Performance of an ethoxyethylacrylate stationary phase for open-tubular liquid chromatography

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Abstract

Fused-silica capillaries of I.D. ca 11.4 μm were coated with polyacrylate layers. The layers consisted of copolymers of silicone acrylate and ethoxyethylacrylate which were polymerized in situ by UV irradiation. Capillaries with phase ratios up to 0.9 were prepared. This results in very high mass loadabilities so that solute amounts suitable for UV detection can be injected without overloading the stationary phase. This is illustrated by the separation of various mixtures containing phenolic and aromatic compounds. The retention behaviour and the kinetic performance were studied with both UV and laser-induced fluorescence detection. On a 5-m column more than 10^6 plates could be generated for anthracene. Further, the possibility of on-column preconcentration and the effect of temperature on the kinetic performance were investigated on this stationary phase.

1. Introduction

In chromatography, the highest separation efficiency and speed can be obtained with open-tubular columns. For liquid chromatography, these attractive features can only be realized by using columns with internal diameters in the range 1–10 μm. In contrast to gas chromatography, where capillary columns are now applied almost exclusively, all routine liquid chromatographic separations are still performed in packed columns. The full exploration of open-tubular liquid chromatography (OT-LC) is hampered mainly by two facts: (i) the lack of sensitive, universal detectors capable of detecting picogram amounts of solute in 40–200-pl peak volumes and (ii) problems encountered with the immobilization of a uniform stationary phase

layer on the inside surface of the capillary with sufficient sample capacity to avoid overloading. Although there has been some progress in the development of miniaturized detection systems, mainly initiated by the interest in capillary zone electrophoresis, detection in OT-LC is still the main bottleneck. In order partly to circumvent this detection problem, it is advantageous to apply a thick stationary phase by which larger solute concentrations can be injected and detection is simplified.

Several methods have been utilized to realize a retentive layer, of which the static coating procedure [1,2] and the creation of a porous structure inside the capillary [3–5] have been studied most thoroughly. One of the most promising procedures in terms of phase ratio and success rate is in situ photopolymerization of acrylates.

In this paper, we describe the results of an investigation to prepare a thick silicone acrylate–

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ethoxyethylacrylate (SiA–EEA) stationary phase in 10-μm capillaries for reversed phase OT-LC.

2. Experimental

2.1. Apparatus

The OT-LC set-up used for the chromatographic measurements is depicted in Fig. 1. It consisted of an HPLC pump (Spectroflow 400; Kratos, Rotterdam, Netherlands), a splitting device used to reduce the flow from ml/min to μl/min and a second splitting device used to diminish the injection volume. The volume of the applied injection valve was 0.2 μl; splitting ratios ranged from 5 in the preconcentration experiments to 1250 under normal conditions. The pressure was monitored with a digital pressure sensor (Type 4041A200; Kistler, Winterthur, Switzerland) combined with a current amplifier (Type 4061; Kistler) and a recorder (Model BD111; Kipp & Zonen, Delft, Netherlands). Two on-column detection systems were employed, a UV absorbance detector (Kratos Model 757) using a laboratory-built on-column detection cell with adjustable slit [6] (the detector rise time was set to 0.1 s) and a helium–cadmium laser (λ_{ex} = 325 nm) (Model 356 XM; Omnichrome, Chino, CA, USA) used as a light source for laser-induced fluorescence detection (LIF) with emission wavelength set at 380 nm. The fluorescence yield was measured with a photomultiplier tube (Type 6225 S; EMI, Hayes, UK).

2.2. Materials

Fused-silica capillaries with I.D. ca. 11.4 μm, having an acrylate outside protective coating, were a kind gift from Philips Research Laboratories (Eindhoven, Netherlands). The outside acrylate coating possessed sufficient UV transparency for in situ photopolymerization. To initiate the polymerization reaction α,α-di-methoxy-α-phenylacetophenone (DMPA) was used (Irgacure 651; Ciba-Geigy, Basle, Switzerland).

HPLC-grade methanol was obtained from Janssen (Beerse, Belgium), ethoxyethylacrylate (EEA) and 3-(methacryloxy)propyltrimethoxysilane (γ-MPS) from Fluka (Buchs, Switzerland) and silicone acrylate (SiA) (Tegomer V-Si2150) from Goldsmidt (Essen, Germany).

