss in retention on the phase ratio and film thickness is exemplified in Table II. In general, a higher efficiency was measured by GC after column rinsing.

A certain mechanical instability was indicated for the thicker films (columns 7 and 8, Table I) during the LC experiments. A variation in retention times was observed, owing to fluctuations in the flow resistance, especially at low flow-rates. This effect was partly caused by an incomplete decomposition of the stationary phase at the ends of the detection zone during removal of the column outer protective polyimide coating. The stationary phase is not attached to the column wall by chemical bonding since it was coated directly on the untreated fused-silica surface. A deactivation layer, containing organic groups that are amenable to covalent linkage to the stationary phase, is expected to increase the mechanical stability of the phase[60].

The effect of aqueous mobile phases on column inertness was monitored by using *n*-octanol as a GC test substance before and after LC use. Symmetrical peaks were observed in all cases for the *n*-alkanes. Thick films exhibit a shielding effect on the fused-silica surface and show symmetric peaks of *n*-octanol. Furthermore, thick film columns show no significant change in peak symmetry after LC use. The situa-

TABLE II

CHANGES IN PHASE RATIO, $\beta = V_m/V_s$, AND FILM THICKNESS, d_f, MEASURED BY GC AFTER SOLVENT RINSING

Column	*Before rinsing*		*After rinsing*	
	β	d_f (μm)	β	d_f (μm)
3	49	0.28	54	0.25
7	8.2	1.56	9.0	1.43

TABLE III

EFFECT OF AQUEOUS MOBILE PHASES ON FUSED-SILICA COLUMN INERTNESS AT VARIOUS FILM THICKNESSES

Kovats' retention indices for *n*-octanol measured by GC at 90°C.

Column	*Stationary phase*	*RI before LC*	*RI after LC*
4	PS-255, 0.57 μm	1057	1060
7	PS-255, 1.56 μm	1054	1054
10	SE-54, 0.54 μm	1074	1080

tion for thin film columns is somewhat different. Firstly, tailing is observed on the freshly prepared columns. Secondly, the retention index of *n*-octanol increases markedly after extensive washing with aqueous solvents (Table III), reflecting a higher silica surface activity.

An attempt was made to examine the presence of acidic sites in the LC column by injection of a basic solute, 2-aminoanthracene, on untreated 50-μm capillaries, as well as on columns with various film thicknesses. This test did not reveal any adsorption, since symmetrical peaks were observed for the amine on all columns tested.

Retention

Farbrot *et al.*[24] reported an increased retention for 0.03–0.6 μm thick films of non-polar polysiloxanes. This range has in the present study been extended to cover film thicknesses up to 2 μm (Table I). Fig. 4a presents LC data on the dependence of k' on the initial phase ratio, representing a film thickness of 0.28–2 μm for PS-255 and of 0.25–0.74 μm for SE-54 in 50-μm columns. A linear increase in retention is observed for these thicker films. It should be noted that the true β values are slightly higher than those presented in Table I (see Table II), owing to removal of the soluble part of the polymer by solvent rinsing before LC testing.

The LC retention data are supported by the data from the GC evaluation of the same columns. The capacity factors in GC (measured on non-rinsed columns) for *n*-dodecane and *n*-tridecane as a function of phase ratio are presented in Fig. 4b. Compared with the LC data in Fig. 4a, for fluorene derivatives, the difference in k' values between SE-54 and PS-255 in GC is less significant. This is reasonable since the phenyl substitution in SE-54 affects the distribution constant to a lesser extent for an alkane than for an aromatic compound.

Retention was also investigated in columns with approximately the same phase ratio, but in different inner diameters (Table IV). A rough agreement was obtained between the 54.7 and 11.6 μm I.D. columns. The 5.3 μm I.D. column shows higher capacity factors, indicating a higher load of stationary phase than expected. Note the change in selectivity with decreasing inner diameter.

In Table V, the retention behaviour for columns 7, 8 and 12 are compared with data from the literature. From the data obtained by Takeuchi *et al.*[38] on cross-linked polysiloxane phases in OTC LC, it can be seen that the capacity factors for the thickest films in the present work are roughly 3–9 times higher. The retention on the thickest PS-255 phase is also higher, by a factor of *ca.* 3, even when compared with their data on a chemically bonded ODS phase. Recently Vargo[19] reported the

