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SWOLLEN POLYSILOXANES AS STATIONARY PHASES IN REVERSED-PHASE OPEN-TUBULAR COLUMN LIQUID CHROMATOGRAPHY

STAFFAN FOLESTAD and MARITA LARSSON*

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

SUMMARY

A new liquid stationary phase was created after swelling cross-linked polysiloxanes with non-polar organic solvents in 11–55 μm I.D. fused-silica open-tubular columns. By choosing different polysiloxane/swelling solvent pairs, the stationary phase film thickness, retention and selectivity could be adjusted within a wide range. Using *n*-heptane as the swelling solvent, the film thickness increased by a factor of 3–4. Mobile to stationary phase volume ratios, $\beta = V_m/V_s$, down to 2 were obtained. An increase in retention of 12–16 times after swelling was observed. Improved stability was obtained as compared with conventional liquid–liquid chromatographic systems with mechanically held stationary phases. The system was stable at very high flow-rates (up to 6.3 cm/s) and showed no mechanical loss of the stationary phase.

INTRODUCTION

There is a continuous interest in achieving higher resolving power and increased separation speed in liquid chromatography (LC). In this respect, the use of open-tubular columns (OTCs) is attractive^{1,2}. Due to recent developments in detection and injection technology, *i.e.*, the use of on-column detection techniques^{3,4} and split injection techniques^{5,6}, open-tubular columns in the range 5–10 μm I.D. may be operated without significant extra-column band broadening. With columns made from fused-silica capillaries, which exhibit good UV-transparency, optimum conditions for on-column laser-induced fluorescence detection can be obtained^{3,7}. However, only a limited number of methods have been presented for preparation of stationary phases in fused-silica capillaries for reversed-phase (RP) open-tubular column LC^{8–10}. The low concentration of metal oxides implies that fused-silica capillaries are less amenable to surface modification than soda lime and borosilicate glass capillaries. Hence, a low density of octadecylsilane (ODS) groups after chemical bonding and low retention was obtained in 30–100 μm I.D. fused-silica capillaries⁸. Immobilization of polysiloxane gum phases has been shown to yield stationary phases well suited for RP-OTC-LC in fused-silica capillaries^{9,10}. The largest capacity factors reported in RP-OTC-LC were obtained on these columns, but were lower than those in packed column LC with ODS phases. Recently Folestad *et al.*¹⁰ reported pre-

liminary results on the use of swollen cross-linked polysiloxanes as stationary phases in RP-OTC-LC. Stationary phases with large film thicknesses could conveniently be prepared and a nearly ten-fold increase in retention was demonstrated. Observations on the swelling of polysiloxane-based stationary phases have also been reported in normal-phase packed column LC¹¹, in capillary supercritical fluid chromatography (SFC)¹² and at sample injection in capillary gas chromatography (GC)¹³.

The mixed solvent-polysiloxane liquid phase formed after swelling has properties that should be attractive for LC. In addition to the increased film thickness, the column selectivity may be varied in a single column by selection of appropriate swelling solvents. Moreover, the mass transfer in the stationary phase is likely to be improved, as has been observed for rubber membranes after swelling¹⁴.

In this study advantage is taken of the swelling of polysiloxane phases with non-polar solvents in order to achieve very thick stationary films in OTC-LC. The aim was to investigate the utility of these swollen phases for RP-OTC-LC and to characterize the effects on retention, selectivity and efficiency compared with non-swollen polysiloxanes.

EXPERIMENTAL

Apparatus

The chromatographic system for OTC-LC was identical with that previously described^{9,10}. Flow-rates in the range 1 nl/min–10 μ l/min were created with a simple split-flow arrangement and an LDC Constametric I pump. The volumetric flow-rates were calculated from the measured column dimensions and the retention time of an unretained solute. Injection volumes of 0.1–20 nl were obtained through a static split and a Valco valve equipped with an external 20- μ l loop. On-column fluorescence detection was performed with a modified Shimadzu Model RF-530 fluorescence detector or a laser-induced fluorescence detector based on a helium-cadmium laser, Model 4210NB from Liconix.

