RETENTION AND RESOLUTION IN DENSITY-PROGRAMMED SUPERCRITICAL FLUID CHROMATOGRAPHY

II. EXPERIMENTAL RESULTS

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SUMMARY

Experimental retention time data have been obtained from density-programmed supercritical fluid chromatography of dodecyl phenyl ether, using Perisorb RP-8 and Spherisorb ODS-2 as stationary phases and carbon dioxide as a mobile phase. They are compared with values calculated from a theoretical treatment of linear sample velocities during a density programme proposed recently. The numerical integration method presented earlier is confirmed for different density gradients. The effect of a density programme on the peak width, σ_L, was also investigated; under the conditions of the experiments, σ_L does not change much with density.

INTRODUCTION

In supercritical fluid chromatography (SFC), optimized separations of components differing considerably in capacity ratios can be obtained by density programming. Here the density programme influences the retention as well as the resolution.

In a previous paper[1], theoretical relationships were derived for the case where detection takes place before pressure release of the mobile phase. From this theory the retention time, t_R, of the components in a density programme can be calculated from easily obtainable parameters. This has been shown for linear density gradients and packed columns, the sample always being injected before the start of the gradient. Smith *et al.*[2], however, developed a theory for capillary columns with detection after pressure release, the relationships derived by Smith *et al.*[2] and us[1] being in accordance, as well as the methods of calculation of the retention times.

In the present work additional results are presented that were obtained in experiments using linear density gradients and packed columns. Here two stationary phases differing in particle sizes were investigated and the samples were injected at different times. From the measured baseline width and the calculated velocity of the sample during elution, the standard deviation, σ_L, of the peak in length units can be obtained. It is the aim of the present investigations to study the influence of different factors on peak compression for packed columns.

EXPERIMENTAL

Apparatus

The SFC apparatus for isobaric measurements has been described in detail elsewhere[1]. Only some modifications made for the density-programmed experiments will be described.

High pressure was created with an Altex 100 A double plunger pump that allows isobaric experiments, the upper pressure limit being adjusted by a built-in pressure feedback unit. This unit can also be operated externally by a microcomputer CBM 8032 SK. Using a program written in BASIC and an adequate equation of state for the mobile phase (here carbon dioxide)[3], optional density gradients can be produced. An 8-bit digital-analog converter was used as an interface between the computer and pump, the pressure ranging from 90 to 200 bar and the resolution being 0.43 bar. For packed columns the pressure drop is taken into account and mean values of the pressure and density are produced by the program.

The pressure was measured before and after the column by high precision strain gauges (Sensotec Super TJE). The column was mounted in an air thermostat (Perkin-Elmer LC 100). Samples were injected with a Rheodyne 7120 injector using a 20-μl loop. In contrast to the isobaric measurements, no buffer volume was used in the density-programmed experiments. Detection was effected with a Kontron Uvikon 720 LC UV/VIS spectrometer using an high-pressure flow cell. The mobile phase was expanded after the detector by a reducing valve, giving an adjustable constant mass flow-rate independent of the inlet pressure. For details see refs. 4, 6.

Substances and columns

Carbon dioxide (purity > 99.995%; Messer-Griesheim, Düsseldorf, F.R.G.) was used as a mobile phase. It was taken from the gas phase in the container and purified mechanically by a 2-μm in-line metal filter. The sample was dodecyl phenyl ether dissolved in heptane (1%). About 1 μl of the solution was injected in each experiment.

A column individually packed with Perisorb RP-8 (Merck, Darmstadt, F.R.G.; for packing procedure see ref. 5) and a commercial column packed with Spherisorb ODS-2 (Kontron-Analytik, Eching, F.R.G.) were used. The stationary phases differed considerably in specific surface area and particle size; for details see Table I.

RESULTS AND DISCUSSION

In the present work investigations were performed with several density gradients differing in steepness and different flow-rates of the carbon dioxide mobile phase.

As an example, the mean density in the column as a function of time obtained in a characteristic experiment is shown in Fig. 1. Whereas the nominal steepness was 0.0507 g cm^{-3} min^{-1}, the real experimental slope was 0.0509 g cm^{-3} min^{-1} with a delay time of 0.15 min. Thus Fig. 1 demonstrates that good results can be obtained with an 8-bit digital–analog converter.

