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Swelling behaviour and kinetic performance of polyacrylate stationary phases for reversed-phase and normal-phase open-tubular liquid chromatography

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Abstract

The effect of the mobile phase composition on the kinetic performance and retention behaviour of polyacrylate films, used as stationary phases in open-tubular liquid chromatography (OT-LC), was investigated. The polyacrylate films studied consisted of the copolymers silicone acrylate–ethylhexylacrylate (SiA–EHA), silicone acrylate–ethoxyethylacrylate (SiA–EEA) and silicone acrylate–hydroxyethylacrylate (SiA–HEA). All polyacrylate films swell significantly in pure organic solvents like methanol and hexane compared to aqueous-organic solvent mixtures. Under the latter conditions the kinetic performance decreased dramatically except for the SiA–HEA stationary phase. The swelling and shrinking of the stationary phase is fast; within one column volume the equilibrium is attained when changing the mobile phase composition. The moderate polarity of the polyacrylate stationary phase allows chromatographic use of the capillaries in the reversed-phase as in the normal-phase mode.

Keywords: Stationary phases, LC; Mobile phase composition; Polyacrylate films; Swelling behaviour; Open-tubular liquid chromatography

1. Introduction

Open-tubular liquid chromatography (OT-LC) performed in capillaries with internal diameters in the range of 2–10 μm offers great separation power. However, OT-LC is still at an experimental stage and exclusively studied and performed in research laboratories. Immobilization of a useful stationary phase in capillaries with an internal diameter $\leq 10 \mu\text{m}$ has been the main problem to broaden the exploitation of OT-LC so far. In OT-LC, columns

with a relatively thick retentive layer are required to obtain sufficient sample capacity [1]. Such conditions can be realized by applying polymeric stationary phases like polysiloxanes [2] and in particular polyacrylates [1,3–5]. The kinetic performance of such polymeric stationary phases strongly depends on the diffusivities in the layer. The diffusivity of polymeric layers and kinetic performance appears to be larger when the layer is swollen by the uptake of an organic solvent [6]. From a chromatographic point of view it is, therefore, valuable to study the swelling behaviour of stationary phases. In this article we report the swelling properties and kinetic performance of different polyacrylate films as

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stationary phases applicable for reversed-phase and normal-phase OT-LC, with various mobile phases.

2. Experimental

2.1. Apparatus

The OT-LC set-up used for the chromatographic measurements is the same as in reference [4]. Briefly it consisted of a HPLC pump (Spectroflow 400, Kratos, Rotterdam, Netherlands), two splitting devices, one is used to reduce the flow from ml min^{-1} to $\mu\text{l min}^{-1}$ and the second splitting device was used for split injection and reducing the flow to nl min^{-1} . The pressure was monitored with a digital pressure sensor (Model 4041A200, Kistler, Winterthur, Switzerland) combined with a current amplifier (Model 4061, Kistler) and a recorder (model BD111, Kipp & Zonen, Delft, Netherlands). Detection was employed at 210 nm with a UV absorbance detector (Kratos 757) using a home built on-column detection cell with adjustable slit [7].

2.2. Materials

Fused silica with an inner diameter of about 11 μm , having an acrylate outside protective coating, was a kind gift from Philips Research Laboratories (Eindhoven, Netherlands). The outside acrylate coating possessed enough UV-transparency for in situ photopolymerization. As a UV photoinitiator α,α -dimethoxy- α -phenylacetophenone (DMPA) was used (Irgacure 651, Ciba-Geigy, Basel, Switzerland).

Methanol, acetone, pentane and hexane were obtained from Janssen (Beerse, Belgium). Toluene,

isopropanol and diethyl ether were obtained from Merck (Darmstadt, Germany). Deionized water was used. Ethoxyethylacrylate (EEA), ethylhexylacrylate (EHA), hydroxyethylacrylate (HEA) and 3-(methacryloxy)propyltrimethoxysilane (γ -MPS) were purchased from Fluka (Buchs, Switzerland). Silicone acrylate (SiA) (Tegomer V-Si2150) was obtained from Goldschmidt (Essen, Germany). All test solutes were purchased from commercial sources.