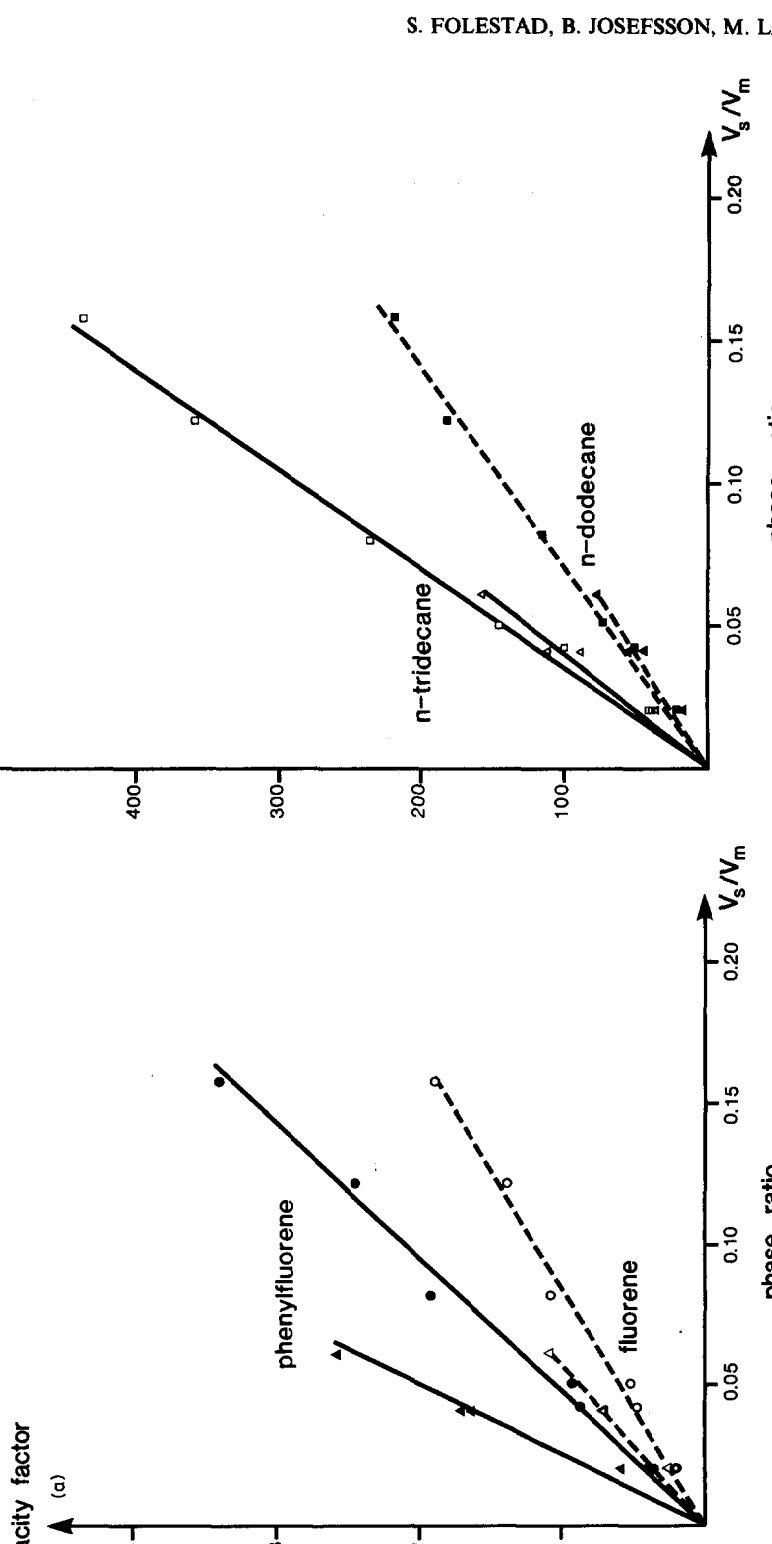

Fig. 4. (a) Dependence of capacity factors on phase ratio in OTC LC for two immobilized polysiloxane stationary phases. Columns, No. 2–12, 2 m × 50 μm I.D.; mobile phase, acetonitrile–water (40:60); solutes, fluorene and phenylfluorene. (b) Dependence of capacity factors on phase ratio in OTC GC for two immobilized polysiloxane stationary phases. k' values were measured at 90°C before column rinsing. Columns as in (a). Solutes, *n*-dodecane and *n*-tridecane. Data points: (a) circles, PS-255; triangles, SE-54; (b) squares, PS-255; triangles, SE-54.

TABLE IV

COMPARISON OF LC RETENTION IN COLUMNS OF DIFFERENT INTERNAL DIAMETERS

Mobile phase, acetonitrile–water (40:60).

Column	*I.D. (μm)*	d_f *(μm)*	*Capacity factor (k')**		*Selectivity (α)*
			Cyano-anthracene	*Anthracene*	
4	54.7	0.57	0.12	0.52	4.3
14	11.6	0.15	0.14	0.46	3.3
16	5.3	0.056	0.39	1.28	3.3

* Normalized to the β-value of columns 4 and 16.

highest capacity factors obtained thus far in OTC LC, using an ODS phase on etched soda-lime columns. Compared with these ODS phase values, a slightly higher retention was obtained for the thickest PS-255 phase (column 8) in the present work. In addition, considering the effect of swelling (see Corollary), a further enhancement in capacity factors of up to 10 times is possible on the cross-linked polysiloxane phases. The retention will then be considerably higher than that obtainable on chemically bonded ODS phases, even in highly etched soft glass columns.