Chemicals

Acetonitrile, chloroform, *n*-heptane, *n*-hexane and 2,2,4-trimethylpentane of p.a. and HPLC grade were obtained from Merck (F.R.G.) and Rathburn Chemicals (Walkerburn, U.K.) and the water was doubly distilled. Different anthracene derivatives were used as model compounds and were kindly supplied by Hans-Dieter Becker, Kjell Andersson and Henrik Sörensen, Department of Organic Chemistry, University of Göteborg.

Column preparation

The static coating technique used for the preparation of narrow bore open-tubular fused-silica columns has previously been described in detail^{9,10}. Capillaries of 12–55 μ m I.D. from SGE (Melbourne, Australia) were coated with PS-255 (methyl, 0.5–1.5% vinyl silicone gum; Petrarch Systems, Bristol, PA, U.S.A.) or SE-54 (methyl, 5% phenyl, 1% vinyl silicone gum; General Electric, Applied Science, PA, U.S.A.). After cross-linking of the phase with dicumyl peroxide, the soluble part of the polymer (typically 8–10%) was removed by rinsing with solvent. The values of the column mobile to stationary phase volume ratio, $\beta = V_m/V_s$, stationary phase film thickness, d_f , and column diameter, d_c , are given in Table I for rinsed columns before swelling.

TABLE I
OPEN-TUBULAR COLUMNS COATED WITH IMMOBILIZED POLYSILOXANE PHASES

| Column | Stationary phase | d_c (μm) | d_f (μm) | Phase ratio, $\beta = V_m/V_s$ |
|--------|------------------|----------------------------|----------------------------|-----------------------------------|
| 1 | PS-255 | 55.3 | 0.25 | 54 |
| 2 | PS-255 | 54.7 | 0.51 | 27 |
| 3 | PS-255 | 53.0 | 0.95 | 14 |
| 4 | PS-255 | 52.6 | 1.43 | 9 |
| 5 | SE-54 | 49.2 | 0.22 | 55 |
| 6 | SE-54 | 54.6 | 0.50 | 27 |
| 7 | SE-54 | 53.7 | 0.49 | 27 |
| 8 | PS-255 | 11.7 | 0.15 | 20 |

Procedures

The mobile phase was saturated prior to use by vigorously shaking the acetonitrile–water mixture with the non-polar solvent. After allowing the two-phase system to settle, the column with the pre-swelled stationary phase was flushed with the saturated mobile phase for 15 min before any separation was performed. Swelling of the stationary phase was accomplished by flushing the column for at least 15 min with the non-polar solvent.

The degree of stationary phase swelling was studied *in situ* by measurements of the column diameter before and after swelling. The average inner diameter was calculated from the column length and the resistance over the mercury filled capillary, as described by Guthrie *et al.*¹⁵. The mercury flow had to be maintained during the measurements to achieve electrical contact between the column ends. The stationary phase film thickness after swelling, d_t^* , was calculated from the change in column radius and the original film thickness. The new mobile to stationary phase volume ratio, β^* , was derived from

$$\beta^* = \frac{1}{(d_t^*/r^* + 1)^2 - 1} \quad (1)$$

where r^* is the column radius after swelling.

RESULTS AND DISCUSSION

Swelling of polymeric films

By dissolution of a non-polar, low-molecular-weight solvent in the polysiloxane phase, a new stationary liquid phase is obtained. The degree of swelling depends on the nature of the solvent–solvent and solvent–polymer interactions, *i.e.*, their respective solution properties. A state of equilibrium swelling is reached when the elastic retractive force from the rubber network is balanced by the dilution force¹⁶ and therefore the cross-link density will affect the degree of swelling. According to Gee¹⁷, maximum swelling should be obtained in a solvent having the same solubility parameter as the rubber itself. Interactions between polysiloxane elastomers and several organic solvents have been investigated by Yerrick and Beck¹⁸, and swelling factors