2.3. Coating procedure

The photopolymerization of acrylates to immobilize a polymer film in fused-silica capillaries has been described before [7]. Briefly, the procedure consists of the following successive steps: etching of the fused silica surface to enlarge the number of silanol groups; silylation of the surface with a silyl reagent containing an acrylate group (γ-MPS), (the acrylate group on the surface is needed to anchor the polymer layer);
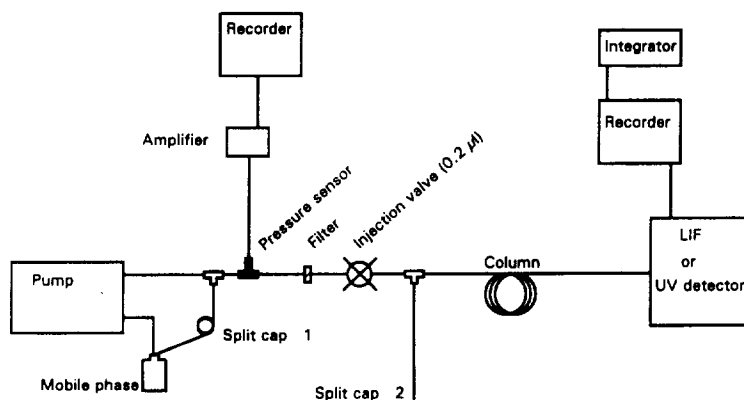

Fig. 1. Schematic diagram of the OT-LC system.

Table 1
Experimental coating conditions and dimensions of the fused-silica columns

Capillary	L (cm)	Monomer (%, v/v)	d_c (μm)	d_f (μm)	V_s/V_m
1	110.3	10% EEA–10% SiA	8.26	1.56	0.90
2	494.0	10% EEA–10% SiA	8.38	1.51	0.85

DMPA concentration, 3.0 mg ml^{-1} for both monomer solutions; solvent, acetone–pentane (1:1); light intensity, 0.14 mW; irradiation time, 350 s; d_c = column diameter; d_f = film thickness; V_s/V_m = phase ratio.

filling the capillary with a mixture of SiA and EEA monomers in acetone–pentane (1:1) containing a photoinitiator, then irradiating the capillary with UV radiation to initiate the polymerization; evaporation of the solvent; and curing at elevated temperature.

Capillaries of length ca. 1 m (capillary 1) and 5 m (capillary 2) were treated according to the aforementioned procedure. The I.D.s of the capillaries were calculated with the Poiseuille equation by measuring the holdup time of an unretained solute. The polymer film thickness, needed to calculate the phase ratio, was determined by subtracting the radius of the coated capillary from the initial capillary radius. The coating conditions and capillary characteristics are given in Table 1.

2.4. Chromatography

Pure methanol, acetonitrile or their mixtures were used as mobile phases. Solutes were dissolved in methanol, except in the preconcentration experiments, where they were dissolved in water. The chromatographic results were evaluated as described previously [7].

3. Results and discussion

3.1. Solvent evaporation

Previously we have reported some problems that can occur during the evaporation step with other acrylate phases [7]. With the present phase such difficulties did not occur during the preparation of the two columns under study, or during

the preparation of columns with smaller diameters. The removal of the solvent from a capillary in time can in principle be derived from Poiseuille's law, resulting in [8]

$$L_c = Cd_c\sqrt{t} \tag{1}$$

where L_c = length of the capillary, C = constant, d_c = I.D. of the capillary and t = time.

The evaporation of the solvent from capillary 2 was followed as a function of time. In Fig. 2 the positions of the meniscus at different time intervals in the evaporation process are plotted against elapsed time. The solid line represents an empirical relationship. For this curve the constant C in Eq. 1 was adapted for the first two
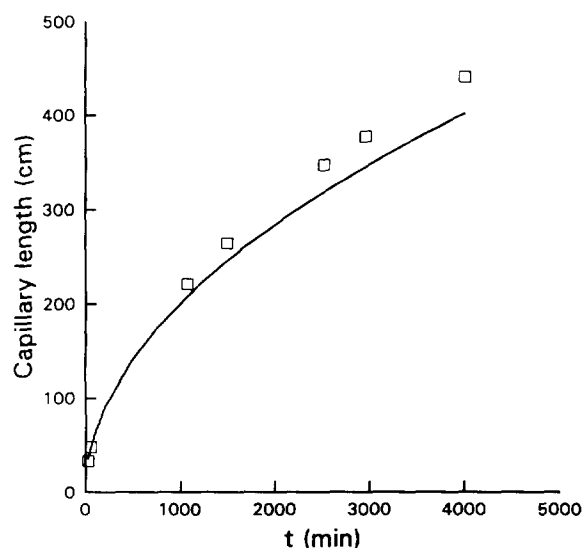