Selectivity

Selectivity factors in LC for fluorene and phenylfluorene are presented for 50 μm I.D. columns in Table VI. A difference in selectivity is found between the siloxanes studied. The highest α values, obtained on the phenyl-substituted phase SE-54 (methyl, 5% phenyl, 1% vinyl silicone), reflect the higher affinity for the solute phenyl group with this phase.

The results from GC measurements of Kovats' retention indices of *n*-octanol and naphthalene are displayed in Table VII. The difference in stationary phase composition is obvious from the higher selectivity obtained for an aromatic substance,

TABLE V

COMPARISON OF LC RETENTION ON IMMOBILIZED POLYSILOXANE LIQUID PHASES WITH OTC LC DATA REPORTED IN THE LITERATURE

Reference	*Column*	*Stationary phase*	*k' in acetonitrile–water**	
			30:70	*50:50*
This work	7	PS-255	7.0	0.51
This work	8	PS-255	9.5	0.71
This work	12	SE-54	5.2	0.38
38	Soda-lime	OV-1	0.78	–
38	Soda-lime	SE-54	1.6	–
38	Soda-lime	ODS	3.31	–
20	Soda-lime	ODS	–	0.65

* Solute anthracene.

TABLE VI

SELECTIVITY FACTORS FOR STATIONARY FILMS LARGER THAN 0.25 μm OF PS-255 AND SE-54 IN OTC LC

Solutes, fluorene and phenylfluorene; mobile phase, acetonitrile–water (40:60); columns, 50 μm I.D.

Column	*Phase*	α
2	PS-255	1.83
3	PS-255	1.82
4	PS-255	1.81
5	PS-255	1.79
6	PS-255	1.78
7	PS-255	1.77
8	PS-255	1.80
9	SE-54	2.36
10	SE-54	2.38
11	SE-54	2.33
12	SE-54	2.37

naphthalene, on the SE-54 columns 9–12 than on the PS-255 columns 4–8. The significantly higher retention index values for *n*-octanol on columns 5 and 9 probably originate from a higher surface activity for one of the batches of fused-silica material. Batch-to-batch variations of the fused silica can be handled by deactivation of the capillaries.

As can be seen from LC data in Table VI, the selectivity is constant for film thicknesses larger than 0.25 μm. In contrast, an unexpected deviation in selectivity is observed for thin film columns. The data in Table IV, for the solute pair anthracene–cyanoanthracene, show significantly lower α values for the 5- and 11.6-μm columns compared with a 50-μm column of the same phase ratio. Furthermore, selectivity changes are also observed for 50 μm columns with thin stationary phase films (Table VIII). The α value for the solutes phenylfluorene–fluorene with the 28-nm film (column 1) is significantly higher than with the 280-nm film (column 2), and an even higher increase in α is observed for a narrow-bore column (column 15). Thus,

TABLE VII

KOVATS' RETENTION INDICES FOR *n*-OCTANOL AND NAPHTHALENE AT 90°C

Sample amount, 40 pg.

Column	*Phase*	*RI (n-octanol)*	*RI (naphthalene)*
4	PS-255	1057	1154
5	PS-255	1064	1155
6	PS-255	1056	1154
7	PS-255	1054	1154
8	PS-255	1053	1154
9	SE-54	1093	1187
10	SE-54	1074	1186
11	SE-54	1074	1186
12	SE-54	1076	1187

TABLE VIII

SELECTIVITY FACTORS FOR STATIONARY FILMS UP TO 0.28 μm OF PS-255

Solutes, fluorene and phenylfluorene; mobile phase, acetonitrile–water (30:70).

Column	*I.D. (μm)*	d_f *(nm)*	β	α
2	55.4	280	49	2.9
1	55.8	28	499	3.5
15	12.2	62	49	4.1

the retention and selectivity behaviour of these polymer films are related not only to the phase ratio: the absolute film thickness and the column inner diameter must also be considered.

