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# Preparation and evaluation of polyacrylate-coated fused-silica capillaries for reversed-phase open-tubular liquid chromatography

Remco Swart, Johan C. Kraak\*, Hans Poppe

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

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## Abstract

By *in situ* photopolymerization of acrylates, thick polyacrylate films can be immobilized in 10- $\mu\text{m}$  fused-silica capillaries. Up to 1.9- $\mu\text{m}$  thick films can be produced in 0.3–1.2 m long capillaries, resulting in phase ratios  $>1.0$  and leading to columns with high mass loadability. Owing to the high mass loadability, application of “on-column” UV detection is possible. The effect of incorporating alkyl acrylates in the film on retention behaviour and efficiency was extensively investigated. The success rate of the preparation of silicone–ethylhexyl acrylate films is almost 100%. The polyacrylate films are stable towards basic solutions up to pH 12. The column efficiency is demonstrated with separations of anthracene derivatives. A fast separation of methyl-substituted benzenes with on-column UV detection is shown.

## 1. Introduction

Chromatographic theory predicts that liquid chromatography can be performed best in tubes in order to produce large plate numbers in an acceptable time, owing to the favourable flow resistance [1]. However, to compete with liquid chromatography in packed columns in that respect, the internal diameter of the tubes must be about the same as the particle size as commonly used in HPLC or preferably smaller (*e.g.*,  $<5\ \mu\text{m}$ ) [1]. This means that the separation system in addition to the detection and injection system has to be miniaturized. Moreover, a uniform retentive layer with sufficient sample capacity has to be immobilized on the inside wall of the

capillary. This last aspect is of paramount importance for two reasons: first for avoiding a decrease in efficiency due to overloading and second for allowing larger injected solute amounts, simplifying detection. Several research groups have recognized this bottleneck in the exploration of open-tubular LC (OT-LC) and have therefore put large efforts into the immobilization of retentive layers in glass and fused-silica capillaries. Efforts have so far been focused on two approaches: the realization of a porous silica layer that can be chemically modified [2–4] and the immobilization of polymeric phases such as cross-linked silicones [5] and acrylates [6,7]. Although some interesting results have been reported with porous silica layers, the retentivity and sample capacity of these layers are still small; as a result, these columns can only

\* Corresponding author.

be used satisfactorily in combination with an extremely sensitive detection technique such as laser-induced fluorescence. More promising are the polymeric phases because thick layers with good sample capacity can be immobilized. A drawback of these polymeric phases is the poorer column efficiency due to small diffusion coefficients of solutes in these retentive layers [5,6].

In this paper, we report the results of an investigation to immobilize thick polyacrylate films in 10  $\mu\text{m}$  I.D. fused-silica capillaries for reversed-phase OT-LC. The study involved the effect of the type of incorporated alkyl chain on the retentive properties, column efficiency, sample capacity and stability of the columns. The favourable properties of thick polyacrylate films for OT-LC is demonstrated with test solutes using laser-induced fluorescence (LIF) and UV detection.

## 2. Experimental

### 2.1. Materials

The applied fused-silica capillaries with an acrylate outside protective coating were a kind gift from Philips Research Labs. (Eindhoven, Netherlands). The outside acrylate coating possesses sufficient UV transparency for *in situ* photopolymerization.

HPLC-grade acetone, methanol and acetonitrile were obtained from Janssen (Beerse, Belgium) and 3-(methacryloxy)propyltrimethoxysilane ( $\gamma$ -MPS), butyl acrylate (BA) and ethylhexyl acrylate (EHA) from Fluka (Buchs, Switzerland). Silicone acrylate (SiA) (Tegomer V-Si2150) was a kind gift from Goldsmidt (Essen, Germany). Lauryl acrylate (LA) was purchased from Merck (Darmstadt, Germany).

$\alpha,\alpha$ -Dimethoxy- $\alpha$ -phenylacetophenone (DM-PA) (Irgacure 651; Ciba-Geigy, Basle, Switzerland) was used to initiate the polymerization reaction. The capillaries were tested with various anthracene derivatives from Janssen. Solutions of these compounds were prepared in methanol.

### 2.2. Apparatus

The experimental set-up of the OT-LC system has been described previously [7]. The mobile phase delivery system consists of a solvent reservoir, which can be pressurized by means of helium. Injections were made with a laboratory-made splitting device connected to a 0.2- $\mu\text{l}$  injection valve (Model 7525; Rheodyne, Cotati, CA, USA). On-column detection was performed by either fluorescence or UV absorption. A helium-cadmium laser,  $\lambda_{\text{ex}} = 325 \text{ nm}$  (Model 356XM; Omnicrome, Chino, CA, USA) was used as a light source for fluorescence detection; the emission wavelength was set at 380 nm. The fluorescence yield was measured with a photomultiplier tube (Type 6225 S; EMI, Hayes, UK).

UV detection was performed with a Model 757 UV detector, Applied Biosystems, Foster City, CA, USA) adapted for on-column detection using a laboratory-made capillary cell with an adjustable aperture [8].

The reservoir for filling the capillaries with coating solutions has been described previously [9]. The silylation reaction and curing of the capillaries were carried out at elevated temperatures in an oven. Irradiation was effected with a UV lamp (Philips, TLD 40W/90N,  $L = 120 \text{ cm}$ ). The light intensity during the polymerization experiments was measured with a laboratory-built UV curing radiometer.

Solvent evaporation from the capillaries was performed with a vacuum pump combined with a thermostated water-bath. Kinematic viscosities of the mobile phase were measured in a water-bath (Model 45; Tamson, Zoetermeer, Netherlands) with a viscometer (Tamson Model, 88233). The densities of the mobile phases, needed to calculate dynamic viscosities, were measured with a digital density meter. Pressures were determined with a digital pressure sensor.

### 2.3. Column preparation

All solutions were filtered through a 0.45- $\mu\text{m}$  filter (Type HV; Millipore, Yonezawa, Japan) prior to use.

The preparation of the polyacrylate-coated capillaries consisted of four consecutive steps: etching of the bare silica capillary, silylation of the etched surface, *in situ* photopolymerization of acrylates and evaporation of the solvent.