Fig. 2. Evaporation process in capillary 2. The capillary length refers to the part of the capillary from which the solvent has been evaporated. □ = Experimental data. The solid line is an empirical relationship based on the first two data points.
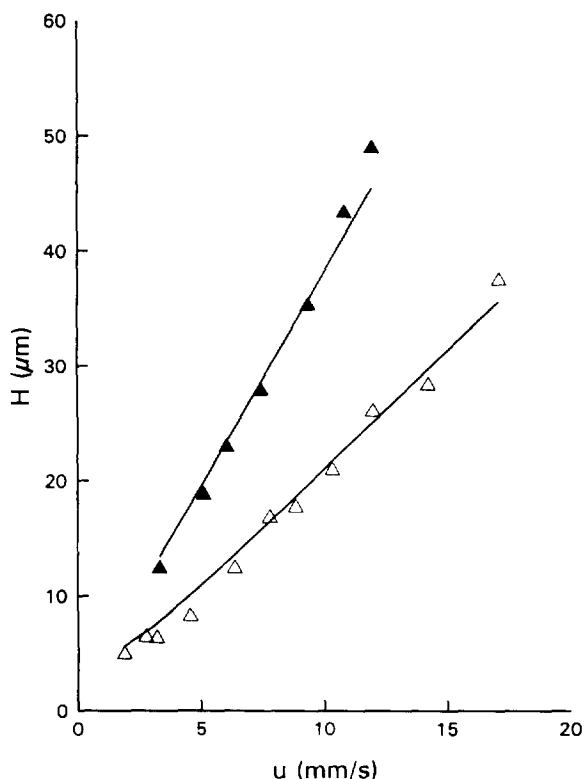
Fig. 3. Experimental and theoretical plate-height curves of anthracene on capillary 2. Mobile phase: ▲ = methanol; △ = acetonitrile. Solid lines represents theoretical Golay curves.

experimental data points. There is good agreement between the curve and the experimental data points. As can be seen from Fig. 2, it takes ca. 3.5 days to empty capillary 2. Eqn. 1 can be used to predict the evaporation time reasonably

Table 2
Diffusion coefficients (10^{-10} m² s⁻¹) of anthracene derivatives in SiA–EEA stationary phases using methanol as mobile phase

Compound	Capillary	
	1	2
Anthracenemethanol	0.62	0.85
Anthracenecarbonitrile	0.95	1.40
Anthracene	1.40	2.20
Fluoranthene	1.10	1.40
9-Phenylanthracene	0.73	0.90
1,2-Benzanthracene	1.10	1.20

well. For instance, the complete evaporation of the solvent from a 10 m × 8.4 μm I.D. capillary under identical conditions will take about 14 days. The same time is predicted to remove the solvent from a 5 m × 4.2 μm I.D. capillary.

3.2. Kinetic performance

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

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

(2)

where u = linear velocity of the mobile phase, d_c = I.D. of the capillary, d_f = film thickness of the stationary phase, D_m = diffusion coefficient in the mobile phase, D_s = diffusion coefficient in the stationary phase and k' = capacity factor.

In the kinetic studies, reliable H values were obtained by using LIF and split injection, leading to negligible extra-column effects and sufficiently low concentrations. To obtain a high mass loadability and thus simplify detection in OT-LC, thick stationary phases have to be immobilized. However, with increasing film thickness the contribution of the third term in the extended Golay equation increases significantly. Therefore, in order to achieve efficient separations it is crucial to select a phase with a large diffusivity.