2.3. Coating procedure

The photopolymerization of acrylates to immobilize a polymer film in fused-silica has been thoroughly described before [1]. Briefly the procedure consists of the following main successive steps:

- Flushing the capillaries with 1.0 M NaOH in order to increase the number of silanol groups on the surface
- Silylation of the etched capillaries with a 5% (v/v) solution of γ -MPS in toluene
- Filling the capillary with a solution of SiA and an acrylate monomer in a proper solvent
- Irradiation of the capillary with UV light to initiate the polymerization
- Removal of the solvent under reduced pressure
- Thermal curing at 120°C for at least 8 h

In Table 1 the experimental conditions of the prepared columns are given.

2.4. Measurement of d_c and d_f

The internal diameters of the capillaries were calculated with the Poiseuille equation by measuring the holdup time of a selected unretained solute at different inlet pressures [1]. When using water and

Table 1
Experimental coating conditions of the prepared fused silica columns

Cap. no.	Length (cm)	Monomer concentration (v/v) ^a	Solvent ^b	Irradiation	
				Light int. (mW)	Time (s)
1	138	10.0% EEA–10.0% SiA	A/P	0.14	385
2	75	10.0% EHA–10.0% SiA	A/P	0.14	385
3	286	6.25% HEA–6.25% SiA	A	0.40	720

^a DMPA concentration was 3.0 mg/ml for all solutions.

^b A, acetone; P, pentane; A/P, 50:50 (v/v, %).

For abbreviations, see Section 2.

methanol or mixtures of these solvents as the mobile phase, salicylic acid functioned as dead time marker and toluene or anthracene is used with hexane as the mobile phase. The polymer film thickness and the phase ratio were calculated by subtracting the radius of the coated capillary from the initial capillary radius.

2.5. Chromatography

For the RP measurements water, methanol and mixtures of methanol–water were used as the mobile phase. As the mobile phase for the NP measurements hexane and mixtures of hexane–isopropanol and hexane–diethyl ether were used. Experimental plate heights of test solutes were calculated using the equation $H=L\cdot\sigma^2/t_r^2$, where L is the column length and σ is half of the peak-width at 0.61 of the peak height.

3. Results and discussion

3.1. Swelling characteristics of polyacrylate films in pure solvents

The polyacrylate stationary phases are prepared by polymerization of a mixture of silicone acrylate and an acrylate monomer. The polarity of the polymeric layer can be changed to some extent by selection of the acrylate monomer.

In order to investigate the influence of the acrylate monomer on the swelling properties of the stationary phase, columns were coated with mixtures of silicone-acrylate and ethoxyethylacrylate, ethylhex-

ylacrylate and hydroxyethylacrylate, respectively, monomers of different polarity.

Film thicknesses of these stationary phases were measured with pure water, methanol and hexane as mobile phases. The film thicknesses, phase ratios and plate heights found for solutes with a capacity factor between 0.7 and 1.0 on the SiA–EEA, SiA–EHA and SiA–HEA stationary phase are given in Table 2. The mobile phase velocity was 9 mm s^{-1} for all measurements and solutes with similar structures were used throughout the measurements. The capacity factors were kept between 0.7 and 1.0. The only exception is the capacity factor of hexamethylbenzene, $k'=0.43$, on the SiA–HEA stationary phase operated with pure methanol as the mobile phase. In that case no compound could be found with larger retention. For that reason the plate height was extrapolated with the theoretical Golay equation for $k'=0.9$ from a measured plate height of $21\text{ }\mu\text{m}$ for $k'=0.43$.

Although with these precautions the results in Table 2 can be compared, a fair comparison is only possible when the internal diameters and film thicknesses are exactly equal. But nonetheless several interesting trends can be deduced from the data in Table 2.

Of the employed solvents, *n*-hexane resulted in the highest phase ratio for the SiA–EEA and SiA–EHA stationary phase. The swelling of the stationary phase is the largest when the polarity, e.g., the solubility parameter of the solvent and of the polymer is similar [9], and can roughly be predicted by comparing the solubility parameters of the polymer and the solvent. As the exact composition of the co-polymer of SiA and EHA is not known an absolute solubility parameter of such a stationary

Table 2

Film thicknesses, phase ratios and plate heights of compounds with k' values between 0.7 and 1.0 on various polyacrylate stationary phases for several pure solvents

	Hexane				Methanol				Water			
	d_c	d_f	V_s/V_m	H	d_c	d_f	V_s/V_m	H	d_c	d_f	V_s/V_m	H
SiA–EEA	7.78	1.81	1.15	33	8.34	1.53	0.87	46	10.02	0.69	0.29	875
SiA–EHA	6.22	2.50	2.16	50	8.82	1.20	0.62	145	9.70	0.76	0.34	1257
SiA–HEA	9.64	0.64	0.29	45	9.30	0.81	0.38	32 ^a	10.00	0.46	0.19	80

All plate heights were measured at a linear velocity of 9 mm s^{-1} ; d_c , internal diameter of the capillary after coating in μm ; d_f , film thickness in μm ; V_s/V_m , ratio of the volumes of the stationary phase and the mobile phase; H , plate height in μm .