#### Etching

A 1 mol/l NaOH solution was pumped through the capillary for 3 h at a pressure of 15 bar. The capillary was then flushed consecutively with distilled water for 1 h, 0.03 mol/l HCl for 1 h and distilled water for 1 h. Next the capillaries were dried overnight at 120°C under a stream of helium.

#### Silylation

The etched capillaries were silylated by pumping through a 5% (v/v) solution of  $\gamma$ -MPS in dried toluene at 120°C for 1 h at 15 bar. The capillary was then flushed successively with toluene for 0.5 h and helium for 4 h at ambient temperature.

#### Photopolymerization

The coating solutions were always prepared just before use by adding the silicone acrylate, alkyl acrylate and photoinitiator (DMPA) to the solvent and vibrationally mixing the solution. The capillary was then filled with this solution and sealed at both ends with grease to fix the solution during the irradiation step. The irradiation of the capillary was carried out by pulling the capillary through a quartz tube along the UV lamp at a constant velocity. By this means, each part of the capillary was exposed to the same curing dose. The light intensity was varied by changing the distance between the lamp and the quartz tube through which the capillary was pulled. The exposure time was set by adjusting the velocity at which the capillary was pulled over the lamp. The curing conditions and capillary dimensions are given in Table 1.

#### Evaporation

After the irradiation, the seal at one of the ends of the capillary was removed and this side was connected to a vacuum pump by means of a

Swagelok fitting with a PTFE ferrule. In order to remove the solvent and possible non-reacted monomers, the capillary was placed in a water-bath and vacuum applied. Then the temperature was raised slowly (in 5–10 min) from ambient to 30°C. The removal of the solvent from the capillary and the acrylate film obtained were checked under a microscope. The capillary was then thermally cured at 120°C for at least 12 h. Finally, the capillary was equilibrated with the mobile phase for 30 min.

#### 2.4. Inner diameter and film thickness

The average inner diameter of the capillaries was measured by a hydrodynamic method, based on a rearrangement of the Poiseuille relationship:

$$r = \sqrt{\frac{8u\eta L}{\Delta p}}$$

where  $u$  is the mobile phase velocity,  $p$  is the pressure drop,  $r$  is the capillary radius,  $\eta$  is the viscosity of the mobile phase and  $L$  is the total length of the capillary.

By measuring the linear velocity of the mobile phase via a non-retarded solute (salicylate) at a given pressure, the diameter of the capillary can be calculated provided that the viscosity of the mobile phase is known. In order to verify that salicylate is unretained in coated capillaries, several other compounds such as trihydroxybenzoic acid, anthranilic acid and several ions (chromate and nitrite) were tested for their retention. Salicylate elutes together with trihydroxybenzoic acid, earlier than any of the other compounds.

The viscosity of the mobile phase was measured at various temperatures between 19.0 and 22.5°C. At least five measurements were carried out over a wide pressure range. The R.S.D. of the determination of the column diameter ranged between 0.1 and 0.5%. The polymer layer thickness was calculated by subtracting the column radius before and after the coating procedure.

Table 1  
Experimental coating conditions for the prepared fused-silica columns

Capillary No.	<i>L</i> (cm)	Monomer (% v/v) (DMPA) <sup>a</sup>	Solvent <sup>b</sup>	Intensity (mW)	Time (s)	<i>d<sub>c</sub></i> <sup>c</sup> (μm)	<i>d<sub>t</sub></i> <sup>d</sup> (μm)	<i>V<sub>s</sub></i> / <i>V<sub>m</sub></i>
1	36.8	30% SiA	A/P	0.13	214	8.50	1.44	0.80
2	88.0	25% SiA	A/P	0.13	302	8.34	1.52	0.86
3	59.3	2.6% LA–30% SiA	A	0.14	400	8.50	1.44	0.79
4	93.0	2.6% LA–30% SiA	A	0.19	480	8.22	1.42	0.81
5	52.2	2.6% LA–30% SiA	A	0.15	522	8.14	1.46	0.85
6	34.1	2.6% LA–30% SiA	A	0.19	390	8.24	1.57	0.91
7	61.2	2.6% LA–30% SiA	A	0.14	285	7.58	1.90	1.25
8	63.0	15% BA–15% SiA	A	0.15	355	8.24	1.43	0.81
9	43.0	15% BA–15% SiA	A	0.15	420	8.02	1.68	1.01
10	52.1	15% BA–15% SiA	A	0.15	458	8.02	1.69	1.01
11	25.2	15% BA–15% SiA	A/P	0.15	304	7.72	1.70	1.07
12	74.5	7.5% EHA–7.5% SiA	A/P	0.13	700	10.44	0.51	0.20
13	79.6	10% EHA–10% SiA	A/P	0.14	345	9.66	0.86	0.39
14	77.1	12.5% EHA–12.5% SiA	A/P	0.13	350	9.72	0.87	0.39
15	115.1	15% EHA–15% SiA	A/P	0.14	385	8.88	1.27	0.65
16	81.3	15% EHA–15% SiA	A/P	0.14	385	8.64	1.39	0.75
17	121.0	15% EHA–15% SiA	A/P	0.15	390	8.32	1.39	0.78
18	56.4	15% EHA–15% SiA	A	0.14	390	8.20	1.43	0.82

<sup>a</sup> DMPA concentration: 3.0 mg/ml for all solutions.

<sup>b</sup> A = Acetone; P = pentane.

<sup>c</sup> *d<sub>c</sub>* = Diameter of the column after coating.

<sup>d</sup> *d<sub>t</sub>* = Thickness of acrylate film.

## 2.5. Chromatography

The chromatographic properties of the coated capillaries were measured using an OT-LC system, as described by Ruan *et al.* [7]. Pure methanol, acetonitrile or aqueous mixtures were used as mobile phases. Detection was carried out either by “on-column” UV detection using a cell with an adjustable slit width or by LIF. For both detection techniques it was necessary to remove the outside coating of the capillary for increased sensitivity. This was done by immersing the detection side of the capillary in methanol and stripping off the protective layer mechanically. All test solutions were prepared in pure methanol.

Experimental plate heights of test solutes were calculated according to  $H = L\sigma^2/t_r^2$ , where *L* is the column length and  $\sigma$  is the peak half-width at 0.61 of the peak height. Asymmetry of the peaks was measured at 10% of the peak height according to Snyder and Kirkland [10].

