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Study of polyorganosiloxanes (native and solvent swollen) for the preparation of narrow (5–15 µm I.D.) and long (1–6 m) open tubular columns in reversed-phase liquid chromatography

Karin Göhlin*

Department of Analytical and Marine Chemistry, Chalmers University of Technology and University of Göteborg, S-412 96 Göteborg (Sweden)

Marita Larsson

Bioanalytical Chemistry, Astra Hässle AB, S-431 83 Mölndal (Sweden)

ABSTRACT

Narrow-bore, (5–15 µm), long (up to 6 m), efficient and stable open tubular columns with four different polyorganosiloxane coatings, were prepared by the static coating and the precipitation coating methods. The polysiloxanes were substituted with phenyl- (OV-17-vi and PS-264), methyl- (PS-255), and octadecyl- ($PMSC_{18}$) groups. They were studied in their regular cross-linked state as well as after swelling with *n*-heptane. Anthracene derivatives were used as model compounds. Column properties such as retention, selectivity and efficiency were investigated in reversed-phase liquid chromatography. GC was used for complementary studies of 50 µm I.D. columns. The highest retention was obtained on the columns coated with OV-17-vi and on the swelled stationary phases. A considerable resolution improvement was obtained in the columns, after swelling with *n*-heptane owing to the increase in retention. More than 10^6 theoretical plates were achieved in 70 min at a k' of 0.46 in a 6 m × 6 µm I.D. column.

INTRODUCTION

In order to reach high separation power (one million theoretical plates and more), *e.g.* for the separation of complex mixtures, open tubular columns (OTCs) are being developed for liquid chromatography (LC). OTCs have a lower flow resistance compared to conventional packed columns. This feature permits the use of very long columns (up to several meters). Consequently, a higher total number of theoretical plates is obtained. The chromatographic theory predicts that OTCs should compete with packed columns in resolution and separation speed, when columns of small I.D. (10 µm or less) are used [1–4]. Recently, our group presented a comparison of narrow-bore (5–15 µm I.D.) OTCs and conventional LC columns with the same type of stationary phase: immobilized polymethyloctadecylsiloxane [5]. These experimental data are in good agreement with the theoretical predictions.

Open tubular LC (OT-LC) columns were in the beginning prepared from soda-lime glass [6–8] and borosilicate glass [9,10]. In 1979, fused silica was introduced as column material [11] and it is now favoured over other materials in gas

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* Corresponding author.

chromatography. Fused silica has some advantageous properties, as compared to other glass capillaries, such as greater flexibility, higher inertness and better UV transparency. These characteristics have made fused silica attractive also for packed and open tubular capillary columns in LC. Naturally, practical difficulties are met with capillaries of a few micrometer I.D. To avoid extra-column band-broadening, the injection and detection volumes should be in the nano- to picolitre range. This has been solved by split injections and various on-column detection techniques.

The most common type of polymeric stationary phases used in open tubular column chromatography are polyorganosiloxanes, which contain a linear backbone of siloxane bonds. Polyorganosiloxane stationary phases have been applied earlier for the preparation of narrow-bore columns for LC [12–18]. They have gained popularity due to properties such as easy formation of uniform and stable cross-linked films, high solute diffusivities, and liquid-like behaviour. The preferred combination of column properties narrow tubes, long columns and thick stationary phase films, all place high demands on the coating procedure. A lot of effort has been made in order to improve the methods for the preparation of narrow-bore OTCs. A major problem has been to achieve sufficient retention, owing to the small ratio of surface area to mobile phase volume in an open tube.

Several methods have been used to prepare small-diameter columns for OT-LC. The dynamic coating method, known from gas chromatography, was first used by Hibi et al. [10]. They coated borosilicate columns with inner diameters down to 30 μm. The dynamic method is a straightforward method, but very high pressures are required to prepare narrow-bore columns with thick films. In addition, it is difficult to control the film thickness and the uniformity along the tube.

With the static coating method it is easy to regulate and control the film thickness. This method is well-established for the preparation of GC columns and it results in more efficient columns than the dynamic method [19]. For the preparation of narrow-bore (30–50 μm) columns in GC, static coating was initially applied by

Schutjes et al. [20]. In OT-LC, the static coating method of polysiloxane stationary phases, was first utilized by Takeuchi et al. [21]. It is a relatively time-consuming method and the coating time increases for narrower columns with thicker stationary phase films. To accelerate the static coating technique for narrow-bore capillaries, different procedures have been employed. It has been performed at elevated temperatures [22,23], including the "free-release" static coating method [24], where the coating is done at higher temperatures, but without applying a vacuum at the column end. Another approach to speed up the static coating method, has been to select more volatile solvents for the stationary phase solution. Different variants have been tried, such as mixed solvents [25], liquefied gases [26,27] and freons [12,28].

Dluzneski and Jorgenson [13] introduced a new method for the preparation of narrow-bore columns. It is known as the precipitation coating method and is based on variable solubilities of a stationary phase polymer in selected solvent systems, at different temperatures. The method is fast but it is difficult to control the film thickness, and it has only been shown to work with the vinyl-modified version of the stationary phase OV-17.

The bonding of monomeric reagents to the column wall is an alternative to the polymeric coatings mentioned above. In order to yield sufficient retention it is necessary to increase the surface area of the capillary wall with an etching procedure. That makes the choice of column material important. The high purity of fused silica gives the material some advantageous properties, as mentioned earlier. However, it is difficult to increase the surface area in fused-silica capillaries. Therefore, borosilicate glass is still used in OT-LC for e.g. bonded ODS phases [29].

Recently, a new procedure supposed to simplify the preparation of narrow-bore OTCs, was presented by Slais et al. [30]. It utilizes a flowing retentive liquid as stationary phase. In addition OT-LC separations have been performed at elevated temperatures in order to obtain high efficiencies in commercially available columns (50–100 μm I.D.) [31,32].

To overcome the problem with low sample

capacity and low column retention in open tubular fused-silica columns, different approaches have been presented in the literature. Tock and co-workers [33,34] used an ammonia solution to convert polyethoxysiloxane films into porous silica layers in fused-silica capillaries. The layer was then modified with silane reagents. Euguchi et al. [35] were able to create thick films of acrylate polymers by photopolymerization in transparent fused-silica tubes, and they achieved considerable retention.

An alternative approach is to swell the polymeric coating, which acts as a stationary phase, with a solvent. Swelling of polysiloxanes with non-polar solvents was accomplished in 11–50 μm I.D. OTCs by Folestad and Larsson [36]. The stationary phase volume increased by a factor of 3 to 4. Besides the increase in retention, swelling also affects column selectivity and the diffusion coefficients in the stationary phase. Horká et al. [37] showed that immobilized Carbowax 20M stationary phase films in OTCs swelled in mobile phases of methanol and water. However, their primary aim was to improve the mass transfer in the stationary phase and not to increase the retention.
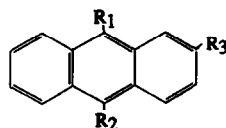
The aim of this work was to prepare and evaluate narrow (5–15 μm I.D.) and long (1–6 m) OTCs for reversed-phase liquid chromatography. The static evaporative and the precipitation coating methods were used to coat the columns with four different polymeric stationary phase films. Polyorganosiloxanes substituted with phenyl, methyl and octadecyl groups were used. In order to increase the retention capacity, which is an obstacle with OTCs, the polymers were also swelled with n-heptane. GC was used as a complementary evaluation method. Differences in the preparation methods and chromatographic performance are discussed.