The different selectivities obtained may have several origins. The thin films discussed have thicknesses ranging from 25 nm to 150 nm. Assuming a uniform film and using 126 Å^3 (calculated from density data) as the volume of a $Si(CH_3)_2O$ group, the corresponding number of dimethylsiloxy units is 50–300. As shown in GC, adsorption of the solute on the fused-silica surface, are diffusion through these thin layers, was observed for *n*-octanol on column 1. This effect is less likely to occur in the LC experiments since no adsorption was observed on uncoated fused-silica capillaries for the test solutes employed in this study. Still, the special behaviour of thin films may be an effect of the fused-silica surface. It is known that a methylsilicone can be adsorbed on a silica surface, possibly via interactions with the silanol groups[61]. A local effect on the polymer structure, such as a certain polymer chain orientation, seems likely. It may be speculated that the helical conformation of dimethylsiloxane chains[62], where the methyl groups are pointing outwards while the siloxane bonds are shielded, may be disrupted close to the silica surface. The apparent selectivity of the phase could then be changed. Whether adsorptive interactions in the stationary phase to mobile phase interphase contribute to the total retention and selectivity behaviour, as has been indicated in packed column LC[63], is not clear. More investigations are needed to reveal the detailed selectivity behaviour of thin polysiloxane films.

Efficiency

The performance of OTCs with different immobilized silicone gum phases is shown as HETP plots in Figs. 5 and 6. For an unretained solute on the 50-μm columns with the PS-255 phase (Figs. 5a and 5b), a close agreement was found between experimental data and values calculated from eqn. 2. This indicates the validity of eqn. 2 for unretained solutes for these column dimensions, and that any extra-column band-broadening, attributed to the on-column detector and to the split injector, was negligible in the present study. This condition was further verified by measuring the band-broadening in untreated 50 μm I.D. tubes. Despite this, column 10 with a SE-54 phase gave higher plate heights for the unretained solute than expected (Fig. 5c), although the same LC system was used. Whether this is due to the unretained solute being in fact slightly retained on that particular column or to some other effect is not clear.

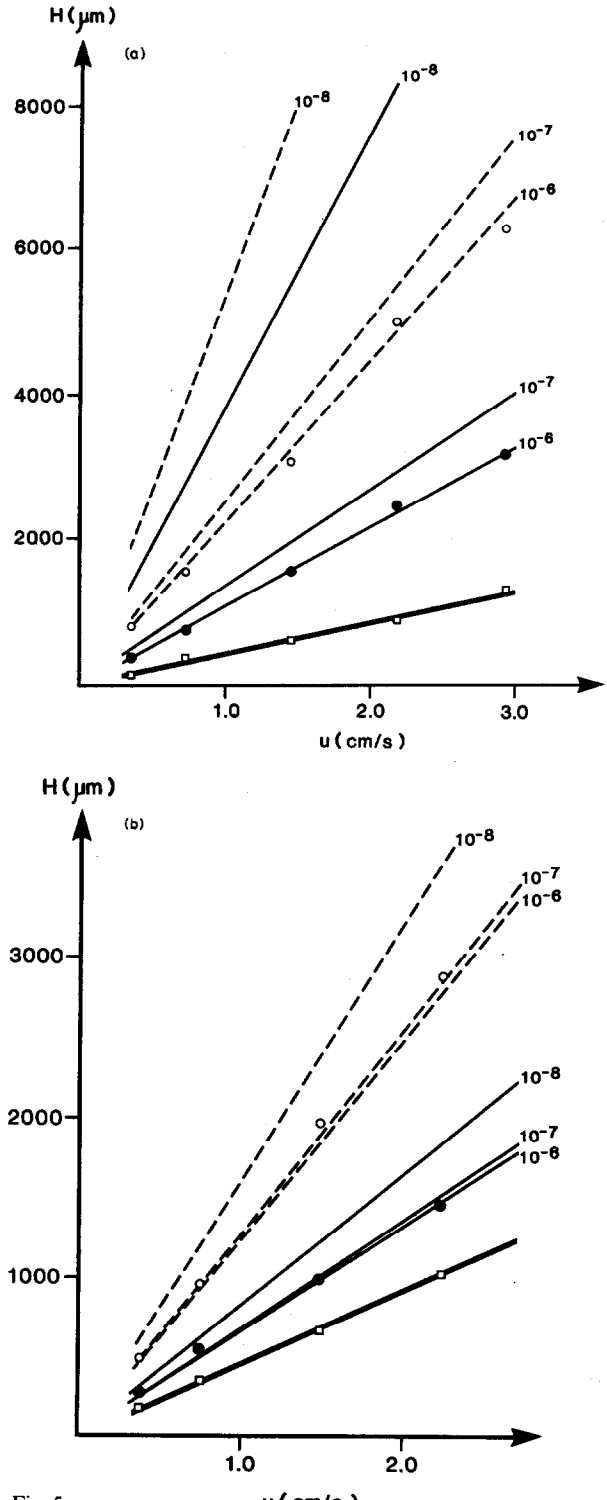
H(μm)
(a)
8000
6000
4000
2000
10⁻⁸
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁷
10⁻⁶
1.0
2.0
3.0
u(cm/s)
H(μm)
(b)
3000
2000
1000
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁸
10⁻⁷
10⁻⁶
1.0
2.0
u(cm/s)

Fig. 5.