The column efficiency was evaluated by comparing the experimental and theoretical plate heights *versus* the linear velocity of the mobile phase. Theoretical values of the plate heights were calculated with the Golay equation.

## 3. Results and discussion

### 3.1. Preparation of polyacrylate films

Of the four main steps in the preparation of polyacrylate layers, the photopolymerization and evaporation of the solvent appear to be the critical steps for obtaining uniform films. During these steps serious problems can occur when no precautions are taken to control the experimental conditions carefully.

#### Photopolymerization

The reaction rate of the polymerization is affected by many variables, *e.g.*, monomer con-

centration, photoinitiator concentration, type of solvent and UV intensity. With given values for these parameters, the exposure time is in principle the sole parameter controlling the conversion and consequently the extent of cross-linking. In order to terminate the polymerization it is therefore not only necessary to stop the irradiation but also to shield the capillary from other light sources by which radicals can be formed. The necessity of shielding the capillary from light after the photopolymerization up to the complete removal of the solvent was experienced when evaporation of the solvent was carried out in daylight. It appears that in the first part of the capillary a regular film is obtained but when the evaporation proceeds a more wavy film arises. The formation of a wavy film is attributed to stress in the gel which occurs at higher percentages of cross-linking [11]. The change in film shape due to increasing cross-linking could be visualized by successively irradiating the same capillary and partly evaporating the solvent: with increasing irradiation time the film shape changes from a “pearl chain” shape to a regular shape into a wavy film. In ref. 7 an illustration of the different film shapes can be found. The formation of a “pearl chain” film is attributed to the Rayleigh instability [12] and occurs when the viscosity of the polymer (related to the extent of cross-linking) is relatively small, as is the case when the irradiation time is short.

The exclusion of light after the irradiation is therefore necessary to avoid undesirable progression of the polymerization during the evaporation of the solvent. However, in a separate experiment an indication was found that even in the dark the reaction is not completely terminated. Capillaries 15 and 16 were initially polymerized as a 2 m long capillary. During evaporation of the solvent in the dark a blockage occurred in about half of the capillary. Therefore, the remaining part of the capillary was cut off and the evaporation was continued. It appears that the second part (capillary 16) had a significantly thicker film (about 10%) than the first part (capillary 15). The reason why the polymerization proceeds in the dark is not clear and therefore additional experiments are being

undertaken to clarify this phenomenon. When this problem cannot be solved satisfactorily, serious problems will occur with the preparation of uniform layers in long capillaries.

#### *Removal of the solvent*

The choice of the coating solvent is very important because after the polymerization the solvent and unreacted monomers have to be removed from the capillary by evaporation under reduced pressure and elevated temperatures. Emptying of the capillary must be achieved in a reasonable time, also to avoid the polymerization reaction progressing as mentioned above. In order to select a suitable coating solvent with favourable evaporation characteristics, tetrahydrofuran (THF), acetone, *n*-pentane and acetone–*n*-pentane mixtures were tested. From these experiments it appeared that with all solvents at 40°C bubbles are formed, which finally leads to irregular films. When keeping the temperature below 30°C no bubble formation was observed. However, at this temperature blockages still frequently occurred with THF and *n*-pentane. Good results were obtained with acetone and particularly with acetone–*n*-pentane (1:1, v/v). With this mixture the speed of evaporation is about four times faster than with the pure solvents. The favourable properties of acetone–*n*-pentane mixtures in the free release coating technique have been reported previously [13]. It is known that the conversion of the polymerization reaction is dependent on the type of solvent and therefore may influence the thickness of the layer [11].

#### *Incorporation of alkyl acrylates*

In order to obtain retentive layers for reversed-phase OT-LC, various alkyl acrylate monomers were incorporated in the acrylate films. The addition of alkyl acrylate monomers on the one hand increases the hydrophobicity of the layer, and on the other it might decrease the cross-link density, because only the silicon diacrylate is responsible for cross-linking. In this study we investigated three reactive diluents: lauryl acrylate (LA), butyl acrylate (BA) and ethylhexyl acrylate (EHA).

In a previous paper [7], we reported the use of polyacrylate films containing a lauryl moiety. In that study a 30% solution of a 1:1 mixture of silicon acrylate and lauryl acrylate was used as coating solution. With this mixture relatively thick layers could be prepared, showing considerable retention with methanol as mobile phase. However, the success rate of the preparation of thick layers was small. For unknown reasons, on several occasions no gel was formed after irradiation. Therefore, this aspect was first studied in more detail. It appeared that by decreasing the lauryl acrylate concentration in the coating solution by a factor of 6 this problem could be satisfactorily solved. However, with butyl and ethylhexylacrylate monomers the aforementioned problems did not occur; SiA-BA and SiA-EHA mixtures could be used up to a ratio of 1:1 without problems.

From the experimental conditions under which the columns were prepared as listed in Table 1, some preliminary conclusions can be drawn. There is no clear relationship between the phase ratio and the irradiation time and intensity. The phase ratio can be changed by varying the monomer concentration in the coating solution as demonstrated with SiA-EHA. For a low monomer concentration it was found necessary to increase the irradiation time to obtain a good film. So far a fair prediction of the layer thickness is not yet possible.

From Table 1, it can also be seen that the repeatability of the coating procedure, with respect to the phase ratio obtained, decreases in

the order SiA-BA > SiA-EHA > SiA-LA. However, the success rate in preparing good films appears to be different and the order SiA/EHA (100%) > SiA-BA (80%) > SiA-LA (50%) was found.