^a Extrapolated value from $H=21\text{ }\mu\text{m}$ for $k'=0.43$ (see text).

phase cannot be given. The solubility parameter, δ , in $\text{cal}^{-1/2} \text{cm}^{-3/2}$, is 7.8–8.0 for the SiA monomer and 7.8 for ethylhexylacrylate [8]. These values are close to the solubility parameter of hexane which is 7.3 [8]. Solubility parameters for methanol and water are 14.5 and 23.4, respectively [8]. According to the solubility parameters the swelling in methanol should be lower than in hexane. This was experimentally confirmed. Water is the worst solvent for all investigated polyacrylate stationary phases. The phase ratio of the SiA–EEA-coated column is a factor 4 lower with water as the mobile phase, compared to *n*-hexane. This behaviour agrees with the expectation that the largest swelling occurs when the polarity, e.g., the solubility parameter, of the solvent and the polymer is quite similar.

The SiA–EEA and the SiA–EHA stationary phases show maximum swelling with hexane, whereas the highest phase ratio for SiA–HEA is found with methanol as the mobile phase.

For all stationary phases a correlation was observed between the phase ratio and plate height; the higher the phase ratio the lower the plate height.

With hexane as the mobile phase the obtained plate heights were small for all three stationary phases. For the hexane–SiA–EHA case this is remarkable, as the film thickness is large. On the other hand, for the hexane–SiA–HEA case the relatively small film thickness may be the cause of the good efficiency.

Using water, however, a significant difference in plate heights occurs between the stationary phases. Despite the fact that the SiA–HEA stationary phase shrinks considerably with water, which might lead to smaller diffusivities, very low plate heights were measured on this stationary phase. The high efficiency is illustrated with the separation of several hydroxybenzenes on column 3 with 100% water as the mobile phase in Fig. 1.

3.2. Swelling and kinetic performance of the SiA–EEA polyacrylate stationary phase with water–methanol mixtures

The swelling characteristics of the SiA–EEA stationary phase was studied in more detail with methanol–water mixtures, typical mobile phases for RP-LC. The film thickness at various methanol–water

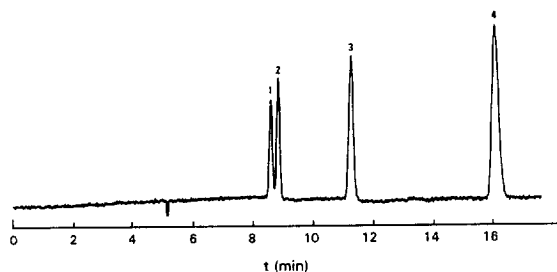


Fig. 1. Separation of hydroxybenzenes on a SiA–HEA-coated column (No. 3). Mobile phase, 100% water; Peaks: 1=1,2,3-trihydroxybenzene; 2=1,4-dihydroxybenzene; 3=1,2-dihydroxybenzene; 4=phenol.

compositions, ranging from 0 to 100% methanol, as well as the corresponding plate height of a selected solute at a fixed linear velocity and capacity factor was determined. The results are given in Fig. 2. As can be seen the film thickness decreases steeply going from 100 to 60% methanol and then becomes more or less constant. The plate height shows an opposite behaviour it gradually increases when going from 100 to 20%, and then steeply increases when further decreasing the methanol content.