By fitting the experimental plate heights with the extended Golay equation, diffusion coefficients in the stationary phase, D_s, can be calculated as all other parameters in Eq. 1 are known or can be calculated. Diffusion coefficients in the mobile phase, D_m, were calculated with the Wilke–Chang equation. Fig. 3 shows H–u curves for anthracene obtained on column 2 using 100% methanol and 100% acetonitrile as mobile phases. The theoretical curves, calculated with the extended Golay equation, are included. Diffusion coefficients of anthracene used for the calculation of the Golay values are $2.2 \cdot 10^{-10}$ and $2.7 \cdot 10^{-10}$ m² s⁻¹ applying methanol and acetonitrile, respectively. The contribution of the third term to the overall plate height is around 50% using acetonitrile and 45% using methanol
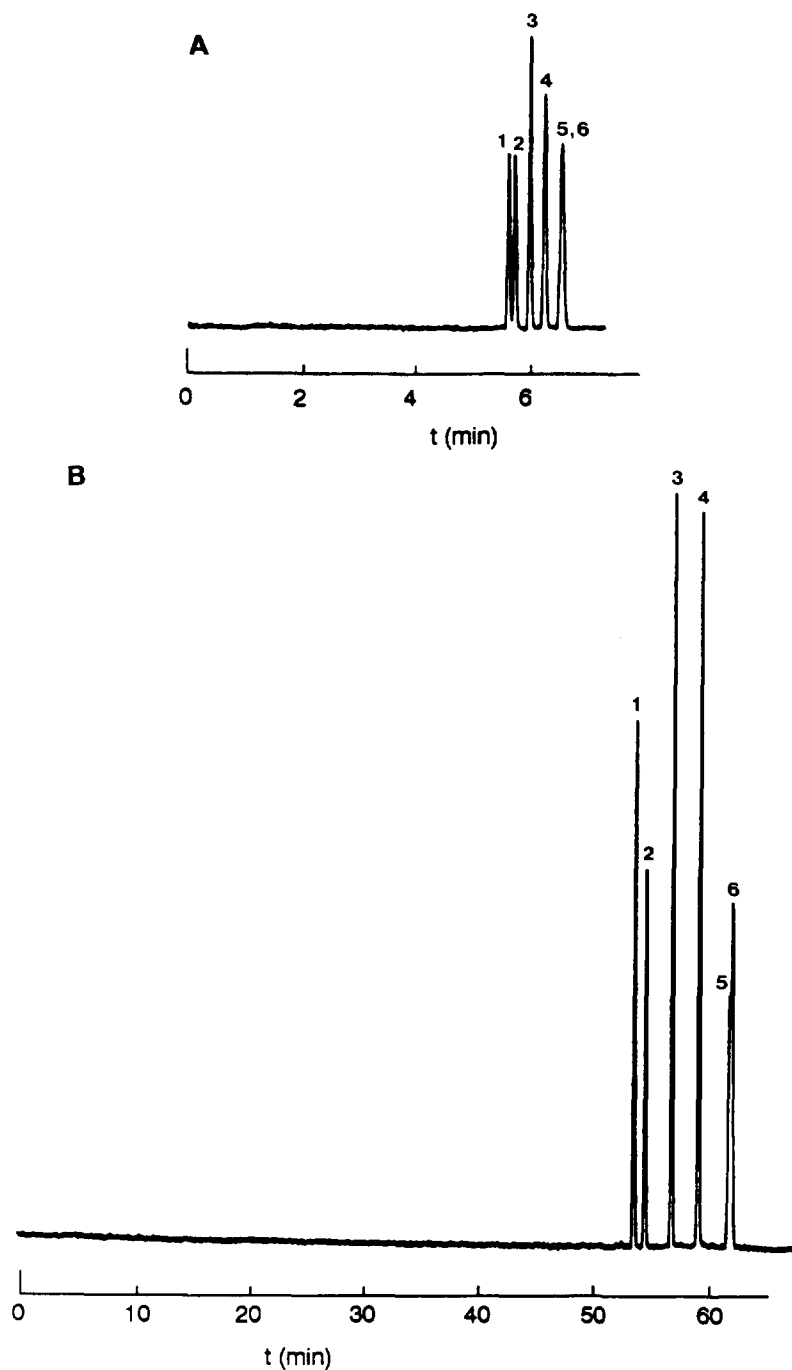
Fig. 4. Chromatograms of anthracene derivatives on column 2 at two mobile phase velocities, u: (A) 18.2; (B) 1.92 mm s^{-1}. Mobile phase: acetonitrile. Solutes: 1 = anthracenemethanol; 2 = anthracenecarbonitrile; 3 = anthracene; 4 = fluoranthene; 5 = 1,2-benzanthracene; 6 = phenylanthracene.

as mobile phase. There is good agreement between the extended Golay equation and the experimental data points. The diffusion coefficients calculated for various anthracene derivatives are given in Table 2.

Compared with previous immobilized stationary phases such as lauryl acrylate (LA), butyl acrylate (BA) and ethylhexyl acrylate (EHA) [7], the diffusion coefficients appear to be larger in the ethoxyethylacrylate polymer layer. On SiA–BA-coated capillaries a diffusion coefficient of $0.77 \cdot 10^{-10}$ m^2 s^{-1} was found for 1,2-benzanthracene using methanol as mobile phase, whereas a value of $1.15 \cdot 10^{-10}$ m^2 s^{-1} was found with SiA–EEA-coated capillaries.

An illustration of the performance of the 5-m capillary at two linear velocities can be seen in Fig. 4. Two solutes, 9-phenylanthracene and 1,2-benzanthracene, have similar capacity factors and cannot be separated at higher velocities (Fig. 4A). However, when the velocity of the mobile is reduced to near the optimum (Fig. 4B), a marginal separation between these compounds can be achieved. Under these conditions 784 000, 1 005 000 and 807 000 plates are generated for anthracenecarbonitrile, anthracene and fluoranthene, respectively.

3.3. Retention and selectivity

The retention behaviour of the SiA–EEA stationary phase was studied with several classes of compounds. The capacity factors of various
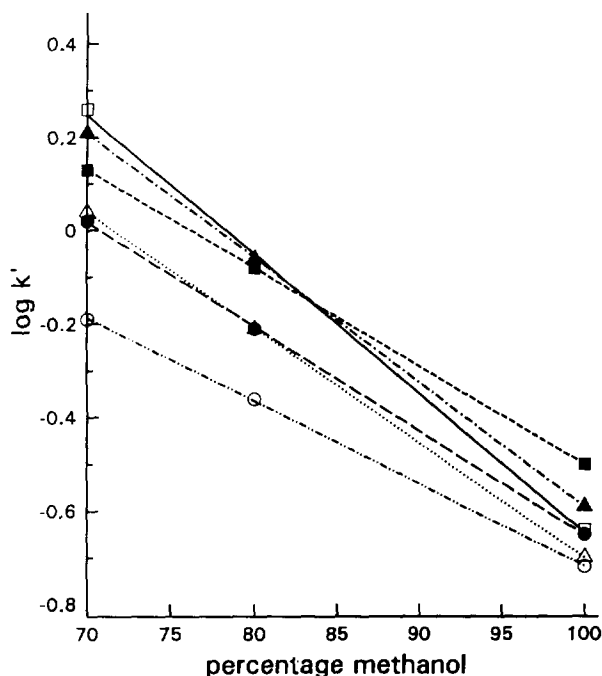