### 3.2. Chromatography

#### Retention and selectivity

In order to characterize the specific nature of the layers, the capacity factors and selectivity factors of some test solutes on the columns were measured with methanol as mobile phase. From the capacity factors,  $k'$ , and the phase ratio,  $V_s/V_m$ , the distribution coefficient  $K$  was calculated according  $K = k'V_m/V_s$ . To determine the effect of the incorporation of an alkyl moiety in the layer, the retention behaviour of films prepared with only SiA were included in the study. The results are given in Table 2. It is evident that, with a selected coating mixture, polyacrylate layers with similar retentive properties can be immobilized irrespective of the film thickness. Moreover, only small differences in selectivity are found between different types of phases. From the large retention of the solutes on columns 1 and 2, it can be concluded that the silicon acrylate matrix itself is hydrophobic. However, incorporation of an alkyl moiety in the films causes an additional increase in retention, as can be seen with the SiA/BA and SiA/EHA columns. The increase in retention is about a factor of two larger with EHA compared to BA. In HPLC it is known that hydrophobic inter-

Table 2  
Distribution coefficients ( $K$ ) and selectivity factors ( $\alpha_{j,i} = K_j/K_i$ ) of anthracene derivatives on acrylate-coated columns

Compound	SiA ( $n = 2$ )		SiA-LA ( $n = 3$ )		SiA-BA ( $n = 4$ )		SiA-EHA ( $n = 7$ )	
	$K$ (R.S.D.)	$\alpha_{j,i}$ (R.S.D.)	$K$ (R.S.D.)	$\alpha_{j,i}$ (R.S.D.)	$K$ (R.S.D.)	$\alpha_{j,i}$ (R.S.D.)	$K$ (R.S.D.)	$\alpha_{j,i}$ (R.S.D.)
Anthracenemethanol	0.28 (0.04)		0.21 (0.05)		0.38 (0.03)		0.39 (0.03)	
Anthracenecarbonitrile	0.57 (0.04)	2.11 (0.21)	0.56 (0.03)	2.76 (0.49)	0.82 (0.02)	2.21 (0.16)	1.02 (0.06)	2.67 (0.09)
Anthracene	0.68 (0.04)	1.20 (0.02)	0.68 (0.02)	1.20 (0.04)	0.90 (0.01)	1.11 (0.01)	1.19 (0.05)	1.17 (0.02)
Fluoranthene	0.80 (0.03)	1.18 (0.01)	0.83 (0.01)	1.23 (0.01)	1.13 (0.01)	1.25 (0.02)	1.51 (0.07)	1.26 (0.01)
9-Phenylanthracene	0.84 (0.03)	1.06 (0.01)	0.90 (0.02)	1.08 (0.01)	1.29 (0.02)	1.14 (0.01)	1.76 (0.08)	1.17 (0.01)
1,2-Benzanthracene	0.91 (0.04)	1.07 (0.01)	1.00 (0.02)	1.11 (0.01)	1.42 (0.02)	1.10 (0.01)	1.88 (0.09)	1.06 (0.01)

Mobile phase, methanol. R.S.D. = relative standard deviation (%).

action increases with increasing alkyl chain length of the stationary phase [14]. The alkyl chain of EHA is longer than that of BA and therefore the interaction of solutes with EHA will be larger, which results in a larger distribution coefficient. From Table 2 it can be seen that the effect of LA on the retention is marginal. This is as expected, because the amount of LA added to SiA is small (about 3%).

The relationship between the phase ratio and retention was studied by measuring the capacity factors of test solutes on a number of EHA–SiA columns with different film thicknesses. As shown in Fig. 1, a linear relationship between the capacity factor and phase ratio is found. This behaviour again confirms that the specific nature of the polyacrylate layers is independent of the layer thickness.

The retention can also be changed by varying the composition of the mobile phase. Fig. 2 shows the effect of the methanol content on the retention of the test solutes. A linear relationship between  $\log k'$  and the percentage of

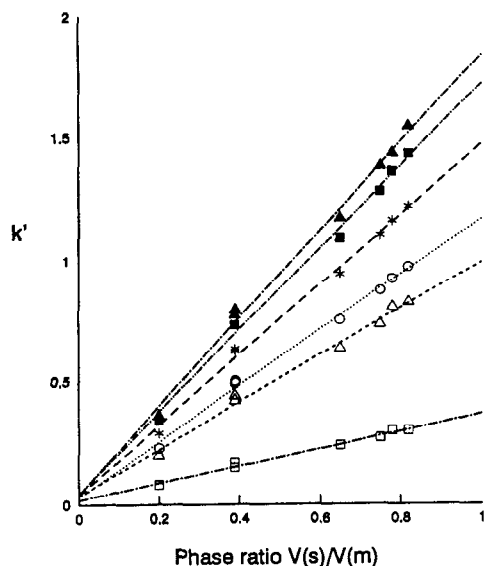


Fig. 1. Capacity factors of anthracene derivatives versus phase ratio on SiA–EHA-coated columns. Mobile phase, 100% methanol.  $\square$  = Anthracenemethanol;  $\triangle$  = anthracenecarbonitrile;  $\circ$  = anthracene; \* = fluoranthene;  $\blacksquare$  = 9-phenylanthracene;  $\blacklozenge$  = 1,2-benzanthracene.

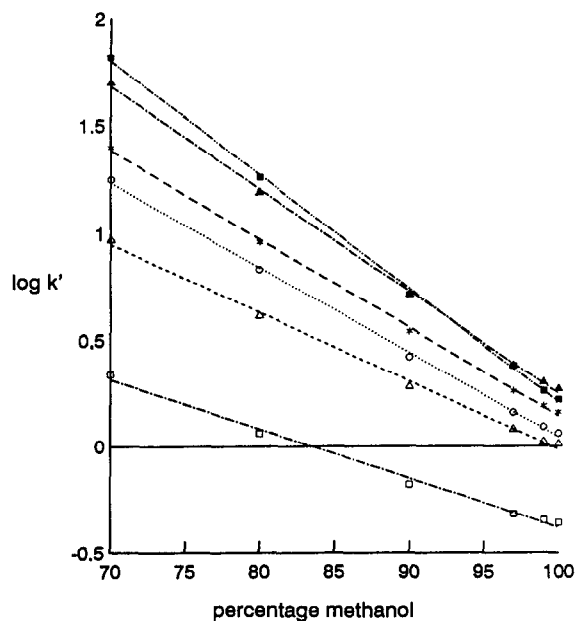


Fig. 2. Dependence of  $k'$  on the mobile phase composition for six anthracene derivatives on capillary 11. Detection, LIF. Symbols and compounds as in Fig. 1.

methanol is found, in agreement with findings in reversed-phase HPLC. This behaviour confirms the hydrophobic nature of the polyacrylate layers. Fig. 3 shows a representative chromatogram of some anthracene derivatives using pure methanol and methanol–water (4:1, v/v) as the mobile phase. From Figs. 2 and 3 it can be noted that the elution order of 1,2-benzanthracene and 9-phenylanthracene reverses on increasing the water content in the mobile phase. Such selectivity changes with the mobile phase composition also occur frequently in reversed-phase HPLC. From Fig. 3, it can be seen that the peak shapes remain very symmetrical on increasing the retention by adding water to the mobile phase.