EXPERIMENTAL

Chemicals

The stationary phases used were $PMSC_{18}$, PS-255, PS-264 and OV-17-vi. $PMSC_{18}$, a polymethyloctadecylsiloxane, was synthesized at Max Planck Institut für Kohlenforschung (Mülheim/Ruhr, Germany), and was a generous gift from G. Schomburg and J. Köhler. PS-255 is a

copolymer of dimethylsiloxane and 1–3% methylvinylsiloxane and PS-264 is a copolymer of 92–96% polydimethylsiloxane, 3–7% diphenylsiloxane and 0.5–1% methylvinylsiloxane, both gum phases from Fluka (Buchs, Switzerland). OV-17-vi is a methylphenylsilicone gum with 50% phenyl and 1% vinyl groups from Alltech (Deerfield, IL, USA). As radical initiators for the cross-linking reaction bis(α,α-dimethylbenzyl)peroxide (dicumyl peroxide) or benzoyl peroxide from Merck (Darmstadt, Germany) were used. The following solvents were applied during the coating procedure and in the evaluation work: n-pentane, dichloromethane, acetone, n-heptane, cyclohexane (analytical-reagent grade) from Merck (Darmstadt, Germany), acetonitrile (LC grade) from Rathburn (Walkerburn, UK), and water from a Milli-Q system from Millipore (Bedford, MA, USA). Anthracene derivatives were used as test compounds (see Fig. 1) and they were dissolved in the mobile phase. The derivatives were synthesized at the Department of Organic Chemistry, University of Göteborg and kindly provided by H.-D. Becker.


	R_1	R_2	R_3
1	-COOH	-H	-H
2	-(CH2)3OH	-H	-H
3	-OCH3	-H	-H
4	-H	-H	-H
5	-CH3	-H	-H
6	-H	-H	-CH3
7	-CH3	-CH3	-H
8	-⟨⟩	-H	-H
9	-(CH2)3-⟨⟩	-H	-H
10	-(CH2)3-CH3	-H	-H

Fig. 1. Anthracene derivatives used as test compounds.

Column preparation

Fused-silica tubing (5 and 10 μm I.D.) from Polymicro Technologies (Phoenix, AZ, USA) was coated mainly by the static coating method as described earlier [14–16]. The stationary phase and the peroxide for the cross-linking reaction were dissolved in n-pentane. When the capillaries were coated with the stationary phase OV-17-vi, the precipitation method as described by Dluzneski and Jorgenson [13] was applied. The dimensions of the columns investigated in this work are presented in Table I. Different phase ratios were obtained by varying the stationary phase concentration in the coating solution. The inner diameter of the columns was measured by the electrical resistance over the mercury filled capillary as described by Guthrie et al. [38]. The stationary phase film thickness, d_f, in static coating was calculated according to eqn. 1

$$d_f = r \cdot \sqrt{\frac{1}{100/c - 1} + 1} - r \qquad (1)$$

where r is the column radius and c is the concentration of the stationary phase in the coating solution (%, v/v). To calculate the volumetric concentration a density of 0.98 g ml^{-1} for PS-255 and PS-264 and a density of 0.9 g ml^{-1} for PMSC$_{18}$ was used.

Instrumental set-up

The chromatographic system was similar to the systems used previously for OT-LC in our laboratory [15,16]. The low volumetric flow-rates and the small injection volumes were created by a simple split arrangement. Splitting ratios up to 1:1 000 000 were used to minimize band broadening from the injection system. The swelling of the stationary phase film was performed according to Folestad and Larsson [36]. During

TABLE I

CHARACTERISTICS OF THE OPEN TUBULAR COLUMNS

Column No.	Internal diameter (μm)	Length (cm)	Stationary phase concentration (%, v/v)	Phase ratio[a]	Film thickness (μm)	Type of stationary phase
1	5.7	176	2.17	0.022	0.031	PMSC$_{18}$
2	5.8	144	1.10	0.011	0.016	PMSC$_{18}$
3	6.0	253	1.10	0.011	0.017	PMSC$_{18}$
4	6.0	212	1.10	0.011	0.017	PMSC$_{18}$
5	6.3	576	1.10	0.011	0.017	PMSC$_{18}$
6	11.1	140	6.25	0.067	0.180	PMSC$_{18}$
7	11.3	146	4.26	0.044	0.124	PMSC$_{18}$
8	12.6	160	2.17	0.022	0.069	PMSC$_{18}$
9	13.3	143	2.17	0.022	0.073	PMSC$_{18}$
10	13.8	475	2.17	0.022	0.076	PMSC$_{18}$
11	6.6	163	1.01	0.010	0.017	PS-255
12	11.7	184	2.97	0.030	0.089	PS-255
13	11.7	176	2.00	0.020	0.059	PS-255
14	11.9	157	2.00	0.020	0.060	PS-255
15	11.6	146	2.00	0.020	0.059	PS-255
16	6.5	187	1.01	0.010	0.017	PS-264
17	13.0	161	2.00	0.020	0.066	PS-264
18	13.0	179	1.01	0.010	0.033	PS-264
19	13.3	170	1.01	0.010	0.033	PS-264
20	6.5	115	1.00	–	–	OV-17-vi
21	9.2	156	2.00	–	–	OV-17-vi

[a] The phase ratio is defined as the stationary to mobile phase volume ratio.

the swelling experiments, the mobile phase was pre-saturated with *n*-heptane. The on-column detection was performed with a laser-induced fluorescence detection system [39,40]. The lasers used were a He–Cd laser Model 4210 NB from Liconix (Sunnyvale, CA, USA) and an argon ion laser Model 2025-05 from Spectra-Physics (Mountain View, CA, USA). The chromatograms were recorded on a Perkin-Elmer 56 strip chart recorder or with a computer system, PC integration pack from Kontron (Zürich, Switzerland).

Procedures

LC capacity factors, k', were measured at ambient temperature, and 9-anthracenecarboxylic acid and nitromethane were used as unretained solutes. The mobile phase used throughout this study was acetonitrile–water (50:50). The peaks were symmetrical in this study and they were treated as Gaussian. Consequently, the theoretical plate height, H for the test solutes was calculated from eqn. 2

$$H = \frac{Lw_{1/2}^2}{5.54t_R^2} \qquad (2)$$

where L is the column length, $w_{1/2}$ is the peak width at half the peak height and t_R is the retention time of the solute. Efficiency measurements at different flow-rates were made. In order to obtain the reduced velocity, ν, the solute diffusion coefficients in the mobile phase, D_m, were calculated from the Wilke–Chang equation [41]. The D_m values in this work are

TABLE II

DIFFUSION COEFFICIENTS IN ACETONITRILE–WATER (50:50), FOR ANTHRACENE DERIVATIVES, CALCULATED ACCORDING TO REF. 41, AT THE TEMPERATURE 298 K

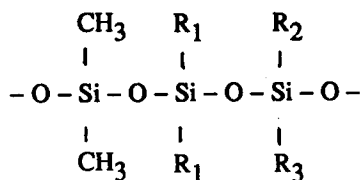| Solute	D_m (cm^2/s)
Anthracene	$8.5 \cdot 10^{-6}$
9-Methylanthracene	$8.0 \cdot 10^{-6}$
9-Phenylanthracene	$6.8 \cdot 10^{-6}$
Propylenphenylanthracene	$6.0 \cdot 10^{-6}$
n-Butylanthracene	$6.8 \cdot 10^{-6}$

Fig. 2. Polysiloxane backbone, the R groups symbolize various organic substituents (see Table III).

presented in Table II. Gas chromatography was used as a complement to study retention characteristics, efficiency and stability of the stationary phase film. Conventional GC equipment is not suited for columns with I.D. of 10 μm or less. Instead 50 μm I.D. columns were coated in an equivalent way. The GC measurements were performed in accordance with earlier work by us [16].