TABLE II
SWELLING FACTORS FOR A POLY(DIMETHYL SILOXANE) IN ORGANIC SOLVENTS

Data from Yerrick and Beck¹⁸

| Solvent | δ_0^* | S_p^{**} |
|------------------------|--------------|------------|
| 2,2,4-Trimethylpentane | 6.6 | 2.02 |
| <i>n</i> -Hexane | 7.3 | 2.22 |
| <i>n</i> -Heptane | 7.5 | 2.25 |
| Cyclohexane | 8.2 | 1.64 |
| Isopropyl acetate | 8.4 | 1.45 |
| Methyl isobutyl ketone | 8.4 | 1.26 |
| Carbon tetrachloride | 8.6 | 2.15 |
| Toluene | 8.9 | 1.69 |
| Benzene | 9.2 | 1.47 |
| Chloroform | 9.3 | 2.01 |
| Acetonitrile | 11.9 | 0.033 |
| Methanol | 14.5 | 0.032 |

* Hildebrand solubility parameter of solvent (cal/cm³)^{1/2}.

** Swelling factor according to eqn. 2.

for a poly(dimethyl siloxane) are exemplified in Table II. Chlorinated solvents show increased swelling power as compared with hydrocarbons of the same solubility parameter, δ_0), while permanent dipoles (ketones, esters) show reduced swelling¹⁸. From their data it can be concluded that with more polar solvents a higher degree of swelling is obtained for methyl phenyl silicones, polymer solubility parameter $\delta_p = 9$ (cal/cm³)^{1/2}, than for dimethyl silicones, $\delta_p = 7.5$ (cal/cm³)^{1/2}.

Exhibiting the largest swelling factor among the hydrocarbons, *n*-heptane was selected for the investigation on swollen polysiloxane phases in RP-OTC-LC. A series of open-tubular columns, coated with PS-255 or SE-54, was studied. The column dimensions before and after swelling are shown in Tables I and III, respectively. The

TABLE III
COLUMN DIMENSIONS AFTER SWELLING WITH *n*-HEPTANE

Column numbers refer to Table I.

| Column | d_c^* (μm) | d_f^* (μm) | β^{**a} | S_p^b | d_f^*/d_f |
|--------|------------------------------|------------------------------|------------------|---------|-------------|
| 1 | 54.2 | 0.79 | 17 | 2.1 | 3.2 |
| 2 | 52.1 | 1.83 | 6.9 | 2.5 | 3.6 |
| 3 | 47.2 | 3.82 | 2.9 | 2.8 | 4.0 |
| 4 | 45.1 | 5.16 | 2.0 | 2.4 | 3.7 |
| 5 | 48.1 | 0.78 | 15 | 2.4 | 3.5 |
| 6 | 52.4 | 1.62 | 7.8 | 2.2 | 3.3 |
| 7 | 51.3 | 1.70 | 7.3 | 2.4 | 3.5 |
| 8 | 11.3 ^c | 0.53 ^c | 5.1 ^c | — | — |

^a Phase ratio from eqn. 1.

^b Swelling factor according to eqn. 2.

^c Calculated from $d_f^*/d_f = 3.5$.

swelling factor, S_v , *i.e.*, the ratio of the volume of the trapped solvent to the original stationary phase volume, was calculated according to:

$$S_v = \frac{\text{volume of the swollen stationary phase} - \text{original phase volume}}{\text{original phase volume}} \quad (2)$$

The method used for measuring the column internal diameter was also applied to the swollen phases and the phase ratio after swelling was calculated from these values. The experimental data for the polysiloxane stationary phases, $S_v = 2.1$ – 2.8 , agree well with the data given in Table II for *n*-heptane, $S_v = 2.25$. A remarkable increase in film thickness is observed, accompanied by a reduction of the column diameter. For column 4 with the thickest film, the mobile phase volume is only twice as large as the stationary phase volume. It should be noted that the inner diameter of column 8 after swelling could not be measured with the present technique. It was therefore calculated using the column diameter measured before swelling and a mean d_f^*/d_f value of 3.5.