Apart from methanol, acetonitrile was also tested as a mobile phase. The elution behaviour with both mobile phases on the same column and same linear velocity is illustrated in Fig. 4. It can be seen that the elution strength of acetonitrile on the polyacrylate column is significantly larger than that of methanol. This is in agreement with the retention theory in RP-HPLC, based on the solubility parameter of the mobile phase [15].

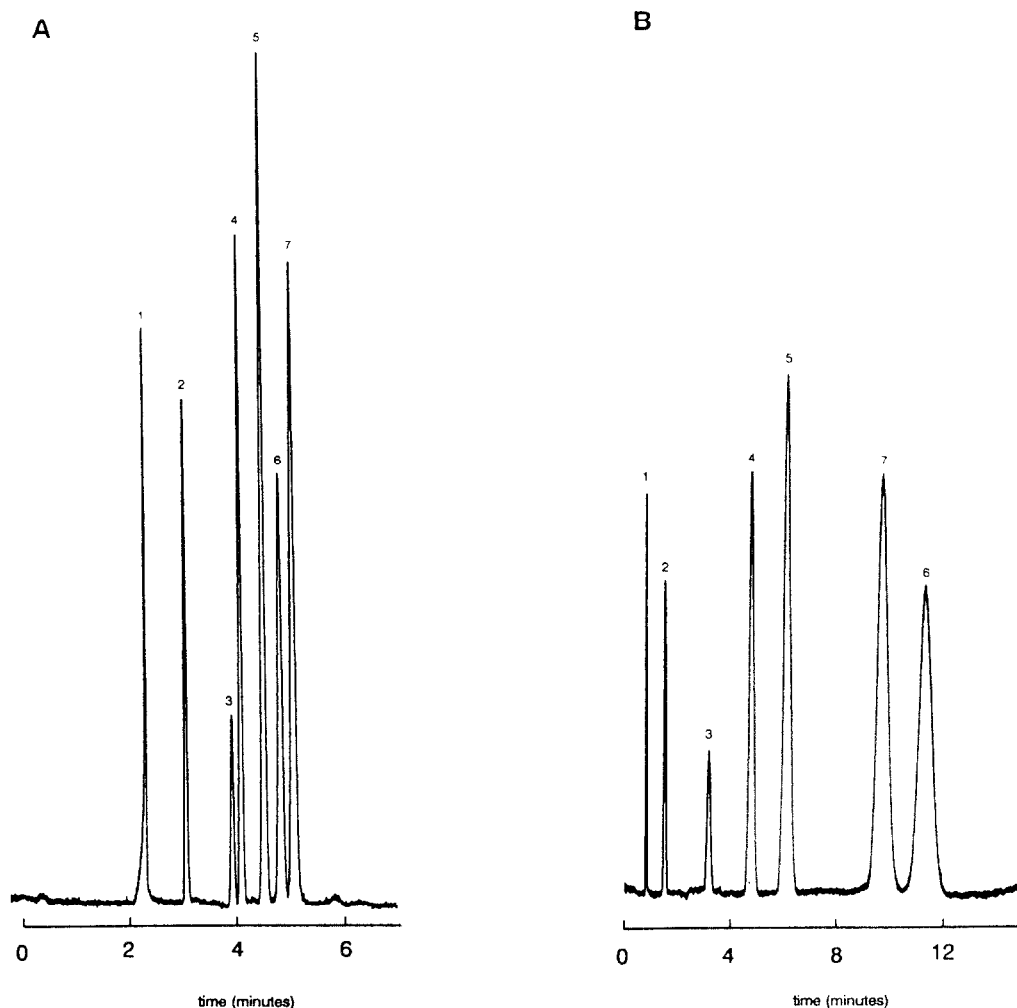


Fig. 3. Chromatograms of anthracene derivatives on column 8. Length, 61.1 cm; stationary phase, SiA-BA;  $V_s/V_m = 0.81$ . Peaks: 1 = salicylate; 2 = anthracenemethanol; 3 = anthracenecarbonitrile; 4 = anthracene; 5 = fluoranthene; 6 = phenylanthracene; 7 = 1,2-benzanthracene. (A) Mobile phase, methanol; pressure, 7.0 bar. (B) Mobile phase, methanol-water (80:20, v/v); pressure, 25.7 bar.

Further, the viscosity of acetonitrile is smaller than that of methanol, so that a smaller pressure drop is required to realize a desired separation speed.

#### Column performance

The theoretical plate height ( $H$ ) in OT-LC can be calculated with the extended Golay equation according to

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

where

$u$  = linear velocity of the mobile phase;

$d_c$  = inner diameter;

$d_f$  = film thickness;

$D_m$  = diffusion coefficient in the mobile phase;