RESULTS AND DISCUSSION

The polyorganosiloxanes have various organic groups attached to the silicon atoms (Fig. 2), and the composition of the stationary phases used in this study is specified in Table III. The chemical and physical properties of polysiloxanes influence the efficiency, selectivity and stability of the coated columns. The column efficiency is a function of solute diffusion rates in the polymer film, as well as uniformity of the coated film. Selectivity is a function of the pendant groups on the polysiloxane backbone. The influence of selectivity is important since the separation of

TABLE III

COMPOSITION OF THE STATIONARY PHASES STUDIED

Numbers refer to the approximate molar proportion of each substituent group bonded to the siloxane backbone.

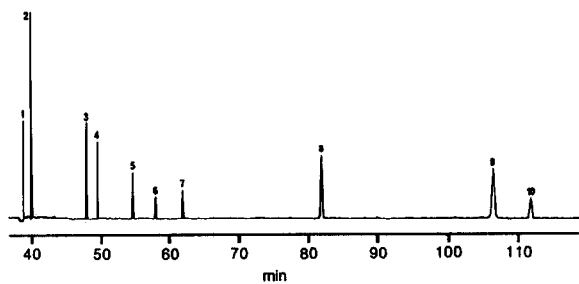| Stationary phase	Composition (%)			
	$-CH_3$	$-C_6H_5$	$-C_{18}H_{37}$	$-CH=CH_2$
OV-17-vi	50	50	–	1
PS-264	94	5	–	1
PS-255	99	–	–	1
PMSC$_{18}$	83	–	17	–

Fig. 3. Separation of anthracene derivatives in column No. 5 (5.8 m × 6.3 μm I.D.) coated with PMSC$_{18}$ and swelled with n-heptane. Mobile phase was acetonitrile–water (50:50). Linear flow-rate: 0.25 cm/s. Volumetric flow-rate: 4.6 nl/min. Peaks: 1 = 9-anthracene carboxylic acid; 2 = 9-propanol-anthracene; 3 = 9-methoxyanthracene; 4 = anthracene; 5 = 9-methylanthracene; 6 = 2-methylanthracene; 7 = 9,10-dimethylanthracene; 8 = 9-phenylanthracene; 9 = 9-propylene-phenylanthracene; 10 = 9-n-butylanthracene.

complex mixtures is facilitated by a variability in selectivity, besides a high efficiency and an adequate retention capacity. The possibility to affect the selectivity in LC with a change in mobile phase composition further increases the potential of high-resolution separations. An example of a high-resolution separation in a polysiloxane-coated OTC is shown in Fig. 3.

Column preparation

The static coating method. The static coating method is the preferred method for polymeric stationary phases in open tubular columns since it is possible to control the film thickness. One of the drawbacks with open tubular fused-silica columns is the low retention capacity. Higher k' values are obtained with thicker stationary phase films. Very thick films in capillary columns were considered in various respects by Grob and Grob [42]. The preferred phase for very thick coatings (up to 5 μm) was according to them, the gum phase PS-255, due to the low viscous solution. SE-54 (5% methylphenyl, 1% methylvinyl siloxane) was less suitable for coating of very thick films. This observation is in agreement with that of Folestad et al. [15]. They were able to coat a 50 μm I.D. column with a PS-255 solution of 13.6% (v/v) while it was impossible to prepare homogeneous SE-54 solutions with a concentration over 6%. The stationary phase PS-264 used in this work is a gum phase and has a

similar composition as the SE-54 phase. We were able to coat a series of 50 μm I.D. columns with a PS-264 concentration of up to 10.1% (v/v). There is a linear relationship between the capacity factor and the phase ratio in these PS-264 columns, when evaluated in GC. The efficiency, expressed as number of theoretical plates for n-alkanes, is comparable to the 50 μm I.D. columns coated with PS-255. Thus, it is possible to create thick stationary phase films with the stationary phase PS-264. However, when the internal diameter of the column is decreased the concentration of the stationary phase solution must be increased to provide similar film thickness. The difficulties in filling and evaporation of the solvent increase accordingly.

The main drawback of the static coating method is that it is relatively time consuming. Long coating times increase the risk of Rayleigh instability, which can result in rearrangement of the stationary phase film [43]. In this study the evaporation time was shorter for columns coated with the stationary phase PMSC$_{18}$ as compared to the two gum phases PS-255 and PS-264. The approximate evaporation time in a 2 m × 5 μm I.D. column coated with a stationary phase solution of 1% (v/v) was 2 h and 45 min for the gum phases, and 1 h and 40 min for the PMSC$_{18}$-coated columns. In addition, the capillaries were easier to fill with the PMSC$_{18}$ solution. Typically, the complete coating procedure of a 5 μm I.D. column took one working day, after the coating solution was homogenized.

To assess the reproducibility of the static coating method under demanding conditions, two long columns (over 5 m in length) of 6 μm I.D. were coated with PMSC$_{18}$ solutions of the same concentration. One of these columns was accidentally broken in halves and was evaluated as two separate columns. The retention and selectivity properties of the "three" columns were equal. These characteristics will be discussed further below. It is generally assumed, that the number of theoretical plates will increase linearly with the length of the column (at constant separation temperature and linear flow velocity). This is exemplified in Fig. 4 where N/m is plotted against the linear flow velocity for the long column and the second half of the
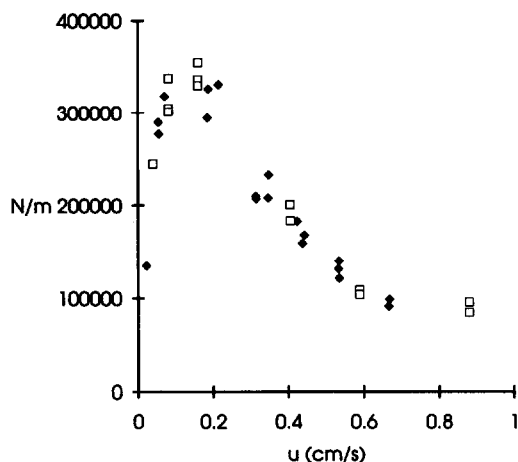
Fig. 4. The number of theoretical plates per meter vs. the linear flow-rate in two 6 μm I.D. PMSC$_{18}$-coated columns, ($\square$) column No. 4 and ($\blacklozenge$) column No. 5. Test solute: 9-phenylanthracene, $k' = 0.26$.

divided column for a retained substance. As can be seen there is good agreement between the two columns. Also, this result is equal to that of column 2, which was coated as a 1.7 m long column. Overall the retention characteristics and efficiency are similar in the columns, which indicates that the reproducibility of the column preparation procedure is appropriate.

To conclude, narrow-bore columns of 5–15 μm I.D. and a length of up to 6 m were successfully prepared. The coating times for all the columns in this work were reasonable compared to for example Van Berkel-Geldof et al. [12,17], who showed that static coating of open tubular columns with PS-255 solutions in n-pentane at 45°C, required several days. Also under demanding conditions, i.e. coating 6 m long columns with an I.D. of 6 μm, the static coating method resulted in efficient columns.

The precipitation coating method. The precipitation coating method [13] is convenient even for very narrow columns. It has resulted in stable columns with inner diameters as narrow as 1.7 μm [44]. The choice of stationary phase is still limited to OV-17-vi, so despite the ease of manipulation and the promptness, the method has not been widespread.

In order to achieve high efficiency in capillary columns it is important to coat the columns with uniform stationary phase films. Originally, it was shown in GC that OTCs coated with polysiloxanes of high phenyl content yielded a relatively low efficiency. This was thought to be related to the low viscosity of the phenyl-containing phases at that time. However, the OV-17-vi has more of a gum like character and is consequently better suited as stationary phase in open tubular columns. Static coating of a similar type of stationary phase in GC columns gave columns of higher efficiency than the original OV-17 phase [45].