The dramatic difference in efficiency between

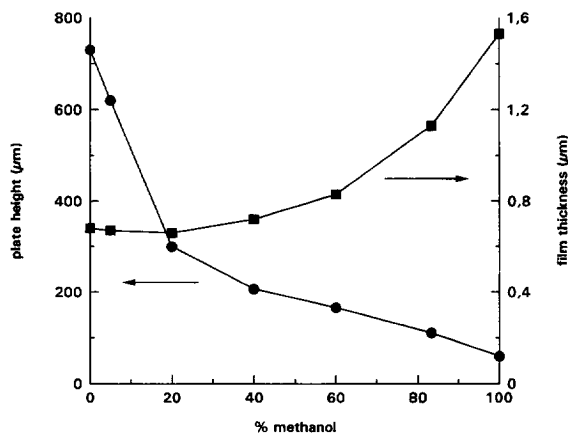


Fig. 2. Plate heights (●) of compounds with $k' = 2.2$ and stationary phase film thickness (■) of a SiA–EEA-coated column (No. 1) for various methanol–water mixtures. Mobile phase composition (methanol–water) (v/v, %) and compounds: (0:100) and (5:95), 1,3-dihydroxybenzene; (20:80), 1,2-dihydroxybenzene; (40:60), phenol; (60:40), 3,5-dimethylphenol; (83.3:16.7), pentamethylbenzene; (100:0), 1,2-benzanthracene.

100% methanol and pure water may be attributed to the contraction of the stationary phase. On the other hand, this assumption does not explain the behaviour between 20 and 0% methanol, when the stationary phase volume is virtually constant and H increases even more strongly. Possibly there are still structural changes in the stationary phase at constant volume. In the contracted stationary phase the diffusivity is significantly smaller [6] and thus the mass transfer impairs the separation efficiency.

A diffusion coefficient of a solute in the stationary phase can be estimated from the H/u curve by fitting the experimental points with the extended Golay equation, using a diffusion coefficient of the solute in the mobile phase, calculated with the Wilke-Chang equation [10].

Experimental and theoretical H/u curves for 1,2-benzanthracene and 1,3-dihydroxybenzene on column No. 1 with methanol and water as the mobile phase are shown in Fig. 3. The reliability of the estimated diffusion coefficients depends on the mobile phase contribution to the overall plate height. In this case these contributions were equal to 10 and 60% for water and methanol, respectively, as the mobile phase. With water as the mobile phase a diffusion coefficient of $1.2 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ was estimated for 1,3-dihydroxybenzene in the SiA-EEA stationary phase, whereas with pure methanol as the mobile phase and 1,2-benzanthracene (a larger mole-

cule) as solute the estimated diffusion coefficient appeared to be 110-times larger, $1.3 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$. A separation of several hydroxybenzenes using methanol–water (5:95, v/v) as the mobile phase is shown in Fig. 4. Separation efficiency is worse compared to the SiA-HEA stationary phase (compare Fig. 1 and Fig. 4).

In a separate experiment the speed of the swelling and shrinking of the polyacrylate stationary phases was investigated. For this purpose the change in film thickness of the SiA-EEA stationary phase was determined as a function of time after changing the mobile phase composition from 90:10 (v/v) methanol–water to 50:50 (v/v) methanol–water by injecting regularly an unretained solute and calculating the internal diameter of the capillary as described in Section 2. In this case the original film (the film at the beginning of the experiment) will shrink considerably. From these measurements it appeared that the new film thickness had become stable after flushing with only one column volume. The same result was found when the 50:50 (v/v) methanol–water mixture was replaced with the 90:10 (v/v) mixture. From these findings it can be concluded that the swelling and shrinking of the stationary phase both are relatively rapid processes and allow gradient elution.

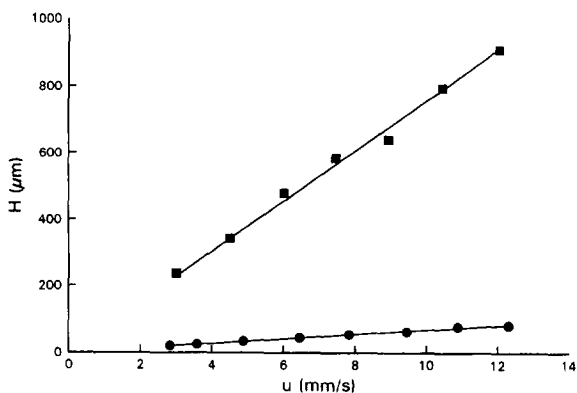


Fig. 3. Experimental and theoretical plate height curves of 1,2-benzanthracene with methanol as the mobile phase (●) and 1,3-dihydroxybenzene with water as the mobile phase (■) on capillary 1. Solid lines represent theoretical Golay curves.