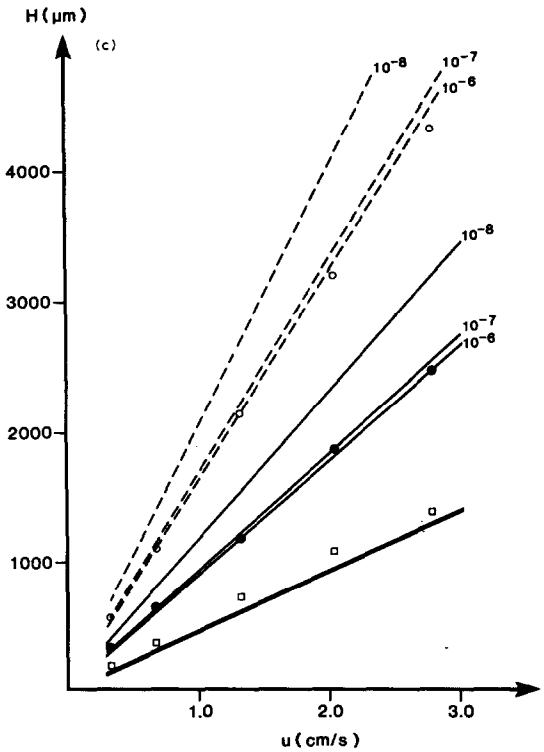

Fig. 5. Plots of experimental and calculated plate heights *versus* linear velocity for different polymer phases and film thicknesses in 50 μm I.D. columns. Calculated plate heights from the Golay equation using D_s values of 10^{-6}, 10^{-7} and 10^{-8} cm²/s. (a) Column 7, PS-255; d_f = 1.56 μm; k'_{CN} = 0.40; k'_{ANT} = 1.70; (b) column 4, PS-255; d_f = 0.57 μm; k'_{CN} = 0.12; k'_{ANT} = 0.52; (c) column 11, SE-54; d_f = 0.55 μm; k'_{CN} = 0.25; k'_{ANT} = 0.85. Experimental plate heights: □ = 9-anthracenecarboxylic acid; ● = 9-cyanoanthracene; ○ = anthracene. Calculated plate heights: ———, ——— and - - - for solute □, ● and ○, respectively. Mobile phase, acetonitrile–water (40:60); temperature, 23°C. Further details in text.

Using eqn. 2 and the experimental values of the tube inner diameter and the film thickness, plate heights were calculated for different D_s values and plotted together with the experimental data in Figs. 5 and 6. These results show that the apparent D_s value is 0.7–2.5 · 10^{-6} cm²/s for column 7 with a 1.56-μm thick PS-255 film, as well as for column 11 with a 0.55-μm SE-54 film. For column 4, with a thinner film of PS-255, 0.57 μm, a lower D_s value, ≃ 10^{-7} cm²/s, was obtained. For column 14, with the PS-255 phase and 11.6 μm I.D., the stationary phase diffusion rate is even lower, 0.9–2.2 · 10^{-8} cm²/s.

The somewhat surprising tendency of achieving lower plate heights for thicker polymer films (compare column 4 and 7 above) have in fact been observed earlier. Both Cramers *et al.*[27], in capillary GC, and Kirkland[63], in LC with polymer-coated particles, observed the same effect. The benefits from this, high performance at high separation speeds, should be clear in the light of the discussion in the theory section. With very thick polymer films on particles, a slow equilibration between the mobile and stationary phase was reported to lead to an increase in peak asymmetry at higher