Fig. 5. Effect of the mobile phase composition on k' of phenolic compounds on capillary 2. $\bigcirc$ = Phenol; $\triangle$ = 3-ethylphenol; $\square$ = 2-sec.-butylphenol; $\bullet$ = 2-methylphenol; $\blacktriangle$ = 3-chlorophenol; $\blacksquare$ = 2-nitrophenol.

anthracene derivatives remained constant for several weeks for all mobile phases. In this period a maximum fluctuation in capacity factors of 5% was observed. Within a day, the fluctuations of the capacity factors were usually 1–3%. As the capillaries were not thermostated, these fluctuations are acceptable. The distribution co-

Table 3
Distribution coefficients, K_i, and selectivity factors, $a_{i,j}$, of anthracene derivatives on SiA–EEA-coated capillaries

Compound	Methanol mobile phase				Acetonitrile mobile phase			
	Capillary 1		Capillary 2		Capillary 1		Capillary 2	
	K_i	$a_{j,i}$	K_i	$a_{j,i}$	K_i	$a_{j,i}$	K_i	$a_{j,i}$
Anthracenemethanol	0.41		0.42		0.29		0.29	
Anthracenecarbonitrile	0.71	1.72	0.75	1.76	0.33	1.14	0.32	1.08
Anthracene	0.78	1.10	0.83	1.11	0.38	1.18	0.38	1.19
Fluoranthene	0.99	1.27	1.06	1.28	0.45	1.17	0.44	1.16
9-Phenylanthracene	1.06	1.07	1.13	1.07	0.52	1.15	0.52	1.18
1,2-Benzanthracene	1.22	1.15	1.30	1.14	0.52	1.00	0.52	1.00

efficients, calculated from the capacity factor and phase ratio according to $K_i = k_i(V_m/V_s)$, and selectivity factors are listed in Table 3. The distribution coefficients with methanol are considerably larger than with acetonitrile, indicating the reversed-phase liquid chromatographic behaviour of the layer. Also, a significantly different selectivity is found with the two mobile phases, as is known from RP-HPLC. The small variations in retention on both columns indicate that the specific nature of the layers is constant.

In a previous paper [7] we reported the retention behaviour of anthracene derivatives on polyacrylate stationary phases using aqueous mobile phases. In this work the effect of the addition of water to the mobile phase on retention was studied with phenolic compounds. In Fig. 5 the logarithms of the capacity factors of phenolic compounds are plotted against the percentage of methanol in the mobile phase. The data points represent mean capacity factors of at least five measurements. As expected for RPLC, a linear relationship between the percentage of water and log k' was observed for all solutes. The correlation coefficients ranged from 0.9978 for 2-sec.-butylphenol to 0.9995 for 3-ethylphenol and 2-chlorophenol. Selectivity changes occur with increasing water content, resulting in a reversal of the elution order of some of the solutes.

3.4. Effect of temperature

According to the Golay equation, the contribution of the second and third terms to the overall plate height decreases when diffusion increases. Larger diffusion coefficients and consequently smaller plate heights can be realized by increasing the temperature, as has been shown by Liu and co-workers [9,10]. In addition, temperature will influence the capacity factors and selectivity.

The effect of temperature was investigated by measuring H–u curves for two phenolic compounds at 20 and 60°C with pure methanol as the mobile phase. For that purpose the OT-LC system represented in Fig. 1 was extended with a thermostated water-bath in which 95% of the
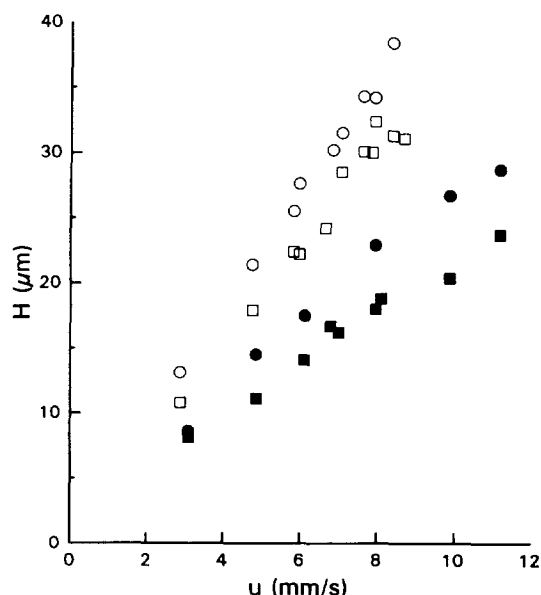