To get a rough estimate of the uniformity of the OV-17-vi films, 50 μm I.D. columns coated with the precipitation method were evaluated, with GC. The efficiency in isothermal separations was evaluated as the number of theoretical plates, N, for n-alkanes and in temperature-programming as Trennzahl (TZ) according to Grob et al. [46]. At the same k' value and the same linear flow-rate, N values in the OV-17-vi coated columns were 4–5 times lower compared to 50 μm I.D. columns coated with PS-255, evaluated earlier by us [47]. The separation efficiency expressed as Trennzahl was approximately 20 and 10 in PS-255 and OV-17-vi coated columns, respectively, at the same elution temperature.

A low efficiency in GC is generally assumed to be associated with an irregular distribution of the stationary phase film. Other parameters that influence the column efficiency with the precipitation method, according to Dluzneski [44] are the precipitation time and the time for removal of solvent. He also observed that there was a large difference in viscosity for different batches of OV-17-vi. However, there was no significant difference in the obtained efficiency in LC, for the narrow-bore OV-17-vi coated columns, as compared to the other stationary phases in this work.

Immobilization and column stability

The degree of stationary phase immobilization was estimated by GC measurements of k' values for n-alkanes, before and after rinsing the column with organic solvents. Such measurements have been performed earlier in our lab-

oratory for the stationary phases PS-255 [15] and PMSC$_{18}$ [16], in 50 μm I.D. columns. It was found that the extractable part of the stationary phase was 8–10% in PS-255-coated columns and somewhat higher, 10–30%, in PMSC$_{18}$-coated columns. A stationary phase similar to OV-17-vi coated with the static coating method and evaluated in GC showed a 20% loss of stationary phase [48]. Dluzneski [44] estimated the extractability of the OV-17-vi stationary phase by the measurement of k' values in LC. He showed that the extractability depended on the percentage of peroxide used for cross-linking. At the recommended concentration, 1% peroxide (w/w of the polymer), the loss of stationary phase was 16–34%.

In this study the stationary phases PS-264 and OV-17-vi were coated in 50 μm I.D. columns and evaluated by GC to estimate the degree of cross-linking. For PS-264 the extractable part was 3–12% and in the OV-17-vi columns coated with the precipitation coating method it was 1–30%. The loss of stationary phase in columns coated with the precipitation method increased with the film thickness. This differs from the observations for the other stationary phases, where the removable part seemed to be independent of d_f.

In order to study the stability of the stationary phases in LC, columns have been stored with mobile phase inside for several years. The columns have also been used in an indirect detection mode with basic dyes as visualizing agents [49]. The mobile phases used were up to 90% buffer of pH 3, and various additives *e.g.* methylene blue, tetramethylammonium chloride, and ion-pairing agents such as n-pentane sulfonic acid. These conditions are comparable to the ageing experiments performed on polymer-coated silica particles by Hetem *et al.* [50]. For example, in our column 17 the k' values for the anthracene derivatives were the same in 1989, when the column was prepared, and in 1992 after storage with mobile phase (acetonitrile and water) and separations under the demanding conditions described above. No influence on the peak shape could be observed. Consequently the stability of the polymer coated columns is high.

Retention and selectivity characteristics

Swelling. To increase the retention in OTCs, the polymeric stationary phase can be swelled with an organic solvent. The extent of swelling depends on the similarity (polarity) of the stationary phase and the solvent, and the degree of cross-linking in the stationary phase. It has been shown [36] that PS-255 and SE-54 coated in 50 μm I.D. columns, swell 3–4 times (by volume in n-heptane). The swollen stationary phases exhibited good stability. Dluzneski [44] tried to swell narrow-bore OTC coated with OV-17-vi. He used n-heptane as a swelling agent, but the k' value for 9-methyl anthracene was lower after the presumed swelling, probably partly owing to extraction of the stationary phase. Pretorius and Lawson [51] studied the swelling of polysiloxane stationary phases in batch experiments. Their results showed that OV-17 swelled 62% in hexane as compared to polydimethylsiloxane which swelled 130% in the same solvent. Additionally, their result showed that dichloromethane was a better swelling agent for OV-17. In order to achieve stable systems the mobile phase needs to be pre-saturated with the swelling agent. The high solubility of dichloromethane in a mixture of acetonitrile and water is unfavourable. A better choice of mobile phase is methanol and water and some preliminary experiments with this combination have been performed [52].

The PMSC$_{18}$ stationary phase has been investigated earlier in OT-LC by us [5,16]. The retention characteristics of the PMSC$_{18}$ stationary phase are favourable but the capacity factors are still comparatively low. In this study swelling of the PMSC$_{18}$ film with n-heptane was performed. The swelling of the stationary phase films in the 50 μm I.D. columns was measured by the difference in I.D. before and after swelling. The film thickness increased with a factor 3–4 and the swelling factor S_v, calculated according to ref. 36, was 2.3–2.5. Thus the swelling of PMSC$_{18}$ is similar to the swelling of PS-255 studied earlier [36]. The increase in retention after swelling of the PMSC$_{18}$ was approximately 5 times and 6 times for fluorene and phenylfluorene, respectively (Table IV). This increase is smaller as compared to the increase of 12–16 times on gum

TABLE IV

SWELLING EFFECTS IN 50 μm I.D. PMSC$_{18}$ COATED COLUMNS

Column I: 58.7 μm I.D., 151 cm long and a film thickness of 0.65 μm. Column II: 49.7 μm I.D., 196 cm long and a film thickness of 0.27 μm. The mobile phase used was acetonitrile–water (50:50). k' values for fluorene derivatives before and after swelling with n-heptane, and selectivity factor $\alpha = (k'_{phenylfluorene})/(k'_{fluorene})$ before and after swelling with n-heptane.

Column	$k'_{fluorene}$	$k'_{phenylfluorene}$	$k'_{fluorene}$, swelled	$k'_{phenylfluorene}$, swelled	α	α, swelled
I	0.22	0.35	1.07	2.02	1.59	1.83
II	0.46	0.72	2.24	4.10	1.57	1.89

phases for the same solutes [36]. Since the degree of swelling is similar the reason could be that the influence of n-heptane in the film is smaller for the more lipophilic PMSC$_{18}$ than for PS-255. The increase in retention for the anthracene derivatives in these 50 μm I.D. columns was 7–10 times. As expected the selectivity in the columns changed after the swelling.

Retention. Capacity factors of anthracene derivatives on cross-linked polysiloxanes of varying composition are presented in Table V. The relative standard deviation was 1–4% for the k' values measured in the flow-rate interval of 0.03–2.5 cm/s. The highest capacity factors for these solutes were observed on columns coated with the stationary phase OV-17-vi, which contains 50% phenyl. Swelling the polymeric stationary phase with n-heptane resulted in increased retention for all types of stationary phases. The relative standard deviation for the k'

value measurements was 2–7% for the swelled stationary phases, over a larger flow-rate interval (up to 5 cm/s).

The gum phases PS-255 and PS-264 coated in 10 μm I.D. columns showed the largest increase in retention for the anthracene derivatives, 10–20 times. The only exception was the column with the thickest film (No. 12), which showed a k' enhancement of 5–10 times. The k' increase for PMSC$_{18}$-coated columns was 3–13 times. In general solutes with higher k' showed a larger increase in retention after swelling, in the same column.

Measurements of the internal diameter of the column before and after swelling with n-heptane in two of the 10 μm I.D. columns (columns 6 and 17) showed that the increase in film thickness was approximately 2.5 times. However, the real phase ratio in the small-diameter columns is unknown (see the part about immobilization). In

TABLE V

CAPACITY FACTORS FOR ANTHRACENE AND 9-PHENYLANTHRACENE IN 10 AND 5 μm I.D. OPEN TUBULAR COLUMNS

The columns were coated with polysiloxane stationary phases of different composition. The concentration of the stationary phase solution was 2% in the 10 μm I.D. and 1% in the 5 μm I.D. columns. Mobile phase acetonitrile–water (50:50).