Stability

In order to achieve stable capacity factors in a dynamic liquid–liquid chromatographic system, the mobile phase must be saturated with the stationary liquid. The rate of *n*-heptane wash-out was investigated with a non-saturated mobile phase on a very thick film column (column 4). Using a mobile phase of acetonitrile–water (65:35) or (50:50), the originally trapped solvent was removed by 43 and 164 column volumes of mobile phase, respectively. At a linear flow-rate of 2 cm/s, the latter case corresponds to a wash-out time of 260 min. The small amount of solvent dissolved in a thin film column (column 5) was removed by only 8 column volumes of acetonitrile–water (65:35).

The good stability of the chromatographic system with the swollen stationary phases, compared with conventional LLC systems with mechanically held stationary liquids¹⁹, was further verified by injecting samples prepared in a non-saturated mobile phase. No effect either on retention or on peak broadening was observed. In addition, the k' values were unaffected when the mobile phase velocities were increased up to 6.4 cm/s, which indicates that even at these very high flow-rates no shear forces were apparent that could remove the stationary phase from the support.

Retention

An increase in retention on cross-linked polysiloxane films of $d_f = 0.03$ – $2 \mu\text{m}$ has been reported for 50 μm I.D. OTC in LC^{9,10}. In Fig. 1, capacity factors are shown for fluorene and phenylfluorene on 50 μm I.D. columns after swelling PS-255 and SE-54 with *n*-heptane. Increased retention is observed, even for the column with the thickest film, $d_f^* = 5.2 \mu\text{m}$ and $\beta^* = 2$ (column 4). As compared with the pure polysiloxane phase, the retention enhancement for this column was a factor of 12 for fluorene and 16 for phenylfluorene.

The high k' values in RP-OTC-LC obtained on non-swollen polysiloxane phases, as compared to OTC with ODS phases, can thus be substantially increased by swelling the stationary phase using non-polar solvents. A comparison was made of the retention on column 4 with that on a conventional packed column¹⁰ using the

capacity factor

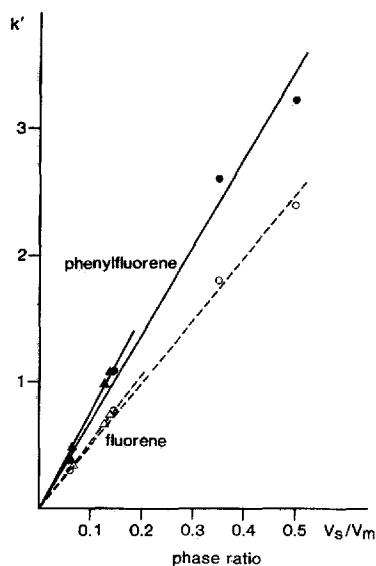


Fig. 1. Dependence of capacity factors on the phase ratio in OTC-LC for two immobilized polysiloxane stationary phases after swelling with *n*-heptane. Columns: 1-7, 2 m \times 50 μ m. Mobile phase: acetonitrile-water (65:35) saturated with *n*-heptane; Solutes: (---) fluorene; (—) phenylfluorene. Stationary phases: (O, ●) PS-255; (Δ , \blacktriangle) SE-54.

same mobile phase composition, acetonitrile-water (75:25). The k' values were 1.15 and 4.9 respectively.

Selectivity

Due to the large uptake of a non-polar solvent in the polysiloxane phase, the column selectivity is changed after swelling. The change in the selectivity coefficient, α , for fluorene-phenylfluorene on SE-54 and PS-255 after swelling is shown in Table IV. The different selectivity on the pure siloxane phases, due to the phenyl groups present in SE-54, is diminished by trapping of *n*-heptane. The selectivity is thus dominated by the nature of the swelling solvent.