Fig. 6. Effect of temperature on the plate height for two phenolic compounds on capillary 2. □ = 2-Ethylphenol at 20°C; ○ = 2-sec.-butylphenol at 20°C; ■ = 2-ethylphenol at 60°C; ● = 2-sec.-butylphenol at 60°C.

capillary could be accommodated. The results of these measurements are shown in Fig. 6. Increasing the column temperature clearly decreases the plate heights. The total effect cannot fully be attributed to larger diffusion, however, since the capacity factors also decreases (by ca. 15% at 60°C).

The capacity factors appear to be more affected by temperature with aqueous mobile phases. Fig. 7 shows the effect of temperature on the separation of alkylphenols. The separation time can be shortened by about 30% at 60°C without significantly changing the resolution. Di-tert.-butylphenol showed the strongest decrease in k', of ca. 70%.

3.5. UV detection

The thick immobilized acrylate layers possess a high mass loadability, which opens the way to apply a less sensitive detection method, such as UV absorbance, without overloading the stationary phase. The use of UV detection will extend the applicability in OT-LC considerably.
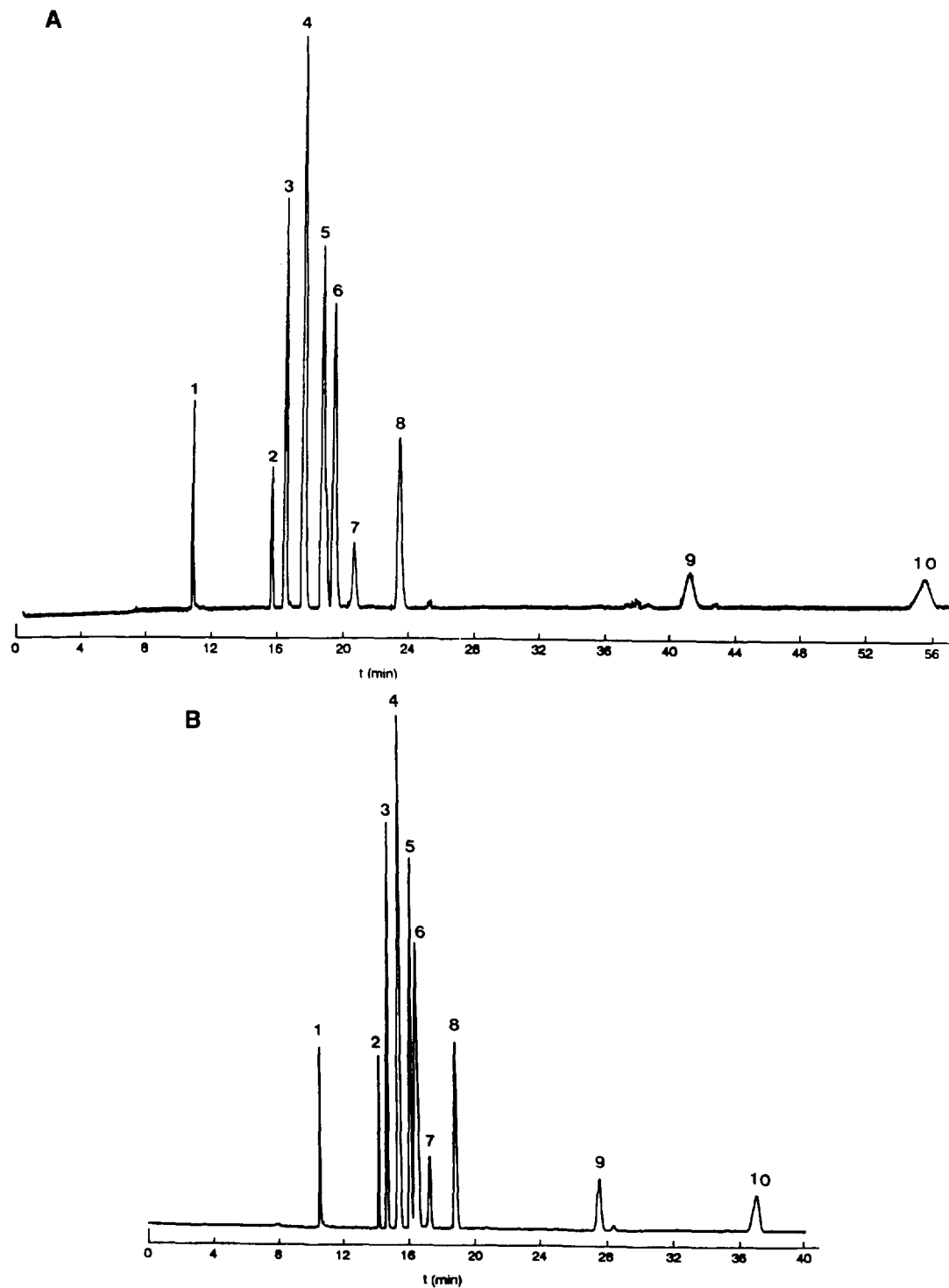
Fig. 7. Separation of a mixture of alkylphenols on capillary 2 at (A) 20 and (B) 60°C. Mobile phase: methanol–water (80:20). UV detection at 210 nm. Solutes: 1 = Salicylate; 2 = phenol; 3 = 3-methylphenol; 4 = 3-ethylphenol; 5 = 2-methylphenol; 6 = dimethylphenols; 7 = n-propylphenol; 8 = 2-sec.-butylphenol; 9 = di-tert.-butylphenol; 10 = tri-tert.-butylphenol.
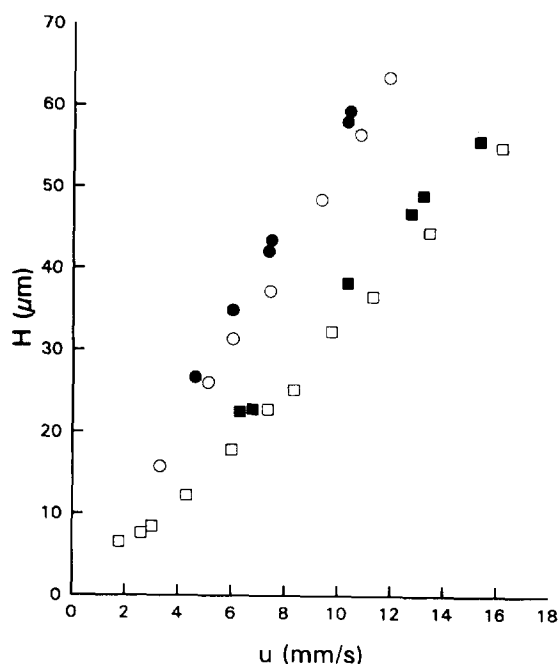
Fig. 8. Experimental plate heights for anthracenemethanol on column 2 using on-column UV and LIF detection. Mobile phase and detection: ● = methanol, UV; ○ = methanol, LIF; ■ = acetonitrile, UV; □ = acetonitrile, LIF.

