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RETENTION AND RESOLUTION IN DENSITY-PROGRAMMED SUPERCRITICAL FLUID CHROMATOGRAPHY

I. THEORY AND SELECTED RESULTS

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SUMMARY

A theoretical treatment of the linear velocities of the eluent and a sample band in the column is given for density-programmed supercritical fluid chromatography (SFC). It is shown that during a density programme the linear velocity of the eluent drops from column inlet to column outlet unless there is a considerable density gradient along the column. A numerical integration method for the calculation of retention times in density-programmed SFC is proposed. Peak compression effects and a reduced resolution can be expected when large decreases in mobile phase density or linear velocity occur along the column.

INTRODUCTION

In recent years there has been a growing interest in supercritical fluid chromatography (SFC) as a supercritical mobile phase may combine favourable transport properties (*e.g.*, low viscosity and high diffusivity) with considerable solvent power for substances of low volatility. The retention of substances in SFC depends strongly on pressure and temperature[1] and, in a more straightforward way, on the density of the eluent[2,3]. Therefore, the retention of different sample components can easily be adjusted by changes in pressure and/or temperature, pressure-programmed SFC thus being comparable to temperature-programmed gas chromatography (GC) and gradient elution in high-performance liquid chromatography (HPLC). For many eluents used in SFC equations of state are known, and with computer-controlled pressure programming it is also possible to create any density programme[4,5].

Much experimental work has been carried out on pressure-programmed SFC[6–9] and the advantages of density programming have been demonstrated[10]. However, few papers have treated density-programmed SFC theoretically that help to explain the experimental findings[11,12]. As density programming not only affects the capacity ratios of sample substances but also the flow of the mobile phase through the column, the prediction of retention and resolution in density-programmed SFC is more complicated than in the related techniques in GC and HPLC. A theoretical treatment

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seems worthwhile because the number of experiments that have to be performed during an optimization procedure can be reduced drastically if the dependence of retention and resolution on the applied programme and on other operating parameters is known.

In this paper, the effect of a density programme on the linear velocities of the eluent and sample substances is considered for a model system and a method for the calculation of retention times in density-programmed SFC is presented. The influences of operating parameters such as density programming rate and eluent flow-rate on the resolution are considered, and the so-called peak compression effect[8,13] is evaluated.

THEORY

Model system

A scheme of the chromatographic system to be considered here is shown in Fig. 1. A pump delivers the eluent into the system with the mass flow-rate $\dot{m}(0)$. The eluent streams in the z-direction through a column with a uniform cross-sectional area A and length L, with the z-coordinate being 0 at the column inlet and L at the column outlet. The mass flow-rates of the eluent at the column inlet and outlet are denoted by $\dot{m}(0)$ and $\dot{m}(L)$, respectively. After having passed through the column the eluent is expanded by a reducing valve RV and leaves the system with a mass flow-rate $\dot{m}_0$. The volume between the column outlet and the reducing valve is V_2.

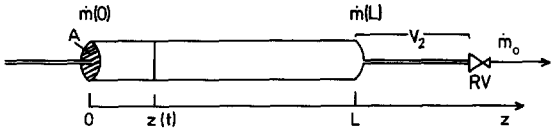


Fig. 1. Scheme of the model system.

Now, for the calculation of retention times the linear velocities of the eluent and sample in the column are of interest.

Linear velocity of the eluent

The mean linear velocity of the eluent in the z-direction is denoted by u_{mob}; in the column it is

$$u_{mob} = \frac{\dot{V}}{A} \tag{1}$$

where $\dot{V}$ is the volume flow-rate of the eluent, which can be replaced according to

$$\dot{V} = \frac{\dot{m}}{\rho} \tag{2}$$

by the mass flow-rate $\dot{m}$ and the eluent density ρ. Combination of eqns. 1 and 2 results in

$$u_{mob} = \frac{\dot{m}}{A\rho} \tag{3}$$

For the simple case when the density in the column is constant with respect to time, the mass flow into the column, $\dot{m}(0)$, is equal to the mass flow out of the column, $\dot{m}(L)$. If the density is constant throughout the column, u_{mob} does not depend on z. Especially in columns packed with small particles, however, there may be a significant pressure drop from the column inlet to the outlet, which results in a density drop in the z-direction that is greater the higher the compressibility of the eluent is. The linear velocity of the eluent then increases from the column inlet to the outlet.