TABLE IV

SELECTIVITY CHANGES OF PS-255 AND SE-54 ON SWELLING WITH *n*-HEPTANE

Columns: SE-54 column 6, PS-255 column 2. Solutes: fluorene and phenylfluorene. Mobile phase: acetonitrile-water (40:60).

| Stationary phase | α |
|------------------|----------|
| SE-54 | 2.34 |
| SE-54/heptane | 2.52 |
| PS-255 | 1.84 |
| PS-255/heptane | 2.35 |

TABLE V
SWELLING OF AN SE-54 OTC WITH VARIOUS SOLVENTS

Column 6. Mobile phase: acetonitrile–water (40:60).

| | <i>Stationary phase</i> | | | |
|--------------------------|-------------------------|-------------------------|-------------------------------|-------------------|
| | <i>SE-54</i> | <i>SE-54 swollen by</i> | | |
| | | <i>n-Heptane</i> | <i>2,2,4-Trimethylpentane</i> | <i>Chloroform</i> |
| <i>k'</i> fluorene | 0.70 | 6.3 | 4.6 | 7 |
| <i>k'</i> phenylfluorene | 1.64 | 15.8 | 11.4 | 21 |
| α | 2.34 | 2.52 | 2.48 | 3 |

The possibility of varying the selectivity on a single column was also studied. In Table V, capacity factors and selectivity factors for the solute pair fluorene–phenylfluorene are presented for a SE-54 column swelled with *n*-heptane, 2,2,4-trimethylpentane and chloroform, respectively. The hydrocarbons show similar selectivities, although *n*-heptane gives higher *k'* values than 2,2,4-trimethylpentane. The increase in both *k'* and α for the chloroform-saturated phase is notable. However, the latter system is less stable due to the high solubility of chloroform in the mobile phase. The PS-255 phase was also investigated by swelling column 4 with *n*-hexane and *n*-heptane. With a mobile phase of acetonitrile–water (50:50) saturated with the respective non-polar solvent, the same selectivity and retention were obtained.

The selectivity of column 4 after swelling with *n*-heptane was compared with the selectivity of an ODS phase in a packed column¹⁰. The α values for the solute pair 9-cyanoanthracene and anthracene were 4.0 and 1.3 respectively, when separated under similar mobile phase conditions, acetonitrile–water (75:25). Moreover, the distribution constants for anthracene on these two stationary phases were compared using data on the phase ratios in these two columns and assuming a liquid–liquid partitioning mechanism. The phase ratio for the packed column was estimated to be $\beta = 6.4$ (properties of Spherisorb ODS-2 5- μ m material stated by the supplier²⁰) and $\beta = 2$ for the OTC, calculated from experimental data. After normalization of the β values, the ratio of the distribution constants, for the packed to the open tubular column was calculated as 13.6. Although the elution orders on these two columns are similar the selectivity of the swelled polysiloxane phase differs from that of an ODS phase.

Efficiency

The performance in terms of the theoretical plate height, *H*, of open-tubular columns with different immobilized polysiloxane phases has recently been investigated¹⁰. Experimental values on plate heights for retained and unretained solutes on columns coated with SE-54 and PS-255 polysiloxane phases were shown to be similar to plate heights calculated from the Golay equation. The stationary phase diffusion coefficients for anthracene derivatives were found to be between 10^{-6} and 10^{-8} cm²/s and varied with the type of polysiloxane phase, the film thickness and the column inner diameter.

A study of the effects of swelling of the PS-255 phase by *n*-heptane on efficiency was performed on the same columns as previously used (columns 2, 4 and 8 are identical to columns 4, 7 and 14 respectively in ref. 10). The experimental plate heights for columns 2, 4 and 8 measured for anthracene after swelling are shown in Fig. 2a-c. These results are plotted together with plate heights calculated from the Golay equation²¹ for different values of the stationary phase diffusion coefficient, D_s . As