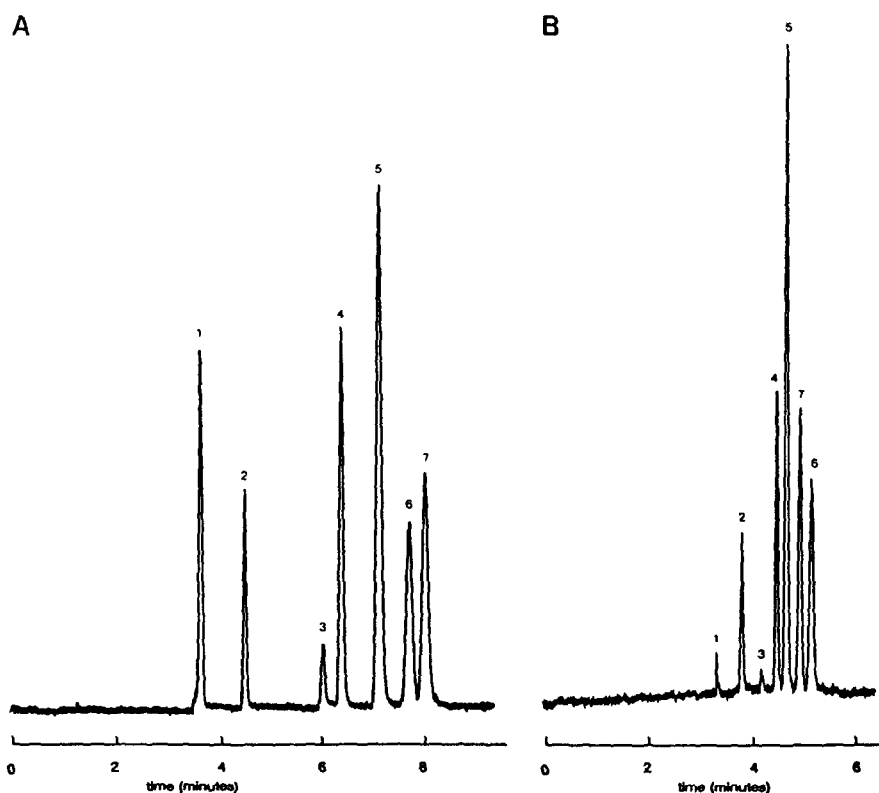


Fig. 4. Chromatograms of anthracene derivatives on column 15. Length, 115.1 cm; stationary phase, SiA-EHA;  $V_s/V_m = 0.65$ , LIF detection; peaks as in Fig. 3. (A) Mobile phase, methanol; pressure, 13.05 bar. (B) Mobile phase, acetonitrile; pressure, 7.5 bar.

$D_s$  = diffusion coefficient in the stationary phase;

$k'$  = capacity factor.

At higher linear velocities the contribution of the first term to the plate height can be neglected in OT-LC. For a given column with known inner diameter, film thickness and capacity factor, the plate height can be calculated provided that the diffusion coefficients in the mobile and stationary phase are known. Diffusion coefficients of solutes in the mobile phase can be calculated reasonably well with the Wilke–Chang equation. However, the diffusion coefficients in the stationary phase, in particular in polymeric layers, are little known. From previous studies with polymer coatings it is apparent that  $D_s$  plays

a decisive role in the selection of polymeric retentive layers for OT-LC.

The efficiencies of the polyacrylate columns were investigated by constructing  $H$  versus  $u$  curves with 1,2-benzanthracene as solute and methanol as mobile phase. Fig. 5 shows the  $H$ – $u$  curves for three different types of columns with approximately the same phase ratio but different capacity factors. As an illustration, the  $H$ – $u$  curve for an SiA–LA (1:1) polyacrylate film taken from a previous paper [7] is included. As can be seen, the experimental curves for columns 4, 8 and 16 are similar, but differ significantly from that for column 4 from ref. 7. The coincidence of the experimental data points is fortuitous and arises from the cancellation of the

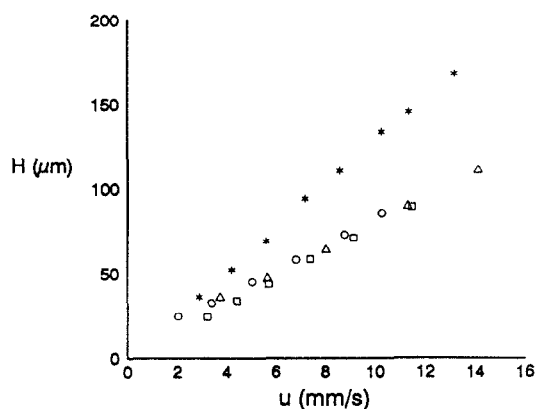


Fig. 5. Experimental plate heights of 1,2-benzanthracene versus mobile phase velocity. □ = Column 4, SiA-LA ( $k' = 0.83$ ,  $V_s/V_m = 0.81$ ); △ = column 8, SiA-BA ( $k' = 1.23$ ,  $V_s/V_m = 0.81$ ); ○ = column 16, SiA-EHA ( $k' = 1.38$ ,  $V_s/V_m = 0.75$ ); \* = column 4 from ref. 7 ( $k' = 1.86$ ,  $V_s/V_m = 0.22$ ).

effect of the magnitude of the diffusion coefficients in the layers ( $D_s$ ) and the value of the capacity factors. By neglecting the peak broadening due to extra-column effects, the  $D_s$  values of 1,2-benzanthracene on several columns were calculated by fitting the experimental curves with the extended Golay equation. Inspection under a microscope showed that all the selected columns had visually a uniform layer. The results of the calculations are given in Table 3. Although the value of  $D_s$  within a set of columns varies considerably, some trends are clearly visible. The diffusivity in the SiA-LA (7:1) layer, which is almost a bare SiA layer, is the largest. Incorporation of alkyl chains in the

polymer network decreases the diffusivity and the decrease is larger the longer is the alkyl chain. From the point of view of column performance, OT-LC columns with bare SiA layers or SiA layers mixed with a short alkyl chain are to be preferred. This last option has our preference because the success rate with the mixed SiA/alkyl acrylate layers is considerably higher than with bare SiA layers.

#### Detection

So far, on-column LIF detection has been applied with OT-LC columns using highly fluorescent anthracene derivatives as test solutes. This detection mode is very convenient for studying OT-LC because it combines an extremely small detection volume with sufficient sensitivity needed to avoid mass overloading. However, to explore OT-LC further a more universal detection principle, such as UV detection, is preferable. On-column UV detection has been applied successfully in capillary electrophoresis and also recently by Crego *et al.* [4] in OT-LC. In OT-LC, the capillary inner diameters are about 5–10  $\mu\text{m}$ , which means that the cell length with on-column detection is very small. This will adversely affect the concentration sensitivity. As a consequence, higher solute concentrations have to be injected with the risk of overloading the column. Therefore, the application of on-column UV detection in OT-LC only becomes attractive when the columns have sufficient sample capacity. As demonstrated in this study, thick retentive layers can be fabricated,

Table 3  
Estimated diffusion coefficients of 1,2-benzanthracene in polyacrylate stationary phases