In order to determine the performance with on-column UV detection, the efficiency obtained with LIF and UV detection was measured on capillary 2. Anthracenemethanol was chosen as the test compound because of its large molar absorptivity and native fluorescence. Fig. 8 shows H–u plots for anthracenemethanol using methanol and acetonitrile as mobile phases. Compared with LIF detection, an extra contribution to the plate height is found when UV detection is applied (solid symbols). If the time constant of the detector were responsible for the extra contribution, the deviation from the LIF data points would be more severe at higher mobile phase velocities, but this was not observed.

Also, the detector cell volume cannot be the cause of the increased plate heights. The solute band width of a peak with 100 000 plates on capillary 2 is 15.8 mm according to $\sigma_z^2 = HL$. As the window width of the detector cell is 1 mm, this cannot be the cause of the extra-column peak broadening. Although we have no explanation yet for this extra peak broadening, the 10%
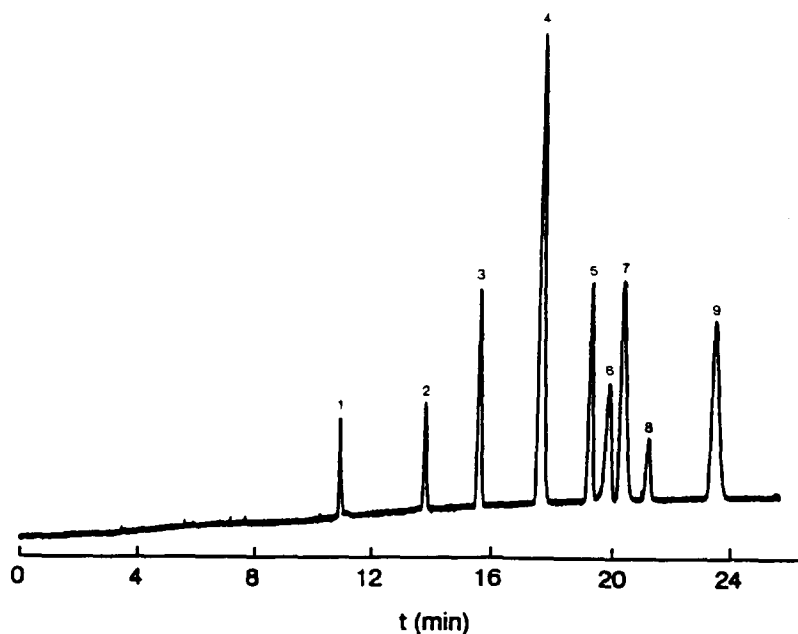