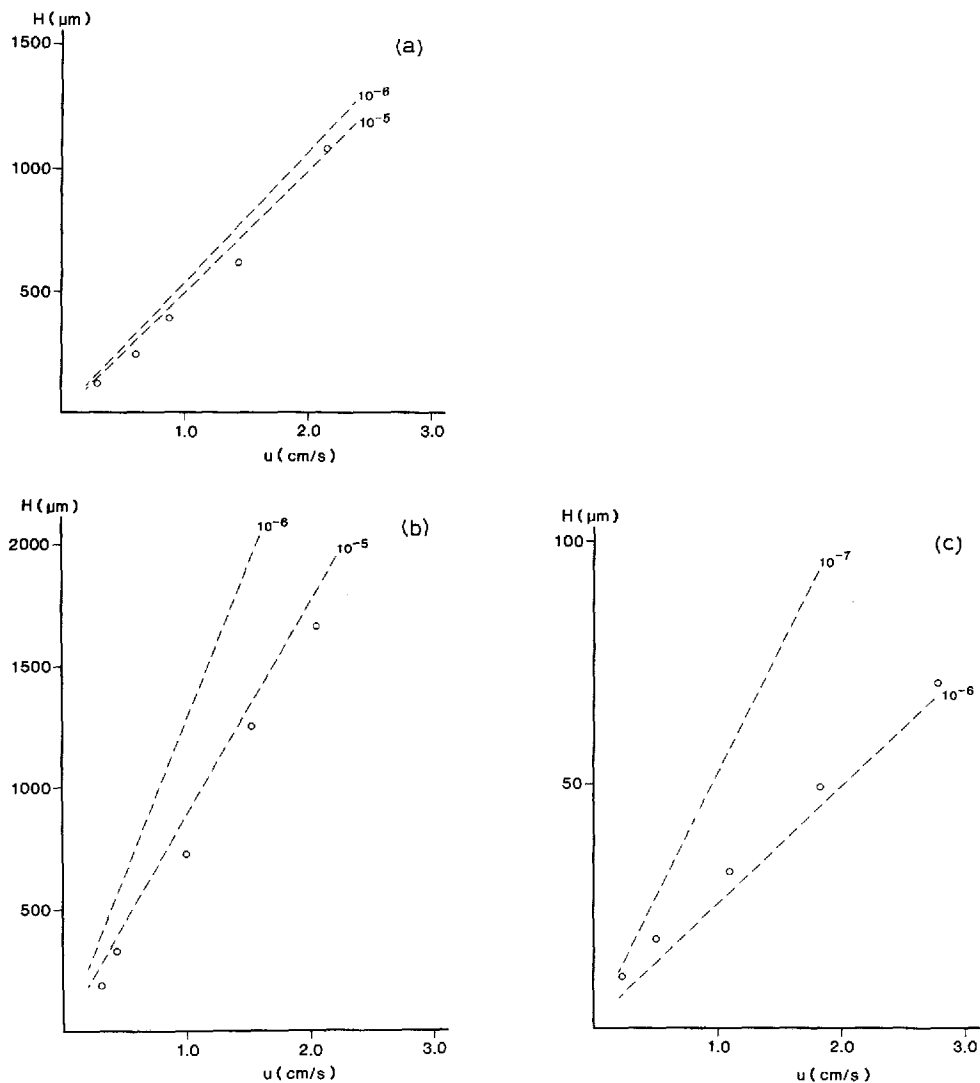


Fig. 2. Plots of experimental and calculated plate heights versus linear velocity for open-tubular columns with PS-255 phases after swelling with *n*-heptane. Calculated plate heights from the Golay equation using D_s values of 10^{-5} , 10^{-6} and 10^{-7} cm^2/s . (a) Column 2, $d_c^* = 52.1$ μm , $d_f^* = 1.83$ μm , $k' = 0.28$; (b) column 4, $d_c^* = 45.1$ μm , $d_f^* = 5.2$ μm , $k' = 1.15$; (c) column 8, $d_c^* = 11.3$ μm , $d_f^* = 0.53$ μm , $k' = 0.25$. Experimental plate heights: o, anthracene. Calculated plate heights: ---, $D_m = 1.24 \cdot 10^{-5}$ cm^2/s (calculated according to ref. 23). Mobile phase: acetonitrile-water (75:25) saturated with *n*-heptane. $T = 23^\circ\text{C}$.