Stationary phase	10 μm I.D. columns		5 μm I.D. columns	
	$k'_{anthracene}$	$k'_{9-phenylanthracene}$	$k'_{anthracene}$	$k'_{9-phenylanthracene}$
PMSC$_{18}$	0.07	0.22	0.08	0.27
PS-255	0.05	0.15	0.05	0.08
PS-264	0.08	0.28	0.03	0.07
OV-17-vi	0.38	1.82	0.50	2.35

addition differences in the cross-linking density could affect the swelling.

Capacity factors, at the same nominal phase ratio, were higher in the 5 μm I.D. columns than in the 10 μm I.D. columns, for PMSC$_{18}$ [16] and OV-17-vi. For example we found that the k' values in the 5 μm I.D. column, coated with a 1% solution, were higher than in the 10 μm I.D. column coated with a 2% solution. However, this has been observed earlier [13], where concentrations of 1% or less of OV-17-vi, gave unexpectedly high k' values.

The 5 μm I.D. columns coated with PS-255 and PS-264 have a lower retention capacity than expected. Furthermore, the selectivity and the increase in retention after swelling in these columns were lower compared to the 10 μm I.D. columns. Grob and Grob [53] noticed that a thick stationary phase film could be almost completely removed by rinsing in spite of cross-linking. They suggested that the rinsing solvent swells the stationary phase and for thick films the expansion could fill a large part of the tubing. Under these conditions the solvent moves with friction, which could be sufficient to tear away a considerable part of the stationary phase. For thinner films there will remain a free space inside the tubing so the solvent can flow with a lower resistance. We always rinse the columns with organic solvents (*e.g.* dichloromethane and *n*-pentane) to remove the extractable part of the stationary phase before LC testing. Sometimes the result is a temporary plugging of the narrow-

bore columns. This occurred *e.g.* in the 5 μm I.D. column coated with PS-264. High pressures were used to remove the plug and it is possible that part of the stationary phase was torn off.

Selectivity. The selectivity factors, for the anthracene derivatives on the different stationary phases used, are presented in Fig. 5a and b. For the phenyl-containing stationary phases, the influence of the phenyl-group in 9-propylphenyl-anthracene was larger compared to the phenyl-group in 9-phenylanthracene. This may be explained by the structure of the two compounds. Intramolecular interactions of the aromatic rings in 9-phenylanthracene are hindered when the phenyl group is located directly on the anthracene molecule. Fig. 5b illustrates the increased similarity between the columns after swelling.

To estimate the contribution from substituents, we calculated the difference in Gibbs free energy ($\Delta(\Delta G)$) from retention data, see eqn. 3

$$\Delta(\Delta G) = RT \ln \alpha \qquad (3)$$

where R is the gas constant and T is the thermodynamic temperature (296 K). Group selectivity calculations for a methyl group, derived from 9,10-dimethylanthracene *vs.* anthracene and from 9-methylanthracene *vs.* anthracene, gave identical values of the selectivity contribution in a column, approximately 900 J/mol.
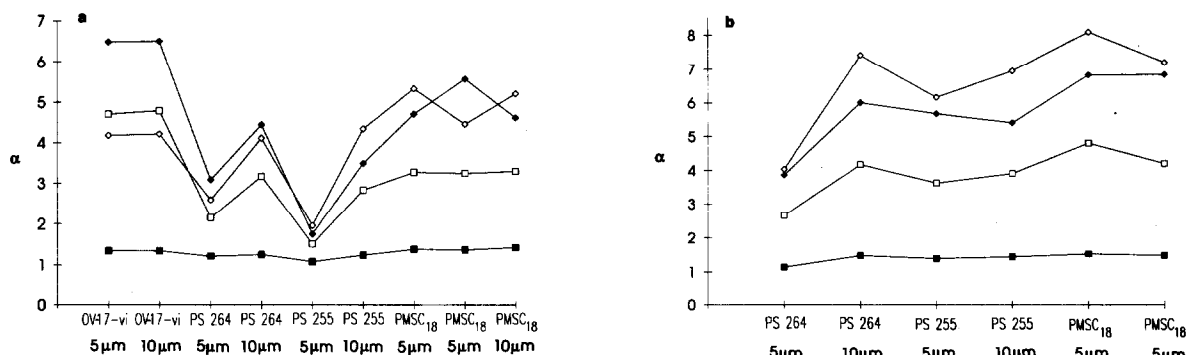
In Fig. 6 the contribution from the phenyl

Fig. 5. Selectivity factors, α, in columns coated with different stationary phases and of different internal diameter. The α-values are calculated against anthracene. ■ = 9-Methylanthracene; □ = 9-phenylanthracene; ◆ = 9-propylenephenylanthracene; ◇ = 9-*n*-butylanthracene. (a) Before swelling with *n*-heptane. (b) After swelling with *n*-heptane.
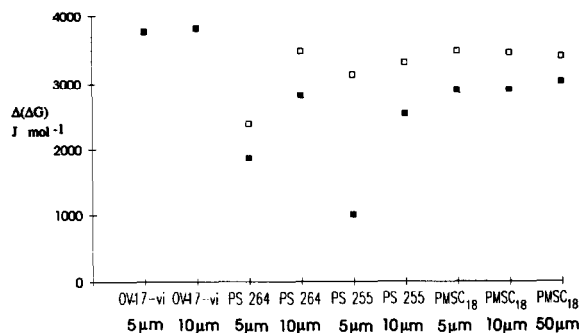
Fig. 6. The difference in Gibbs free energy, $\Delta(\Delta G)$ (J/mol), for a phenyl group in the different columns. $\Delta(\Delta G)$ was calculated from 9-phenylanthracene and anthracene. ■ = Before swelling, □ = after swelling.

group in 9-phenylanthracene is shown. The phenyl groups in the stationary phase OV-17-vi show a higher selectivity towards the phenyl substituted anthracene. Whereas the PS-264 stationary phase, containing a smaller fraction of phenyl groups, has about the same selectivity as the PMSC$_{18}$ stationary phase.

The methylene group selectivity for columns with different stationary phases and internal diameters is shown in Fig. 7. Different values were obtained when the methylene group selectivity was calculated from 9-butylanthracene and 9-methyl anthracene compared to propylphenyl-anthracene and 9-phenylanthracene. This may be explained by the different environment for the methylene group units. Evidently the selectivity effect of a methylene unit is different when it is
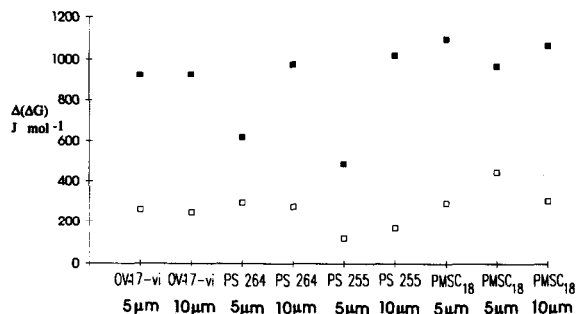

Fig. 7. The difference in Gibbs free energy, $\Delta(\Delta G)$ (J/mol), for a methylene group in the different columns. $\Delta(\Delta G)$ was calculated in two ways: (□) from 9-propylenephenyl-anthracene and 9-phenylanthracene and (■) from 9-n-butyl-anthracene and 9-methylanthracene.

located on an aromatic ring as compared to an alkyl chain.

Columns coated with the same stationary phase in general show the same selectivity. Notable in Figs. 5–7 is the anomalous behaviour of the 5 μm I.D. columns coated with gum phases as discussed above. Also, the order of 9-propylenephenylanthracene and 9-n-butylanth-racene was reversed in two of the 5 μm I.D. columns coated with PMSC$_{18}$. These two columns were the last to be prepared and the dissimilarity could be caused by a change in the polymer composition owing to ageing.