For each gradient the sample was injected at different times before, during and after the pressure increase. A typical chromatogram is shown in Fig. 2. Here the

TABLE I

COLUMN DATA

No. of column	1	2
Length (mm)	125	256
Inner diameter (mm)	5.0	4.5
Packing	Spherisorb ODS-2	Perisorb RP-8
d_p (μm)	5	30–40
S_{BET} (m^2 g^{-1})*	110.2	7.3
Mass (g)	1.82	6.12
Void volume, V_0 (cm^3)	1.404	1.68
Porosity, ε	0.413	0.572

   * Present measurements.

detector baseline decreases with increasing density of carbon dioxide. In spite of the fact that the same amount was always injected, the peak areas differ considerably. The peaks eluted at constant density have about the same area; the peaks detected during the increase in density, however, are by far too small. This indicates that, in density-programmed SFC, normal UV detection is not applicable for quantitative analysis.

In Table II the measured retention times for dodecyl phenyl ether on Perisorb RP-8 are compared with values calculated from the known column and gradient data. According to a theory published recently[1], the linear velocity, u_i, of a sample zone was taken as

$$u_i = \frac{1}{1 + k_i'} \cdot \frac{\dot{m}_0 + [(L - z)A + V_2] \cdot d\rho/dt}{A\rho} \tag{1}$$

where k_i' = capacity ratio of sample i, $\dot{m}_0$ = mass flow of eluent out of the system, L = column length, z = position of sample zone in the column, A = free internal area of the column and V_2 = volume between the column outlet and reducing valve. The capacity ratio was calculated from
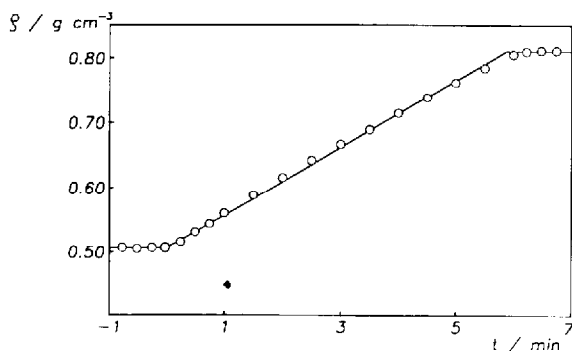
$$k_i' = a(\rho/\rho^0)^{-b} \tag{2}$$

Fig. 1. Density gradient obtained in the experiments. Mobile phase: carbon dioxide. $\bar{p}_A$ = 88.6 bar, $\bar{p}_E$ = 161.8 bar, $\overline{\Delta\rho}$ = 0.302 g cm^{-3}, dρ/dt = 0.051 g cm^{-3} min^{-1}, $\dot{V}$(NTP) = 312.1 cm^3 min^{-1}, T = 38.9°C. Stationary phase: Spherisorb ODS-2. ———, theoretical; $\bigcirc$, calculated.

TABLE II

EXPERIMENTAL AND CALCULATED RETENTION TIMES FOR DODECYL PHENYL ETHER ON PERISORB RP-8 USING CARBON DIOXIDE AS A MOBILE PHASE

$\dot{V}(NTP)$ = cm³ of CO_2 per minute at outlet (NTP). $\bar{p}_A$ and $\bar{p}_E$ = mean pressures before and after density programme respectively; ρ_A and ρ_E = mean densities of CO_2 before and after density programme respectively; t_g = duration of density programme; $\Delta\rho/t_g$ = steepness of density gradient during density programme; t_a = delay time; t_R = retention time.

Exp. No.	$\dot{V}(NTP)$ (cm³ min⁻¹)	$\bar{p}_A$ (bar)	$\bar{p}_E$ (bar)	ρ_A (g cm⁻³)	ρ_E (g cm⁻³)	$\Delta\rho/t_g$ (g cm⁻³ min⁻¹)	t_a (min)	t_R (exptl.) (min)	t_R (calc.) (min)	Deviation in %
1	484.4	85.1	162.6	0.399	0.807	0.198	9.5	4.34	4.27	-1.6
							5.06	4.34	4.27	-1.6
							3.06	3.76	3.50	-6.8
							1.55	2.57	2.33	-9.2
							0.05	1.26	1.06	-15
							-0.94	0.59	0.72	-24
							-1.61	1.00	1.05	+5.0
							-3.00	1.62	1.62	0
2	487.0	83.3	163.3	0.435	0.808	0.093	7.00	3.37	3.33	-1.2
							2.51	3.20	3.02	-5.6
							1.24	2.57	2.32	-9.6
							0.01	1.76	1.56	-11
							-1.57	1.01	1.10	+8.9
							-2.87	0.97	1.00	+3.0
							-7.5	1.67	1.70	+1.7
3	489.2	86.3	163.0	0.438	0.808	0.046	5.51	3.25	3.18	-2.2
							2.50	3.20	3.07	-4.1
							0.01	2.31	1.97	-14
							-2.01	1.58	1.37	-13
							-4.00	1.20	1.22	+1.7
							-5.96	1.22	1.23	+0.8
							-9.00	1.61	1.62	+0.6