expected, a large increase in the stationary phase diffusion after swelling was observed for each column. The D_s value was found to be equal in magnitude, $1 \cdot 10^{-5}$ – $3 \cdot 10^{-5}$ cm^2/s , for the two $50 \mu\text{m}$ I.D. columns with a thin ($1.8 \mu\text{m}$) and a thick ($5.2 \mu\text{m}$) stationary phase film of PS-255. This deviates from the results obtained on the same columns with non-swollen polysiloxanes where the column with the thicker film exhibited a ten-fold larger D_s value. Column 8 with a calculated inner diameter of $11.3 \mu\text{m}$ after swelling showed a ten-fold larger D_s value than in the non-swollen state. This is still a smaller D_s value, $3 \cdot 10^{-7}$ – $6 \cdot 10^{-7}$ cm^2/s , than for the $50 \mu\text{m}$ I.D. columns.

The largest efficiencies, measured as the number of plates, were obtained for column 8 at a flow velocity of 0.23 cm/s for the retained solutes 9-cyanoanthracene ($k' = 0.07$) and anthracene ($k' = 0.25$) with acetonitrile–water (75:25) as mobile phase, 253 000 and 179 000 respectively. From these values it can be calculated that the experimentally determined mass transfer in these columns is only about 1.1 times larger than the values predicted by the Golay equation assuming a negligible stationary phase mass-transfer term. Even higher plate numbers are expected when using lower flow-rates.

An interesting feature of columns with swollen polysiloxane phases, with their relatively high D_s values and concurrently flat slopes of the curves of height equivalent to a theoretical plate at high flow velocities, is the possibility of rapid separations with high efficiencies. A separation of six anthracene derivatives performed with acetonitrile–water (70:30) as the mobile phase at the high flow velocity of 5.5 cm/s is shown in Fig. 3. The separation was carried out within 70 s and demonstrates that more than 220 plates/s (peak 4, anthracene, $k' = 0.4$) can be produced in RP-OTC-LC. This performance is in the same range as the best reported for high-speed packed columns with 150–450 plates/s²².

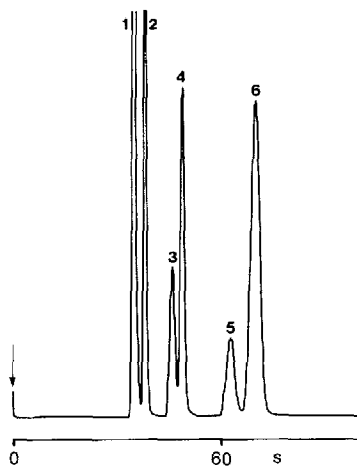


Fig. 3. Rapid separation of anthracene derivatives on an PS-255 OTC swollen by *n*-heptane. Column: 8, $193 \text{ cm} \times 11.3 \mu\text{m}$ Mobile phase: acetonitrile–water (70:30) saturated with *n*-heptane Splitting ratio: 1:33 000. Flow velocity: 5.5 cm/s . Solutes: 1 = 9-anthracenecarboxylic acid; 2 = 9-cyanoanthracene; 3 = 9-methoxyanthracene; 4 = anthracene; 5 = 9,10-dimethylanthracene; 6 = 9-phenylethynyl-anthracene.

CONCLUSIONS

Swelling of polysiloxanes with non-polar solvents is a practical approach to achieving thick stationary phase films in open-tubular fused-silica columns for reversed-phase LC. Full advantage can be taken of these thick films due to an efficient mass transfer in the stationary phase and a high mechanical stability of the stationary liquid film. Using cross-linked polar polysiloxane phases and polar swelling solvents, it is possible to extend the technique to LC methods other than reversed-phase LC.

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