To conclude, it was seen that polysiloxane stationary phase composition affects the selec-tivity for the anthracene derivatives. Overall, the same selectivity factors were obtained in the polysiloxanes after swelling with n-heptane. This indicates that the organic solvent dominates the retention mechanism in the swelled columns.

Retention and selectivity, comparison with other publications. Even if conventional liquid chromatography has a high accuracy, precision and repeatability within one laboratory, reten-tion results between laboratories, or even on nominally identical systems, can vary significant-ly. Differences in the stationary phase materials, and methods to measure the void volume are two of the problems.

The gum phases PS-255 and PS-264 have been evaluated earlier in OT reversed-phase LC [12,15,17]. Comparison of k' values and selec-tivity factors for anthracene derivatives from different groups are presented in Table VI. It can be seen that the obtained retention in the columns coated by Van Berkel-Geldof et al. [12] is higher compared to columns prepared in our laboratory. In our work and in ref. 15 the columns were rinsed with pentane and dichloro-methane while the columns in ref. 12 were rinsed with a mixture of acetonitrile and water, before LC tests. Consequently, a larger part of the stationary phase could be extracted in the former case, which leads to a lower retention. It is however important to rinse out the extractable part of the stationary phase, especially to dimin-ish the risk of plugging when the columns are swelled with n-heptane. The mobile phase composition was not the same in the different

TABLE VI

COMPARISON OF THE RETENTION AND SELECTIVITY DATA ON THE GUM PHASES PS-255 AND PS-264 FROM DIFFERENT PUBLICATIONS

The columns were coated with the static coating technique. In this work acetonitrile–water (50:50) and in refs. 12 and 15 acetonitrile–water (40:60) was used.

Stationary phase	I.D. (μm)	Phase ratio[b]	$k'_{anthracene}$		Selectivity[a]	Ref.
			Acetonitrile–water (40:60)	Acetonitrile–water (50:50)		
PS-255	11.7	0.030		0.077	1.2	This work, column no. 12
PS-255	10.5	0.032	0.97		1.3	12
PS-255	11.6	0.050	0.14			15
PS-255	28.5	0.042	1.3			12
PS-264	13.0	0.020			1.3	This work, column no. 17
PS-264	25.1	0.059			1.3	12

[a] Selectivity factors for 9-methylanthracene vs. anthracene.
[b] Stationary to mobile phase volume ratio.

papers. However, the selectivity factors for 9-methylanthracene shown in Table VI are similar. This agrees with our previous observations with 50 μm I.D. columns where acetonitrile–water (50:50) and (40:60) gave the same α for 9-methylanthracene vs. anthracene.

The phase ratios in the columns coated with OV-17-vi are unknown, but according to Dluzneski and Jorgenson [13] higher stationary phase concentrations will result in columns with a larger amount of stationary phase. The k' values can be compared to those obtained for 9-methylanthracene in 10 μm I.D. columns in the method description [13]. The k' value for 9-methylanthracene in a column coated with a 2% solution is approximately 1.2 with a mobile phase composition of acetonitrile–water (35:65). In our 10 μm I.D. column k' was 0.5 for the mobile phase composition 50:50, which agrees reasonably well.

Kinetic performance efficiency

To determine the kinetic performance in chromatographic columns it is necessary to use solutes that show the column at its best, *i.e.* give symmetrical peaks [54]. The anthracene solutes are a good choice in this respect.

A rough estimate of the diffusion coefficients in the different stationary phases (D_s value) was

obtained by comparing experimental data with the Golay equation by use of a curve-fitting procedure. D_s values in 10 μm I.D. columns coated with PMSC$_{18}$ were $1 \cdot 10^{-8}$–$4 \cdot 10^{-8}$ cm^2/s and in the PS-255 coated columns they were $5 \cdot 10^{-9}$–$8 \cdot 10^{-9}$ cm^2/s for 9-phenylanthracene. In the 5 μm I.D. columns coated with PMSC$_{18}$ the obtained D_s values were smaller. In column 5 the D_s value was approximately $1 \cdot 10^{-9}$ cm^2/s.
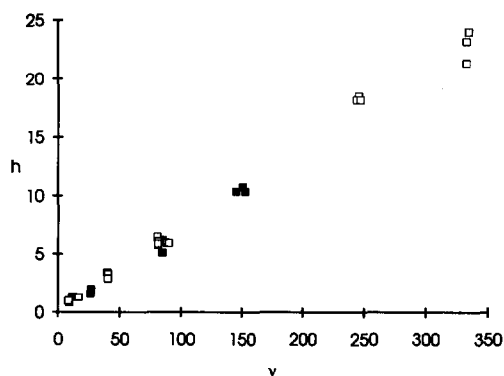

Fig. 8. Reduced plate height, h, vs. reduced flow-rate, ν, in the columns coated with OV-17-vi. ■ = column No. 20 with an internal diameter of 6.5 μm. Test solute was 9-phenylanthracene with a k' value of 2.4. □ = Column No. 21 with an internal diameter of 9.2 μm. Test solute was 9-propylenephenylanthracene with a k' value of 2.5. D_m values used to calculate the reduced velocity are shown in Table II.

After swelling and assuming an increase in film thickness of 2.5 times in column 6 the obtained D_s value was $1 \cdot 10^{-8}$ cm^2/s. Again the real film thickness in these columns is unknown. However, estimating the loss in film thickness to 10% gave approximately the same D_s value in the columns.

In OV-17-vi coated columns the nominal film thickness is unknown. However, Fig. 8 shows the experimentally obtained reduced plate height ($h = H/d_c$, d_c = column diameter) vs. the reduced velocity ($v = ud_c/D_m$, u = linear velocity) for the two columns coated with OV-17-vi, for solutes of approximately the same k' value. The major advantage of reduced parameters rather than absolute ones, is that they readily allow comparisons of columns with different internal diameters and for solutes with different diffusion coefficients. As can be seen there is an excellent agreement between the efficiency in the two columns.

In Fig. 9, h is plotted vs. v for three of the stationary phases: PMSC$_{18}$, OV-17-vi and one of the gum phases, PS-264. Different solutes were chosen to obtain the same k' value ($k' = 0.36$). A h_{min} of 0.5 was achieved, which corresponds to 180 000 plates/m in the PMSC$_{18}$ column, 217 000
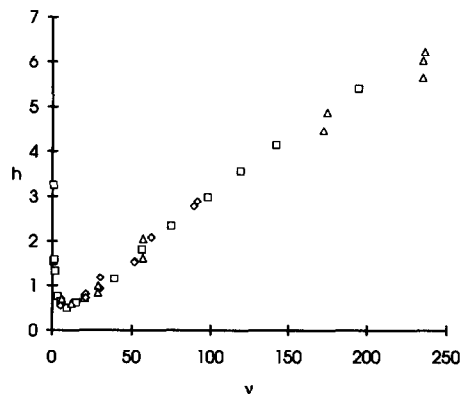

Fig. 10. The number of theoretical plates/m vs. k'. The linear velocity of the mobile phase was 0.2 cm/s. PMSC$_{18}$-coated columns. ■ = Column No. 5 (5.8 m × 6.3 μm I.D.) before swelling and □ = after swelling with n-heptane. ◆ = Column No. 6 (1.4 m × 11.1 μm I.D.).

plates/m in the OV-17-vi column and 154 000 plates/m in the PS-264-coated column.

Certainly, the obtained number of theoretical plates and the low h values in the OTCs are impressive. However, one must not forget the dependence of efficiency on k' at these low k' values. The rapid drop in N with increased k' values is illustrated, by experimentally obtained data, in Fig. 10.