4	490.2	86.4	162.7	0.443	0.807	0.023	10.4	4.79	4.72	−1.5
							3.71	4.78	4.70	−1.7
							0.22	3.70	3.38	−8.6
							−2.79	2.85	2.68	−6.0
							−5.79	2.16	2.13	−1.4
							−8.80	1.95	2.03	+4.1
							−11.8	2.00	2.05	+2.5
							−16.69	2.55	2.57	+0.8
5	489.2	86.3	163.0	0.438	0.808	0.046	5.51	3.25	3.18	−2.2
							2.50	3.20	3.07	−4.1
							0.01	2.31	1.97	−14
							−2.01	1.58	1.37	−13
							−4.00	1.20	1.22	+1.7
							−5.96	1.22	1.23	+0.8
							−9.00	1.61	1.62	+0.6
6	317.2	86.2	163.3	0.436	0.808	0.047	9.08	5.26	5.17	−1.7
							3.08	4.80	4.40	−8.3
							0.09	3.04	2.70	−11
							−1.93	2.07	2.02	−2.4
							−3.92	1.53	1.82	+19
							−5.90	1.68	1.78	+6.0
							−9.00	2.57	2.58	+0.4
7	124.4	87.9	167.5	0.479	0.812	0.043	16.28	10.25	10.50	+2.4
							4.26	7.47	6.78	−9.2
							0.29	4.45	4.03	−9.4
							−2.70	2.55	3.23	+26
							−4.73	2.55	3.25	+27
							−9.00	6.40	6.41	+0.2

TABLE III

EXPERIMENTAL AND CALCULATED RETENTION TIMES FOR DODECYL PHENYL ETHER ON SPHERISORB ODS-2

For symbols see Table II.

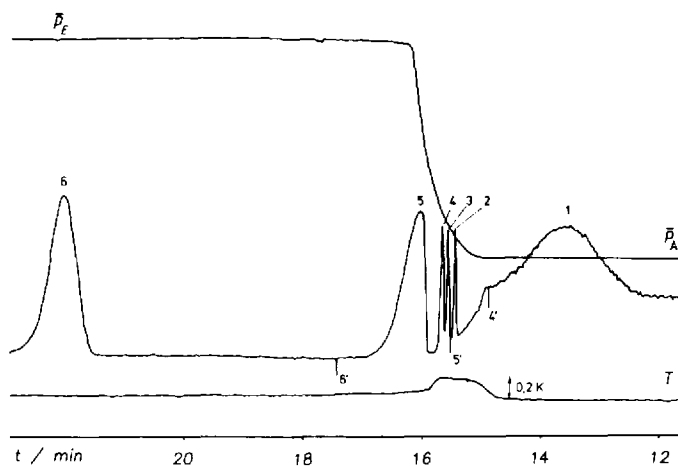| No.	$\dot{V}(NTP)$ (cm³ min⁻¹)	$\bar{p}_A$ (bar)	$\bar{p}_E$ (bar)	ρ_A (g cm⁻³)	ρ_E (g cm⁻³)	$\Delta\rho/t_g$ (g cm⁻³ min⁻¹)	t_a (min)	t_R (exptl.) (min)	t_R (calc.) (min)	Deviation in %
8	475.0	83.9	161.4	0.376	0.807	0.027	24.0	31.46	31.43	−0.1
							6.96	16.2	16.19	−0
							0.42	10.5	10.43	−0.7
							−4.37	6.60	6.60	0
							−7.84	4.72	4.88	+3.3
							−18.0	3.60	3.58	+0.6
9	477.1	85.9	161.9	0.437	0.808	0.048	36.25	26.92	26.92	−0.1
							16.19	20.24	20.21	−0.
							3.26	9.25	9.41	+1.7
							2.06	4.67	4.78	+2.4
							−5.25	3.30	3.47	+5
							−10.0	3.61	3.60	−0.3
10	475.3	85.7	161.9	0.422	0.807	0.099	35.0	25.18	25.10	−0.3
							20.19	22.04	21.93	−0.5
							8.19	11.29	11.63	+3
							−0.30	3.81	4.16	+9
							−2.31	2.85	3.18	+11
							−10.00	3.61	3.60	−0.3
11	477.8	85.1	161.2	0.407	0.806	0.204	33.78	30.36	30.13	−0.8
							20.08	21.33	21.61	+1.3
							10.05	12.05	11.41	+0.3
							1.11	3.93	4.38	+11
							−1.70	2.45	3.02	+23
							−4.00	3.61	3.60	−0.3
12	476.5	84.4	159.9	0.395	0.806	0.302	37.0	30.72	32.6	+6.1
							24.98	26.00	26.0	0
							15.31	16.36	16.9	+3.3
							3.51	7.16	6.78	−5
							−0.06	1.78	3.03	+7
							−3.00	3.69	3.68	−0.3