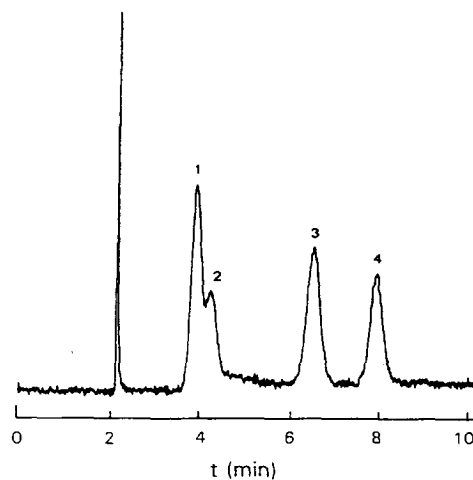


Fig. 4. Reversed-phase separation on a SiA-EEA-coated column (No. 1). Mobile phase, methanol–water 5:95 (v/v); Peaks: 1 = 1,2,3-trihydroxybenzene; 2 = 1,4-dihydroxybenzene; 3 = 1,3-dihydroxybenzene; 4 = 1,2-dihydroxybenzene.

3.3. Dual retentive character of the SiA–EEA stationary phase

The SiA–EEA phase is build up from non-polar SiA monomer and the more polar ethoxyethylacrylate and swells in non-polar as well as in polar organic solvents. For chromatography this implies that the layer can be used in the RP mode but also in the NP mode. This was investigated by measuring the retention behaviour of 1,2-dihydroxybenzene in methanol–water and hexane–isopropanol mixtures. The results are given in Fig. 5.

In the RP mode retention increases with increasing water content in the mobile phase. At the same time the film thickness of the stationary phase decreases which results in higher plate heights. In the NP mode, however, the retention and film thickness increase simultaneously with increasing hexane content in the mobile phase. The performance of the SiA–EEA in NP mode is illustrated by two separations of several hydroxybenzenes in Fig. 6. Toluene acts as dead-time marker in both separations. With a mixture of hexane–isopropanol as the mobile phase the elution order of the hydroxybenzenes is completely reversed compared to the RP mode (see Fig. 4). The separation efficiency is under these conditions comparable to the RP separation. Using a hexane–diethylether mixture as the mobile

phase the separation can be performed more efficiently, as can be seen in Fig. 6B. An example of a NP separation with hexane–isopropanol (95:5) as the mobile phase is shown in Fig. 7. Plate heights ranged from 20 to 60 μm for compounds with capacity factors of 0.2 to 2.2.

4. Conclusions

Due to the stability and the swelling properties of the polyacrylate stationary phases in various solvents, selectivity can be altered to a great extent. Depending on the polarity of the solutes either a RP or a NP chromatography mode can be chosen. For the RP mode it holds that the higher the percentage of organic solvent in the mobile phase, the larger the swelling of the polymer layer. This favours the mass transfer in the stationary phase and higher separation efficiencies can be achieved, despite the larger film thicknesses.

From the plate heights in Table 2 it may be clear that the choice of the best stationary phase depends on the applied mobile phase. For high chromatographic efficiency maximum swelling of the stationary phase should be aimed for. All stationary phases act well in NP chromatography with hexane as the mobile phase.

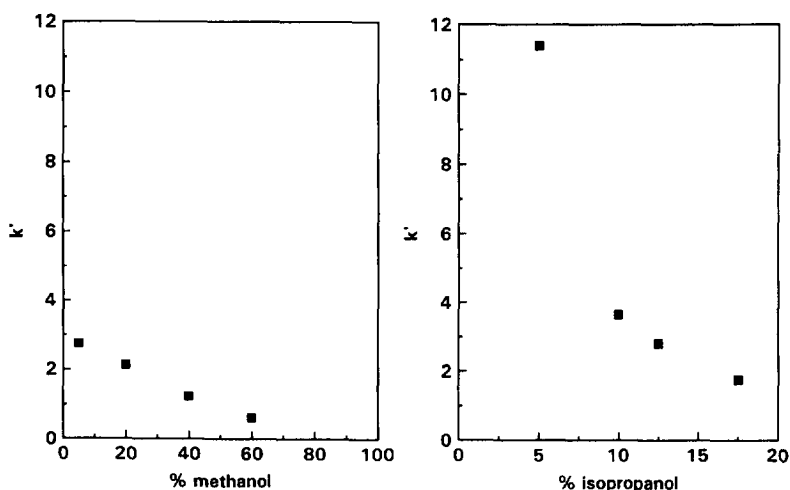


Fig. 5. Capacity factors of 1,2-dihydroxybenzene on a SiA–EEA-coated column (No. 1) under (left) reversed- and (right) normal-phase conditions.