Capillary No.	Monomer	$D_s$ ( $\times 10^{-10} \text{ m}^2/\text{s}$ )	Mean $D_s$ ( $\times 10^{-10} \text{ m}^2/\text{s}$ )
3	LA	0.95	} 0.88
4	LA	0.65	
6	LA	1.05	
8	BA	0.75	} 0.77
9	BA	0.70	
10	BA	0.85	} 0.53
13	EHA	0.43	
14	EHA	0.51	
16	EHA	0.65	

which should give good sample capacities. Therefore, we investigated the application of on-column UV detection with the prepared polyacrylate columns. For this purpose we adapted the adjustable aperture used by Bruin *et al.* [8] for on-column UV detection in capillary electrophoresis. With this aperture the slit width can easily be adjusted under a microscope. The best signal-to-noise ratio was found when the slit width was the same as the inner diameter of the capillary. The length of the detection window was adjusted to 1 mm. In order to determine the extra peak broadening of the UV cell, the plate height of anthracene on column 17 was measured with LIF and UV detection (see Fig. 6). From previous studies it has been found that the extra peak broadening with on-column LIF detection is negligibly small. It can be seen that the plate heights with UV detection are about 10% larger than with LIF detection. On decreasing the width of the window, the plate heights with LIF and UV detection coincide but the signal-to-noise ratio decreases dramatically. As a decrease

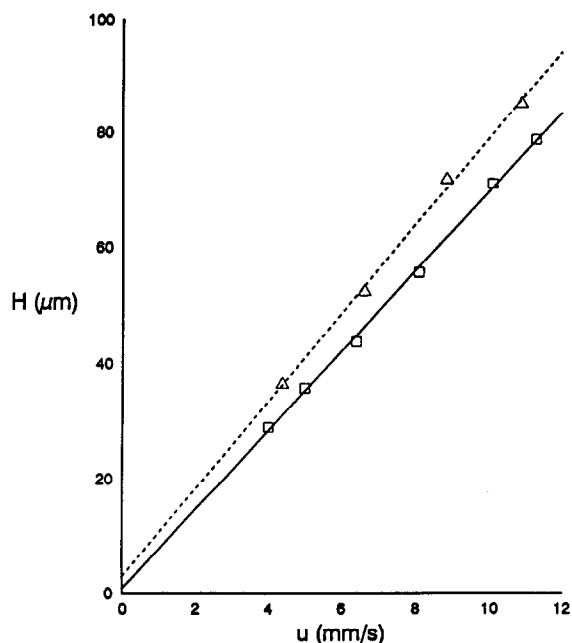


Fig. 6. Experimental  $H$  versus  $u$  plot for anthracene on column 17. Mobile phase, methanol; detection,  $\square$  = LIF and  $\triangle$  = UV.

in efficiency of 10% is acceptable, a 1 mm window is a good compromise. Fig. 7 shows the chromatograms of anthracene derivatives with the same column and conditions using LIF and UV detection. The applicability of on-column UV detection in OT-LC is well demonstrated in Fig. 8, showing a rapid separation of alkylbenzenes.

#### Mass loadability

The mass loadability of polyacrylate columns was investigated by calculating the plate number and peak asymmetry from chromatograms obtained with different solute concentrations and methanol as the mobile phase. Toluene was

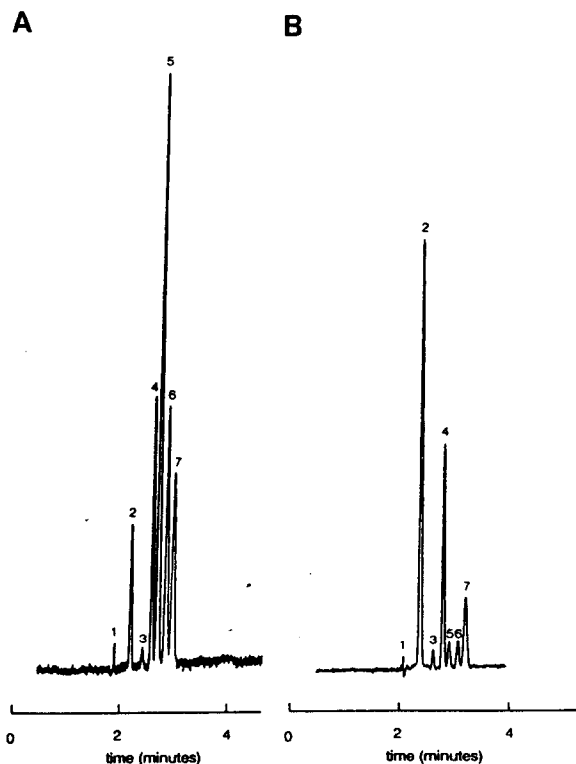


Fig. 7. Chromatograms of anthracene derivatives on column 15. Length, 115.1 cm; stationary phase, SiA-EHA;  $V_s/V_m = 0.65$ ; mobile phase, acetonitrile. Peaks: 1 = salicylate; 2 = anthracenemethanol; 3 = anthracenecarbonitrile; 4 = anthracene; 5 = fluoranthene; 6 = 1,2-benzanthracene; 7 = 9-phenylanthracene. (A) Detection, LIF; pressure, 13.8 bar. (B) Detection, UV (258 nm); pressure, 13.0 bar.

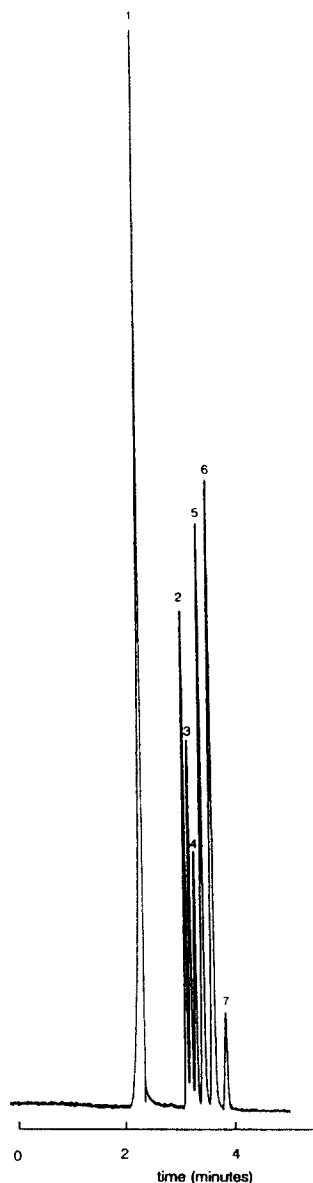


Fig. 8. Rapid separation of methyl-substituted benzenes on column 8. Detection, UV (210 nm); mobile phase, methanol. Peaks: 1 = salicylate; 2 = benzene (0.16 mol/l); 3 = toluene (0.14 mol/l); 4 = dimethylbenzene; 5 = trimethylbenzene; 6 = tetramethylbenzene; 7 = pentamethylbenzene.