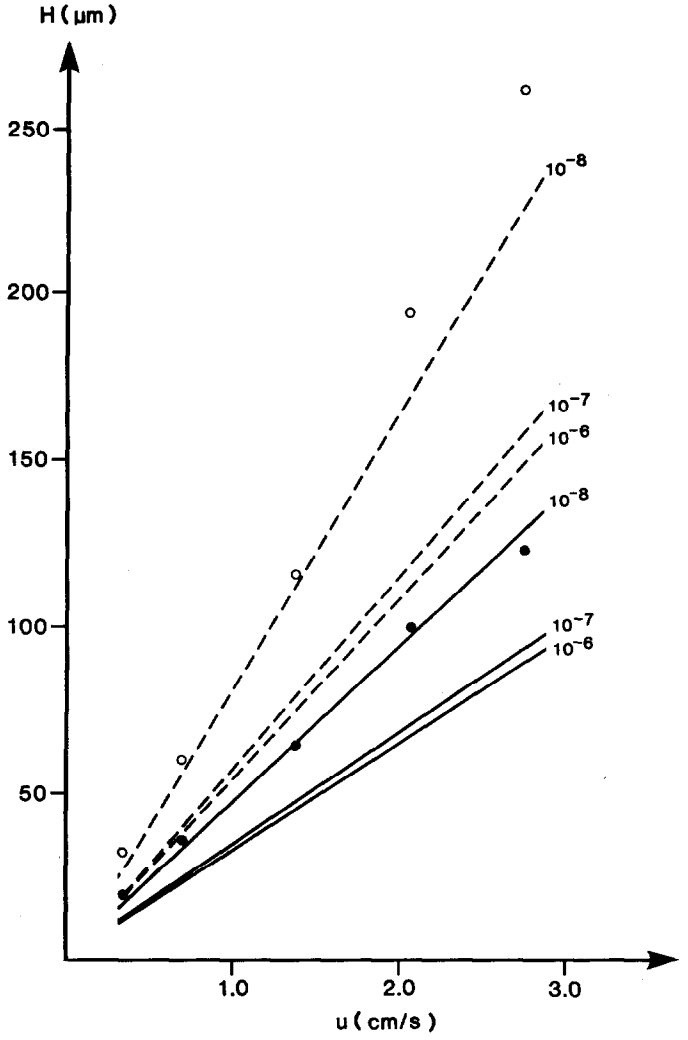

Fig. 6. Plot of experimental and calculated plate heights *versus* linear velocity for column 14, PS-255; d_f = 0.15 μm; 197 cm × 11.7 μm I.D.; k'_{CN} = 0.16; k'_{ANT} = 0.50. Other details as in Fig. 5.

velocities[62]. It should, however, be noted that symmetrical peaks were obtained throughout these investigations, even for the thickest films and at high mobile phase velocities.

The differences in column performance, even with the same type of polymer but in different film thicknesses, as well as in different column inner diameters, may have various origins. The differences in the final polymer film structure, when varying the absolute film thickness, as discussed above (see Selectivity and Tables IV and VIII) may also affect the apparent stationary phase diffusion rates. However, existence of irregularities in the stationary phase, due to drop formation, seems unlikely since in GC no corresponding difference in efficiency was observed between these columns.

Slow interfacial mass transfer[64] may also be the cause of differences in performance, an effect that should increase in importance with smaller column inner diameters. The lower D_s value obtained for the 11.6-μm column, compared with the 54.7-μm column, which has approximately the same phase ratio, supports the occurrence of this effect. A slow interfacial mass transfer should also cause a larger effect for solutes with larger k' values. However, the experimental uncertainty in the plate heights does not permit any observation of significant variations in D_s with k' for these columns.

The highest efficiency, in number of plates, was obtained for column 14 (1.97 m × 11.7 μm I.D.). A standard separation of anthracene derivatives on this column is shown in Fig. 7, as well as a comparison of the performance of the OTC with that of a packed column. The linear velocity during the OTC LC separation was 0.35 cm/s, which is a factor of *ca.* 10 higher than the assumed optimal flow-rate, whereas for the packed column, the rate was near the optimal. Operating column 14 close to optimal conditions (flow-rate 0.042 cm/s) yielded very high plate numbers: 351 000 ($k' = 0.16$) and 266 000 ($k' = 0.5$) plates for peak 1 and 3, respectively. Furthermore, for the corresponding plate heights at this low flow-rate an excellent agreement was obtained between the experimental values, H_{exp}, and those calculated from the Golay equation, H_{Gol}, using the experimentally determined D_s values obtained at high flow-rates. For peak 1, $H_{exp} = 5.6$ and $H_{Gol} = 5.2$, and for peak 3, $H_{exp} = 7.4$ and $H_{Gol} = 7.4$.

The relative performance of different column types can be compared by using the Knox separation impedance parameter[2], $E = h^2\varphi$, where h is the reduced plate height and φ the flow-resistance parameter. The optimal value for a packed column should ideally be 2000, whereas the value for an OTC operated under optimal conditions is substantially lower, *e.g.* as low as *ca.* 13 for column 14 ($k' = 0.5$). The E values for peak 1 in Fig. 7 are 1800 and 3300 for the OTC and the packed column, respectively. A comparison of the performance of OTCs and packed columns based solely on the separation impedance can, however, be somewhat misleading. For packed columns the efficiency, expressed as the plate number, is roughly the same for all k' values in a chromatogram, whereas for OTCs there is a decrease in efficiency at higher k' values, and thereby only a narrow capacity factor range gives optimal conditions in an OTC separation. In addition, the hold-up time of an OTC, with a bore larger than the particle size in the packed column, is long compared with that of a packed column of similar efficiency. A straightforward approach is to run the same separation on the two column types. In Fig. 7 no thorough optimization was made of the respective separation conditions. Nevertheless, the elution order of the anthracene derivatives is the same on the OTC with the PS-255 phase as on the packed column with the ODS phase. It is also shown that with the same total separation time the hold-up time for the OTC is much longer. Furthermore, owing to the different selectivity, an enhanced resolution was obtained for peaks 1–3. It should be noted that as a result of the different retention characteristics, the acetonitrile content of the mobile phase was lowered for the OTC. Still, the k' value of the last eluting peak is smaller on the OTC.