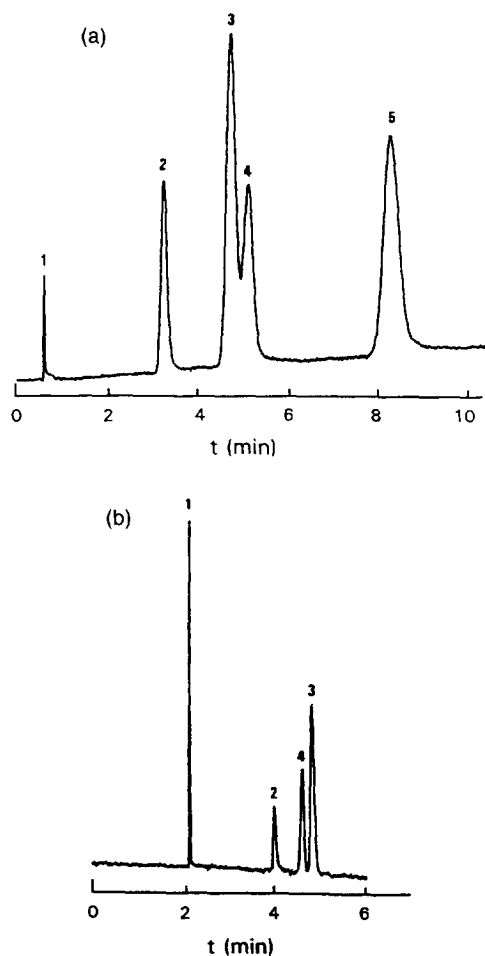


Fig. 6. Normal-phase separations on a SiA-EEA-coated column (No. 1). Column length, 98 cm; mobile phase, (a) hexane-isopropanol 87.5:12.5 (v/v); (b) hexane-diethylether 30:70 (v/v). Peaks: 1=toluene; 2=1,2-dihydroxybenzene; 3=1,3-dihydroxybenzene; 4=1,4-dihydroxybenzene; 5=1,2,3-trihydroxybenzene.

ary phase should be aimed for. All stationary phases act well in NP chromatography with hexane as the mobile phase.

For separations of non-polar compounds like polycyclic aromatic hydrocarbons in the RP mode, the SiA-EEA or the SiA-HEA stationary phases with highly organic mobile phases give the best performance. If polar compounds are to be separated the SiA-HEA stationary phase is preferred.

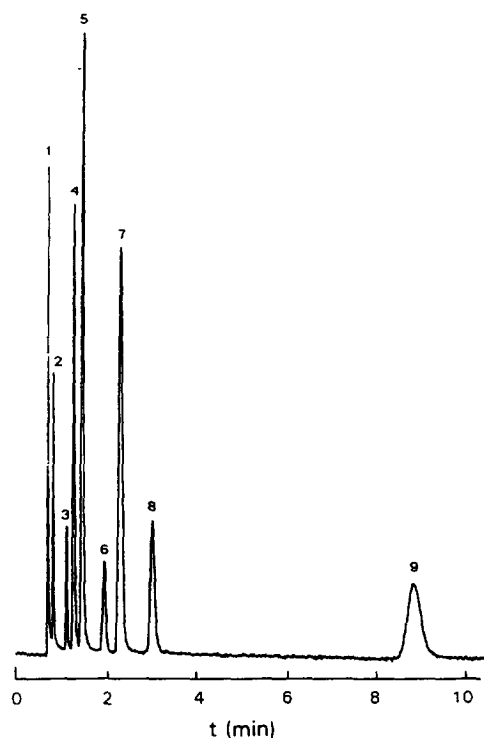


Fig. 7. Normal-phase separation on a SiA-EEA-coated column (No. 1). Mobile phase, hexane-isopropanol (95:5 v/v); peaks: 1=toluene; 2=methoxybenzene; 3=benzaldehyde; 4=nitrobenzene; 5=*o*-nitrophenol; 6=phenylpropanol; 7=3-ethylphenol; 8=phenol; 9=catechol.

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