Fig. 9. Separation of a mixture of aromatic compounds using on-column UV detection. Mobile phase: methanol–water (80:20). Solutes: 1 = salicylate; 2 = phenylpropanol; 3 = phenol; 4 = 3-ethylphenol; 5 = methoxybenzene; 6 = 2-nitrophenol; 7 = 3-chlorophenol; 8 = toluene; 9 = 2-sec.-butylphenol.

extra broadening is acceptable in practice as the resolution changes with the square root of the plate height. In order to show the applicability of on-column UV detection, the separation of a mixture of aromatic compounds using UV detection is presented in Fig. 9.

3.6. Preconcentration

The justification for studying on-column preconcentration in OT-LC is twofold: on the one hand, the concentration detectability can be improved, and on the other, split injection, which may be less reproducible, could be avoided by using this technique.

For this preconcentration experiment, increasing volumes of solutions of phenol in water and in the mobile phase were injected and the plate numbers measured. The mean plate numbers of at least three measurements are given in Fig. 10 as a function of injected volume. It can be seen that when phenol is dissolved in the mobile phase, up to 160 pl can be injected without changing the plate number. With larger injection volumes the plate number decreases steeply.

However, on injecting phenol dissolved in water, the plate number remains approximately constant up to 40 nl. Under these conditions a preconcentration factor of 250 can be realized for phenol. Larger preconcentration factors can be expected for less polar, more retained compounds.

Fig. 11 shows the separation of an aqueous solution containing catechol and phenol present at concentrations of $9.8 \cdot 10^{-4}$ and $8.5 \cdot 10^{-4}$ mol^{-1} l, respectively. These concentrations are approximately one order of magnitude above the detection limit. The injected solvent, 22.6 nl, fills the first 41 cm of the capillary while the solutes were concentrated at the head of the column.
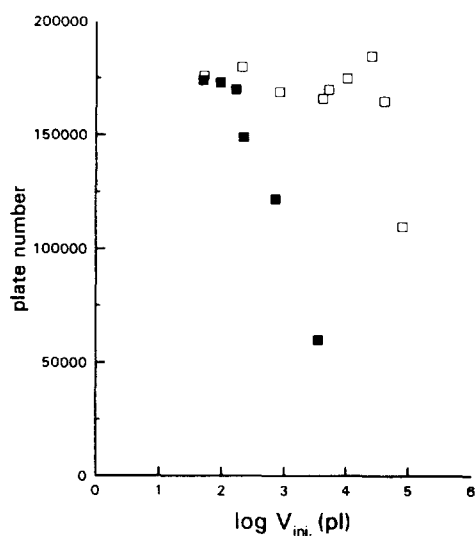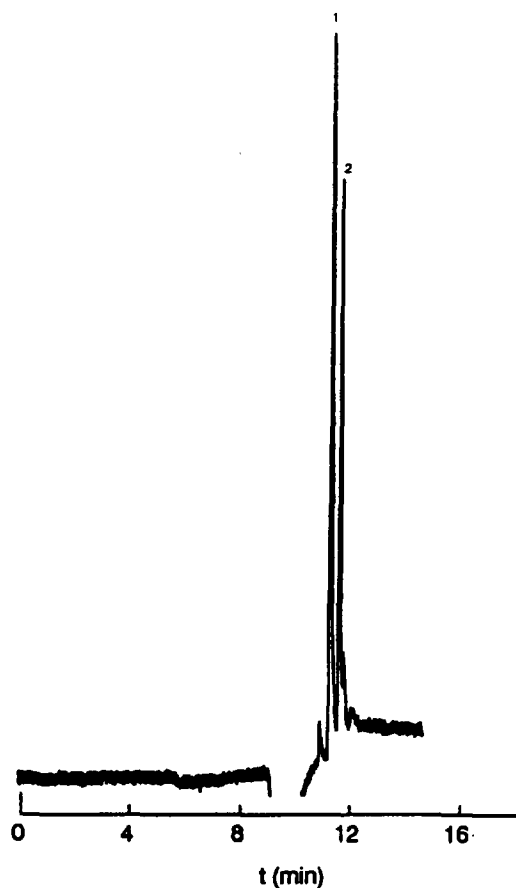
Fig. 10. Plate number versus injection volume on capillary 2, (□) with and (■) without preconcentration conditions. Mobile phase, methanol; UV detection at 210 nm; amount of phenol injected, 6.8 pmol.

Fig. 11. Separation of (1) catechol ($9.8 \cdot 10^{-4}$ mol^{-1}) and (2) phenol ($8.5 \cdot 10^{-4}$ mol^{-1}). Mobile phase, methanol; UV detection at 210 nm; volume injected, 22.6 nl.

The water plug can be seen as a dip in the baseline.

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