Operating column 14 near the optimal flow-rate, 0.042 cm/s, a drastic increase in separation efficiency was obtained (from 22 500 to 351 000 plates for peak 1), but at the cost of a much longer separation time (a factor of *ca.* 8.3). For the packed

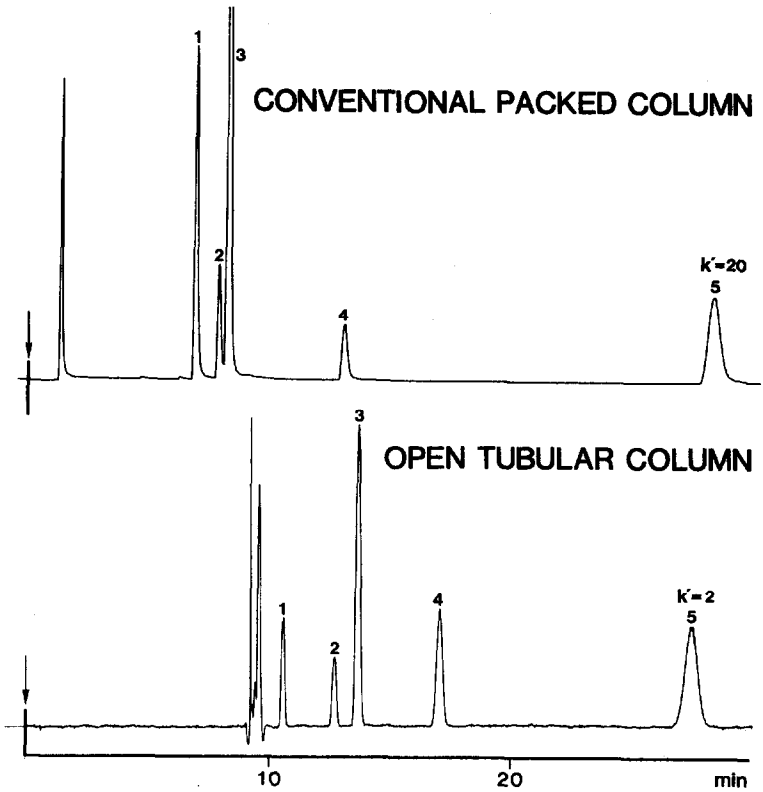

Fig. 7. Comparison of performance of an open tubular column with that of a packed column. Column 14, 197 cm × 11.7 μm I.D.; acetonitrile–water (40:60) at 22 nl/min; split, 1:24 600. Packed column (150 × 4.6 mm I.D.) Spherisorb ODS2, 5 μm; acetonitrile–water (75:25) at 0.7 ml/min. Solutes: 1 = 9-cyanoanthracene; 2 = 9-methoxyanthracene; 3 = anthracene; 4 = 9,10-dimethylanthracene; 5 = 9-phenylacetyleneanthracene.

column, the only way to improve the resolution under isocratic conditions is to increase the column length, which also will increase the separation time. Alternatively, a decrease in the particle size will improve the resolution but, simultaneously, increase the pressure drop. It is interesting to note that with an 8.3 times longer packed column, yielding a corresponding 8.3 times increase in the plate number and the same separation time as with the OTC, the increase in number of plates is only about half of that for the OTC (a factor of 15.6). Concurrently, the pressure drop will increase for the packed column but decrease for the OTC.

Application

A preliminary attempt was made to evaluate the potential of OTC LC with polysiloxane phases for use in routine LC work. An intraveneous solution containing 17 of the common protein amino acids was treated with the *o*-phthaldialdehyde (OPA)–mercaptoethanol precolumn reagent and separated on the OTC No. 14 (1.97 m × 11.6 μm I.D.). The separation was performed at two different flow-rates by gradient elution as shown in Fig. 8a and b. The mobile phase conditions are similar to those described by Jones and Gilligan[51] for conventional LC except for the simple