Fig. 2. Chromatogram for density-programmed SFC. Mobile phase: carbon dioxide. $\bar{p}_A$ = 86.3 bar, $\bar{p}_E$ = 164.6 bar, $\Delta\rho$ = 0.434 g cm^{-3}, $\dot{V}$(NTP) = 124.6 cm^3 min^{-1}, T = 38.9°C. Injection times: peak 1, 0.0 min; peak 2, 8.0 min; peak 3, 12.1 min; peak 4, 14.8 min (= 4′); peak 5, 15.7 min (= 5′); peak 6, 17.5 min (= 6′). Stationary phase: Perisorb RP-8. Sample: 1 μl dodecyl phenyl ether in hexane (1%).

where ρ^0 is a standard density of 1 g cm^{-3} and the parameters a = 0.024 and b = 5.96 for the sample on Perisorb RP-8. In order to calculate retention times, t_R, the integral

$$L = \int_0^{t_R} u_i \, dt \tag{3}$$

was solved numerically[1].

In each of the experiments in Table II the peak corresponding to the first injection left the column before the start of the density gradient, and the last injection was made after the density had reached the final value. For these isobaric experiments the calculated retention times agree very well with the times found experimentally. However, discrepancies between calculated and experimental retention times occur when the sample is injected near the start of the gradient. These deviations become larger with increasing steepness of the gradient (experiments 1–4) as well as with decreasing eluent flow-rate at the outlet (experiments 5–7). This means that the eluent velocity at the sample position after the start of the gradient differs more and more from the value before the increase in density. In the range of velocities used in this work and in a previous publication[6], for the isobaric measurements (90–1200 cm^3 min^{-1}) the capacity ratios were scattered by less than 5%. Therefore the discrepancies are probably not produced by disturbance of the partition equilibrium of the sample between the mobile and the stationary phases, respectively, but by a disturbance of the equilibrium of mobile phase velocities along the column.

In Table III our results for the stationary phase Spherisorb ODS-2 are com-

TABLE IV

COMPARISON OF BASELINE WIDTHS, B, DURING DIFFERENT DENSITY GRADIENTS

For symbols see Table II and text.