In density-programmed SFC the density of the eluent is increased with time. This requires the mass flow into the system to be higher than the mass flow out of the system. The mass flow through the column then becomes a function of z, and it will now be divided into a part which does not depend on z and equals $\dot{m}_0$, and a z-dependent part $\dot{m}(z)$:

$$\dot{m} = \dot{m}_0 + \dot{m}(z) \tag{4}$$

Here $\dot{m}(z)$ is the mass flow that is necessary to increase the density in the volume V_z between z and the reducing valve RV by $d\rho$ in the time interval dt; it is given by

$$\dot{m}(z) = V_z \cdot \frac{d\rho}{dt} \tag{5}$$

The volume V_z can be written as the sum of the volume between z and the column outlet plus the volume between the column outlet and reducing valve V_2:

$$V_z = (L - z)A + V_2 \tag{6}$$

Using eqns. 4–6, eqn. 3 becomes

$$u_{mob} = \frac{\dot{m}_0 + [(L - z)A + V_2]d\rho/dt}{A\rho} \tag{7}$$

With eqn. 7 the momentary values of u_{mob} during a density programme can be calculated for any point in the column. Here A, L and V_2 are constants; $\dot{m}_0$ does not depend on z but may depend on time. For a density programme, of course, ρ depends on time, as is the case for $d\rho/dt$ if the programme is not linear. If there is a significant density drop along the column, both ρ and $d\rho/dt$ also depend on z. Several important features of eluent flow in density-programmed SFC can be deduced from eqn. 7:

(a) u_{mob} decreases linearly from the column inlet to the outlet if the density drop along the column can be neglected. With a finite density drop, however, the decrease in u_{mob} becomes less pronounced.

(b) u_{mob} is higher for a given system the more rapidly the density is increased.

(c) u_{mob} is higher for a given density programme the larger is the volume V_2.

(d) u_{mob} decreases with time for a linear density programme if $\dot{m}_0$ is held constant.

Linear velocity of the sample

The mean linear velocity of a sample i in the z-direction, denoted by u_i, is related to the linear velocity of the eluent as follows:

$$u_i = \frac{1}{1 + k_i'} \cdot u_{mob} \tag{8}$$

where k_i' is the capacity ratio of the sample, and the factor $1/(1 + k_i')$ gives the fraction of the time a sample spends in the mobile phase during its passage through the column. In SFC the capacity ratio depends strongly on the density of the mobile phase, and it is the aim of density programming to decrease the capacity ratios of the sample components one after another, because usually k' decreases with increasing density. Often a linear relationship between log k' and density is assumed[11,12]. For the supercritical region, however, we prefer the two-parameter exponential form[4,14]

$$k_i' = a(\rho/\rho^0)^{-b} \tag{9}$$

where ρ^0 is a unit density of 1 g cm^{-3}, and a and b are fitting parameters that can easily be determined from a few measurements at different densities. The parameter a gives the value of k_i' at the unit density ρ^0, and the parameter b can be related to the size ratio of the molecules of the sample and the eluent[4].

The combination of eqns. 7 and 9 with eqn. 8 yields the following expression for the linear velocity of a sample:

$$u_i = \frac{1}{1 + a(\rho/\rho^0)^{-b}} \cdot \frac{\dot{m}_0 + [(L - z)A + V_2]\mathrm{d}\rho/\mathrm{d}t}{A\rho} \tag{10}$$

Therefore, in density-programmed SFC u_i is a function of time and also of the distance z the sample has already travelled in the column. For a linear density programme and constant $\dot{m}_0$, u_i will usually increase with time because the effect of the decrease in the capacity ratio is mostly more pronounced than the effect of decreasing mobile phase velocity caused by the increased density.