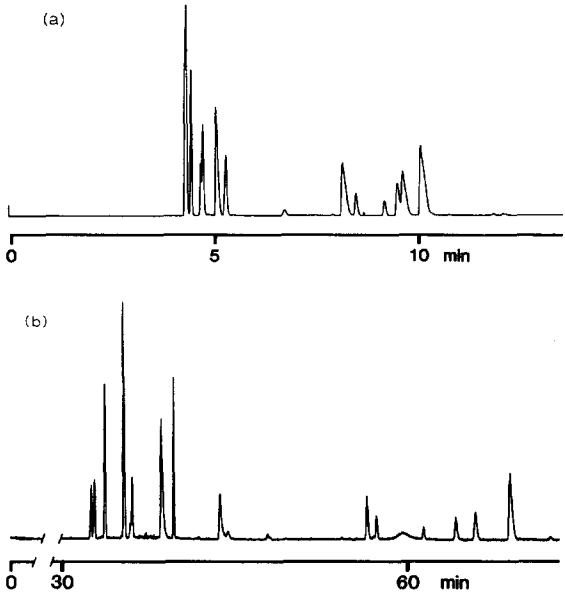

Fig. 8. Separation of OPA–mercaptoethanol derivatized amino acids. Column 14, 197 cm × 11.7 μm I.D.; mobile phase, methanol–buffer (0.1 *M* sodium acetate, pH 7.2); sample, 13–30 μ*M* of each amino acid. (a) Flow-rate, 24 nl/min; linear gradient, 0–40% methanol from 1.0 to 8.0 min; injection volume, 0.3 nl. (b) Flow-rate, 6.5 nl/min; linear gradient, 0–40% methanol from 5.0 to 60 min; injection volume, 0.1 nl.

linear gradient employed for the present OTC separations. Several of the seventeen amino acids could be baseline-separated, although a 100% mobile phase buffer concentration was needed to increase the resolution of the most hydrophilic amino acid derivatives. However, to resolve all the amino acids in the first part of the chromatogram, an increase in column retention is desirable. A change in phase ratio by increasing the film thickness, or alternatively increasing the retention by swelling the stationary phase with non-polar solvents (see below) may both improve the separation conditions. Still, it is clearly demonstrated that it is possible to separate and detect the majority of the common protein amino acids efficiently, even in the concentrations commonly found in physiological fluids. Furthermore, as can be seen in Fig. 8, and in contrast to the properties of packed columns, the same OTC can be used for both fast separations with moderate resolution (Fig. 8a) and for high resolution separations (Fig. 8b).

COROLLARY

The effect of stationary phase swelling on retention

It is well known that organosiloxanes swell in various solvents. The solvent is trapped in the polymer matrix, yielding a considerably larger stationary phase volume than in the unswollen state[29]. The degree of swelling depends on the type of solvent, the polymer composition and the degree of cross-linking. Methyl silicones show the highest degree of swelling in hydrocarbons and in chlorinated solvents. A dimethyl silicone rubber is able to swell to more than three times its initial volume in these

TABLE IX

EFFECT OF SWELLING OF AN IMMOBILIZED METHYLPHENYLVINYL SILICONE GUM PHASE WITH *n*-HEPTANE

Stationary phase, SE-54, column 11.

	Acetonitrile–water (40:60)		*Acetonitrile–water (40:60) saturated with n-heptane*	
	Fluorene	*Phenylfluorene*	*Fluorene*	*Phenylfluorene*
k'	0.70	1.64	6.3	15.8
α	2.33		2.51	

solvents[29]. In chromatography, only a few observations have been reported on this phenomenon. Swelling of the stationary phase has been observed at sample injection in capillary GC[32,65]. Recently, the effects of swelling of non-polar siloxanes in SFC were discussed[30,66]. In straight-phase LC with polar siloxanes, coated on particles, the mobile phase caused swelling of the stationary phase[63].

A stationary phase, consisting of a non-polar solvent trapped in an immobilized layer of a non-polar silicone, have properties that are attractive for use in OTC reversed-phase LC. The main advantage of this approach is the convenient preparation of stationary phases with very large film thicknesses. In addition, swelling with low molecular weight solvents is likely to affect the stationary phase diffusion. The D_s value can be expected to increase, improving the mass transfer in the stationary phase and thereby the column efficiency.

Preliminary results demonstrate that cross-linked siloxane phases swollen by non-polar solvents can be used for reversed-phase LC. In Table IX, k' data (column 10) obtained after the saturation of both the stationary and mobile phases with *n*-heptane, demonstrate a nearly ten-fold increase in retention and a parallel change in selectivity. The characteristics and the utility of this type of OTC liquid–liquid chromatographic system are presently under investigation[67].

ACKNOWLEDGEMENTS

This work was financially supported by the Swedish Natural Science Research Council, Carl Trygger's Fund for Scientific Research and Adlerbert's Research Foundation. The anthracene derivatives were kindly supplied by Kjell Andersson, Hans-Dieter Becker and Henrik Sörensen. Jan-Erik Rosenberg is acknowledged for valuable discussions on polymer chemistry. Discussions on the manuscript with David Dyrssen and Derek Biddle are acknowledged.

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