When the quality of a separation should be judged, both the performance and the time must be considered along with the experimental conditions. To consider time, the rate of production of effective theoretical and theoretical plates (N_{eff} and N, respectively) was studied in the columns. N per unit time is facilitated by high flow-rates and low k' values. In the 10 μm I.D. columns this number is approximately 300 plates/s, whereas in the 5 μm I.D. columns up to 2000 plates/s could be reached at low k' values. N_{eff} depends on the k' value and the highest N_{eff} was, consequently, obtained in the swelled stationary phases, and in the columns coated with OV-17-vi. Concurrently the retention time increases yielding N_{eff}/s in the same order of magnitude as for the columns with lower k' values. In addition the obtained resolution vs. the retention time for the last eluting component in the pair could be plotted, as recommended by Ettre and March [55]. Such plots are shown in Fig. 11. The solute

Fig. 9. Comparison of the reduced plate height vs. reduced velocity for columns coated with the different stationary phases. □ = Column No. 6 (1.4 m × 11.1 μm I.D.) coated with PMSC$_{18}$, 9-methylanthracene, $k' = 0.36$. △ = Column No. 21 (1.6 m × 9.21 μm I.D.) coated with OV-17-vi, anthracene, $k' = 0.38$. ◇ = Column No. 17 (1.6 m × 13 μm I.D.) coated with PS-264, 9-n-butylanthracene, $k' = 0.36$. D_m values used to calculate the reduced velocity are shown in Table II.

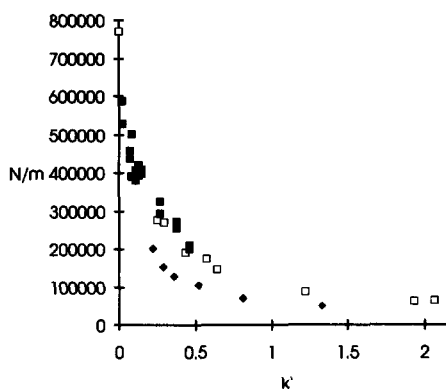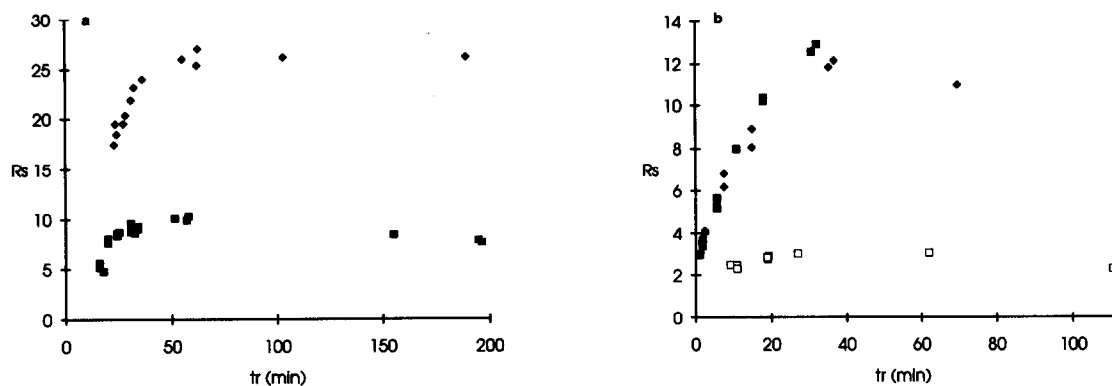Fig. 11. Resolution for the solute pair 9-methylanthracene and anthracene *vs.* the retention time of 9-methylanthracene. (a) Column No. 5 (5.8 m × 6.3 μm I.D.) coated with PMSC$_{18}$ (■) before swelling and (◆) after swelling with *n*-heptane. (b) Column No. 17 (1.6 m × 13 μm I.D.) coated with PS-264, (□) before swelling and (◆) after swelling with *n*-heptane. (■) Column No. 21 (1.6 m × 9.2 μm I.D.) coated with OV-17-vi.

pair is 9-methylanthracene and anthracene, which has approximately the same α value for all the stationary phases. The swelling of the stationary phase increases the resolution considerably due to the increase in k' value (Fig. 11a and b). The obtained resolution in the 6.3 μm I.D. column (Fig. 11a) is higher as compared to the 13 μm I.D. column (Fig. 11b). In Fig. 11b the high resolution obtained in a OV-17-vi coated column, possessing a high retention

capacity, is also shown. This also illustrates the large influence on resolution from the retention capacity.

CONCLUDING REMARKS

Chromatographers have explored Golay's model of open tubular columns, aiming at an improved resolution. In gas chromatography and supercritical fluid chromatography open tubular
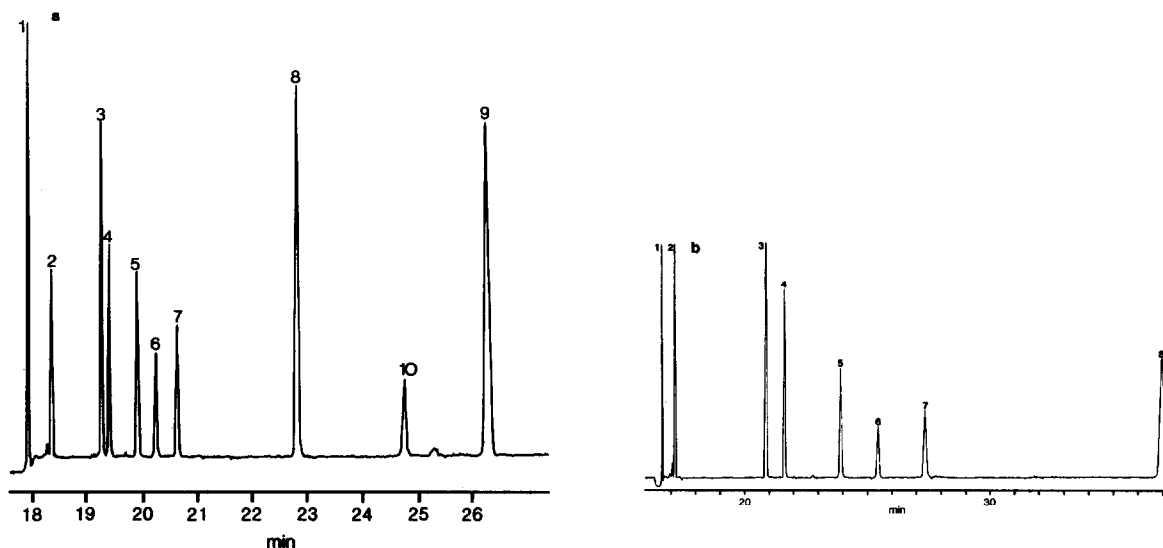

Fig. 12. Separations of anthracene derivatives. PMSC$_{18}$-coated column No. 5 (5.8 m × 6.3 μm I.D.) at approximately the same mobile phase velocity. Linear flow-rate: 0.55 cm/s. Volumetric flow-rate: 10 nl/min. Other separation conditions as in Fig. 4. (a) before swelling and (b) after swelling with *n*-heptane.

columns are widely used nowadays. In liquid chromatography however, the columns need to be very narrow to be able to show the excellent performance suggested in theory. Analytical requirements of the stationary phase and the columns are defined polarity, variable phase ratio, long-term chemical stability and mechanical strength. The columns prepared and evaluated in this work agree well with the requirements stated above. It is shown that the static coating method (the preferred method for coating OTCs) works well even for very long (6 m) and narrow (6 μm) columns. High efficiency was achieved in our columns, e.g. more than 10^6 theoretical plates. The time of the complete coating procedure is reasonable. The swelling of the stationary phase to increase the k' values was here shown to work well in narrow-bore OTCs and stable systems were achieved. The swelling of the stationary phase also influences the selec-

tivity of the column. Fig. 12 shows the efficient separation obtained in a long narrow column and the differences in resolution before and after swelling with n-heptane. In Fig. 13 the excellent retention properties of OV-17-vi columns for these solutes is shown. The influence of the high amount of phenyl groups in the stationary phase film is visualized by the elution order of the anthracene derivatives substituted with phenyl groups. Also, it illustrates that the efficient mass transfer in these columns can be used to achieve fast separations.