selected as the test solute and on-column UV detection at 210 nm was applied. The change in plate number and peak asymmetry with increas-

ing solute concentration is shown in Fig. 9. The plate number is almost constant up to a concentration of 0.1 mol/l toluene. With an injection volume of 133  $\mu$ l this corresponds to an injected amount of about 13 ng. Surprisingly, the peak symmetry is still well preserved up to 1 mol/l toluene. From this mass loadability study,

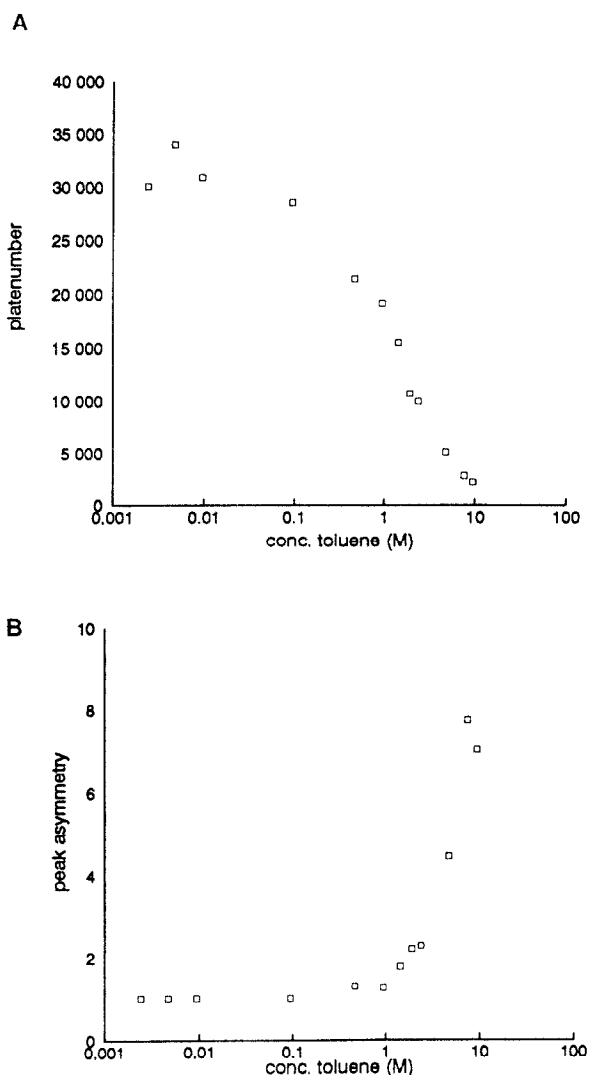


Fig. 9. (A) Plate number and (B) peak asymmetry versus injected toluene concentration on capillary 8. Mobile phase, methanol; detection, UV (220 nm); phase ratio, 0.81; injection volume, 133  $\mu$ l. Asymmetry of the peaks was calculated as described under Experimental.

it can be concluded that the thick polyacrylate layers have sufficient sample capacity to avoid decrease in efficiency by overloading and for on-column UV detection to be applied.

#### *Long-term stability of the columns*

The long-term stability of the various types of polyacrylate columns was investigated by measuring the efficiency and retention as a function of time with anthracene and 1,2-benzanthracene. With methanol and methanol–water mixtures as mobile phase no significant changes were observed over several months. In order to test the stability under more aggressive conditions, column 16 was flushed with 0.01 mol/l KOH for 3 h with methanol for 12 h. The capacity factors before and after the flushing with potassium hydroxide did not alter. Surprisingly, the plate height of anthracene was about 20% smaller after the flushing whereas that of 1,2-benzanthracene did not change.

As the columns appeared to be very stable with 0.01 mol/l KOH, the effect of a still higher potassium hydroxide concentration was investigated. For that purpose column 17 was flushed with 0.1 mol/l KOH for 1 h and then with methanol. In order to follow the equilibration of the column with methanol after the KOH flush, the capacity factors of the test solutes were measured at fixed time intervals. After 3 h of flushing the capacity factors of anthracene and 1,2-benzanthracene became constant; however, they were 15 and 19% smaller, respectively, than on the original column. By that time the film thickness appeared to have been reduced by 15%. This reduction results in a 20% decrease in the phase ratio, and this largely explains the decrease in the capacity factors. Further, the plate heights of the test solutes after the KOH flushing were found to be smaller (*ca.* 20%) than on the original column. The reason why the film thickness decreases is not yet clear, but probably some loosely bound constituents in the layer are extracted into the strong alkaline solution. No further deterioration of the layer was observed after flushing for an additional 4 h with 0.1 mol/l KOH.

#### 4. Conclusions

Polyacrylate layers 1–2  $\mu\text{m}$  thick can easily be prepared in 10  $\mu\text{m}$  I.D. fused-silica capillaries. The layers appear to be very suitable for reversed-phase OT-LC. By using acetone–pentane as the coating solvent, the duration of the evaporation of the solvent is considerably decreased. With this solvent mixture the success rate of the preparation of SiA–EHA-coated capillaries was almost 100%.

The columns show a high mass loadability, which allows “on-column” UV detection. The possibility of using UV detection substantially increases the applicability of OT-LC. Extra peak broadening can be kept reasonably small by using a UV cell with an adjustable aperture. The polyacrylate films are extremely stable and can even withstand basic solutions of pH 12.

Current research is focused on the immobilization of thick polyacrylate layers in 5- $\mu\text{m}$  capillaries. Other acrylates will be used in order to change the selectivity of the stationary phases.

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