Stationary phase	$\dot{V}(NTP)$ $(cm^3\ min^{-1})$	ρ_A $(g\ cm^{-3})$	ρ_E $(g\ cm^{-3})$	$\Delta\rho/t_g$ $(g\ cm^{-3}\ min^{-1})$	t_a (min)	B (min)	u_i $(cm\ min^{-1})$	Bu_i (cm)
Spherisorb ODS-2	475	0.376	0.807	0.027	24.0	2.10	0.40	0.83
					6.96	0.51	1.68	0.86
					0.42	0.30	2.34	0.70
					−4.37	0.28	2.76	0.77
					−7.84	0.23	3.24	0.75
					−18.00	0.21	3.60	0.76
Spherisorb ODS-2	475.3	0.422	0.807	0.099	35.0	1.81	0.49	0.89
					20.19	0.40	2.28	0.91
					8.19	0.19	4.20	0.80
					0.30	0.18	4.02	0.72
					−2.31	0.20	4.02	0.80
					−10.00	0.20	3.66	0.73
Spherisorb ODS-2	476.5	0.395	0.806	0.302	37.0	2.18	0.41	0.89
					24.98	0.19	4.80	0.91
					15.31	0.14	4.14	0.58
					3.51	0.25	3.48	0.87
					−0.06	0.23	3.48	0.80
					−3.00	0.22	3.48	0.77
Spherisorb ODS-2	168.8	0.511	0.807	0.025	34.64	0.95	0.96	0.91
					25.51	0.71	1.26	0.89
					19.48	0.60	1.50	0.90
					3.50	0.50	1.80	0.90
					−2.50	0.68	1.26	0.86
					−12.75	0.78	1.26	0.98
Spherisorb ODS-2	168.6	0.509	0.810	0.100	35.12	0.40	2.44	0.98
					27.96	0.37	2.58	0.95
					19.54	0.60	1.32	0.79
					15.52	0.62	1.20	0.74
					−0.91	0.70	1.20	0.84
					−5.15	0.78	1.26	0.98

Sphrisorb ODS-2	167.7	0.537	0.810	0.258	35.0	0.30	2.94	0.88
					27.01	0.27	3.00	0.81
					20.1	0.52	1.68	0.87
					9.5	0.61	1.44	0.88
					2.0	0.65	1.28	0.83
					−3.5	0.70	1.20	0.84
Perisorb RP-8	314.8	0.491	0.804	0.240	10.0	0.75	6.5	4.88
					2.51	0.15	28.0	4.20
					0.05	0.15	32.3	4.85
					−1.02	0.40	11.0	4.40
					−10.0	0.42	11.0	4.62
Perisorb RP-8	319.0	0.456	0.790	0.148	12.1	0.91	5.3	4.82
					2.60	0.24	19.6	4.70
					0.01	0.18	23.0	4.14
					−1.53	0.43	11.2	4.82
					−2.41	0.45	11.0	4.95
Perisorb RP-8	371.9	0.370	0.789	0.046	7.45	0.57	6.8	3.88
					0.01	0.39	11.1	4.33
					−2.95	0.32	13.6	4.35
					−6.50	0.31	14.9	4.62
					−8.05	0.43	11.0	4.73
					−14.3	0.43	10.9	4.69
Perisorb RP-8	2.18.1	0.439	0.747	0.016	11.3	1.71	2.8	4.79
					4.0	0.83	5.0	4.15
					−6.0	0.56	7.6	4.26
					−11.0	0.50	8.5	4.25
					−16.0	0.53	8.8	4.66
					−20.0	0.60	7.4	4.44
Perisorb RP-8	381.9	0.424	0.745	0.015	10.0	1.01	4.7	4.75
					3.0	0.70	6.2	4.34
					0.01	0.55	8.6	4.77
					−4.0	0.43	10.7	4.60
					−8.0	0.35	12.8	4.48
					−14.0	0.33	13.3	4.39
					−23.0	0.35	13.3	4.66

piled. Here we used the parameters $a = 0.682$ and $b = 4.841$ in eqn. 2. The conclusions are the same as for Table II.

Our experiments were also designed to study the effect of a density gradient on the peak width of a sample in the column. As is seen from Fig. 2, the peaks recorded during a gradient are much narrower than those recorded at constant densities. The width of a sample zone in the column in length units, σ_L, is related to the width of the detected peak in time units, σ_t

$$\sigma_L = \sigma_t u_i \tag{4}$$

where u_i is the linear velocity of the sample at the end of the column, which was calculated according to eqn. 1.

As we were not interested in the absolute values of σ_L but rather in the relative variations, we did not determine σ_t from the chromatograms but measured the baseline widths, B, of the peaks; B is proportional to σ_t, e.g., for a Gaussian peak the baseline width is $4\sigma_t$. The results for both columns tested are given in Table IV.

If values at low and high densities are compared, σ_L does not change much with density under the conditions of the experiments. During the gradients, however, smaller values of the products are found; since the capacity ratios are rather large and the density increase is rather small, this finding is not very conclusive. Smith et al.[2], however, performed their elution experiment at very small values of k' and large density gradients; there, peak compression by compression of the mobile phase evidently played a more important rôle.

ACKNOWLEDGEMENTS

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