Fig. 13. Fast separation of anthracene derivatives in an OV-17-vi coated column No. 21 (1.6 m × 9.2 μm I.D.). Linear flow-rate: 2.2 cm/s. Volumetric flow-rate: 84 nl/min. Other separation conditions as in Fig. 4.

REFERENCES

1 J.H. Knox and M.T. Gilbert, J. Chromatogr., 186 (1979) 405.

2 G. Guiochon, Anal. Chem., 53 (1981) 1318.

3 F.J. Yang, J. Chromatogr. Sci., 20 (1982) 241.

4 J.W. Jorgenson and E.J. Guthrie, J. Chromatogr., 255 (1983) 335.

5 K. Göhlin, A. Buskhe and M. Larsson, Chromatographia, in press.

6 D. Ishii, T. Tsuda and T. Takeuchi, J. Chromatogr., 185 (1979) 73.

7 T. Tsuda, K. Tsuboi and G. Nakagawa, J. Chromatogr., 214 (1981) 283.

8 D. Ishii and T. Takeuchi, J. Chromatogr. Sci., 22 (1984) 400.

9 T. Tsuda, K. Hibi, T. Nakanishi, T. Takeuchi and D. Ishii, J. Chromatogr., 158 (1978) 227.

10 K. Hibi, D. Ishii, I. Fujishima, T. Takeuchi and T. Nakanishi, J. High Resolut. Chromatogr. Chromatogr. Commun., 1 (1978) 21.

11 R. Dandeneau and E.H. Zerenner, J. High Resolut. Chromatogr. Chromatogr. Commun., 2 (1979) 351.

12 O. van Berkel-Geldof, J.C. Kraak and H. Poppe, J. Chromatogr., 499 (1990) 345.

13 P.R. Dluzneski and J.W. Jorgenson, J. High Resolut. Chromatogr. Chromatogr. Commun., 11 (1988) 332.

14 A. Farbrot, S. Folestad and M. Larsson, J. High Resolut. Chromatogr. Chromatogr. Commun., 9 (1986) 117.

15 S. Folestad, B. Josefsson and M. Larsson, J. Chromatogr., 391 (1987) 347.

16 K. Göhlin and M. Larsson, J. Microcol. Sep., 3 (1991) 547.

17 O. van Berkel, H. Poppe and J.C. Kraak, Chromatographia, 24 (1987) 739.

18 J.W. Jorgenson, E.J. Guthrie, R.L. St. Claire III, P.R. Dluzneski and L.A. Knecht, J. Pharm. Biomed. Anal., 2 (1984) 191.

19 G.A.F.M. Rutten and J.A. Luyten, J. Chromatogr., 74 (1972) 177.

20 C.P.M. Schutjes, E.A. Vermeer, J.A. Rijks and C.A. Cramers, J. Chromatogr., 253 (1982) 1.

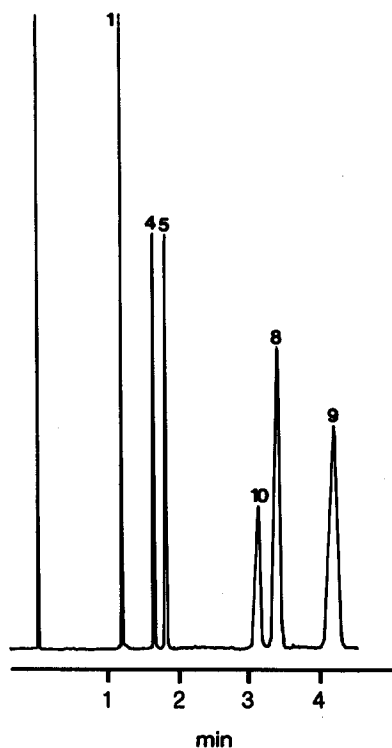21 T. Takeuchi, H. Kitamura, T. Spitzer and D. Ishii, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 666.

22 R.C. Kong and M.L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 319.

23 F.I. Onuska, *J. Chromatogr.*, 289 (1984) 207.

24 B. Xu and N.P.E. Vermeulen, *Chromatographia*, 18 (1984) 520.

25 R.C. Kong and M.L. Lee, *Chromatographia*, 17 (1983) 451.

26 T. Wännman, L. Blomberg and S. Schmidt, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 32.

27 K. Janák, V. Kahle, K. Tesarik and M. Horká, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 843.

28 S.R. Sumpter, C.L. Woolley, E.C. Huang, K.E. Markides and M.L. Lee, *J. Chromatogr.*, 517 (1990) 503.

29 R.L. St. Claire III, D.M. Dohmeier and J.W. Jorgenson, *J. Microcol. Sep.*, 3 (1991) 303.

30 K. Slais, M. Horká and K. Kleparnik, *J. Chromatogr.*, 605 (1992) 167.

31 G. Liu, N.M. Djordjevic and F. Erni, *J. Chromatogr.*, 592 (1992) 239.

32 G. Liu, N.M. Djordjevic and F. Erni, *J. Chromatogr.*, 598 (1992) 153.

33 P.P.H. Tock, G. Stegemann, R. Peerboom, H. Poppe, J.C. Kraak and K.K. Unger, *Chromatographia*, 24 (1987) 617.

34 P.P.H. Tock, C. Boshoven, H. Poppe, J.C. Kraak and K.K. Unger, *J. Chromatogr.*, 477 (1989) 95.

35 S. Euguchi, J.G. Kloosterboer, C.P.G. Zegers, P.J. Schoenmakers, P.P.H. Tock, J.C. Kraak and H. Poppe, *J. Chromatogr.*, 516 (1990) 301.

36 S. Folestad and M. Larsson, *J. Chromatogr.*, 394 (1987) 455.

37 M. Horká, K. Janák and M. Krejcí, *J. Microcol. Sep.*, 3 (1991) 217.

38 E.J. Guthrie, J.W. Jorgenson, L.A. Knecht and S.G. Bush, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 566.

39 S. Folestad, L. Johnson, B. Josefsson and B. Galle, *Anal. Chem.*, 54 (1982) 925.

40 S. Folestad, B. Josefsson and B. Galle, *J. Chromatogr. Sci.*, 23 (1985) 273.

41 C.R. Wilke and P. Chang, *Am. Inst. Chem. Eng. J.*, 1 (1955) 264.

42 K. Grob and G. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 133.

43 K.D. Bartle, C.L. Woolley, K.E. Markides, M.L. Lee and R.S. Hansen, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 128.

44 P.R. Dluzneski, *Ph.D. Thesis*, The University of North Carolina at Chapel Hill, Chapel Hill, NC, 1987.

45 B.W. Wright, P.A. Peaden and M.L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 413.

46 K. Grob, G. Grob and K. Grob, Jr., *J. Chromatogr.*, 156 (1978) 1.

47 K. Göhlin and M. Larsson, *J. Chromatogr.*, 634 (1993) 31.

48 P.A. Peaden, B.W. Wright and M.L. Lee, *Chromatographia*, 15 (1982) 335.

49 S. Folestad, K. Göhlin, A.P. Larsson and A. Wallin, in preparation.

50 M.J.J. Hetem, J.W. de Haan, H.A. Claessens, C.A. Cramers, A. Deege and G. Schomburg, *J. Chromatogr.*, 540 (1991) 53.

51 V. Pretorius and K. Lawson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 335.

52 K. Göhlin, unpublished results.

53 K. Grob and G. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 153.

54 P.A. Bristow and J.H. Knox, *Chromatographia*, 10 (1977) 279.

55 L.S. Ettre and E.W. March, *J. Chromatogr.*, 91 (1974) 5.