Calculation of retention times

At the retention time $t_{R,i}$ a sample travelling through the column with a linear velocity u_i has reached the column outlet. The equation

$$\int_0^{t_{R,i}} u_i \, \mathrm{d}t = L \tag{11}$$

generally holds. If the linear velocity of the sample is constant with respect to time and position in the column, $t_{R,i}$ can easily be calculated from

$$t_{R,i} = \frac{L}{u_i} \tag{12}$$

This way of determining $t_{R,i}$ is no longer possible when u_i changes during the elution of the sample, as in density-programmed SFC. From eqn. 10 it becomes obvious that the exact solution of the integral in eqn. 11 for the conditions given by density programming may often not be feasible. However, a simple and universal approach for the calculation of retention times is the computer-aided numerical integration of eqn. 11. In this procedure the time is increased by small intervals Δt and the linear velocity of the sample for time t and position z is calculated. From the product of Δt and u_i the distance, Δz, the sample travels during the time interval Δt is determined. The increments of time and distance are summed, and when the sum of all increments Δz equals the column length L the retention time $t_{R,i}$ is given by the sum of all time intervals Δt. A similar calculation method has recently been proposed for the determination of retention time in gradient HPLC[15].

An important advantage of this numerical integration method is that any time and z dependence of the variables $\dot{m}_0$, ρ and $d\rho/dt$ in eqn. 10, which can be expressed mathematically, can be accounted for by the calculation. So, *e.g.*, a comparison of the effects of different density programmes on retention times can be made without additional experiments, once the fitting parameters for the density dependence of k' and the system parameters A, L and V_2 are known.

Peak compression and resolution

In a chromatogram that is taken at a constant mobile phase density and velocity the substance peaks mostly become broader the longer the retention time of a substance. If the density of the mobile phase is increased during an analysis, peaks eluted later, however, can be of the same width or even be narrower than preceding peaks.

The width of a peak on the recorder trace is characterized by σ_t, which is half the width (in time units) of a Gaussian peak at 67% of the maximum height. In density-programmed SFC, therefore, a decrease in σ_t can be observed.

It has been stated that a "peak compression effect" should play an important role in pressure- and density-programmed SFC[8,13]. This effect has been explained as follows[8]. During a density programme there is a density drop from the column inlet to the outlet leading to an increase in the capacity ratio of a sample inside the sample zone in the column from the column inlet to the outlet. Hence the parts of the sample zone that are nearest to the column inlet are moving faster in the z-direction than the parts further down the column, resulting in a compression of the sample zone in the column.

However, if there is no density drop, peak compression should also occur according to eqn. 10 because of the gradient of the eluent velocity along the column. This gradient also causes the parts of a sample zone to move faster the nearer they are to the column inlet. Generally, the peak compression should increase with increasing decrease in the sample velocity along the column.

The dimension of a sample zone in the column is characterized by σ_z, the standard deviation of the peak in length units. At the column outlet, the standard deviation in length units is σ_L. The standard deviation σ_t that can be observed on the recorder is related to σ_L by

$$\sigma_t = \frac{\sigma_L}{u_i(L,t_{R,i})} \tag{13}$$

where $u_i(L,t_{R,i})$ is the linear velocity of the sample as it is eluted from the column at the retention time $t_{R,i}$.

As it can be seen from eqn. 13, the narrowing of peaks by density programming can either be a result of a decrease in σ_L (which itself could be caused by the peak compression effect) or could be caused by an increase in $u_i(L,t_{R,i})$ for substances eluted one after another. This second factor certainly plays a very important role, and as the application of eqn. 10 allows the calculation of $u_i(L,t_{R,i})$ it is also possible to determine σ_L from experimental values of σ_t and to evaluate the extent of the compression of the sample zones in the column quantitatively. We shall report experimental work concerned with this aspect elsewhere[5].

Up to now only the factors that lead to a decrease in σ_L as a consequence of a density programme have been considered. However, increasing the density also can result in an increase in σ_L. The standard deviation in length units is closely related to the plate height, H, which is a measure of the efficiency of a column, by

$$H \equiv \frac{\sigma_L^2}{L} \tag{14}$$

From theory it follows that H increases with increasing viscosity of the mobile phase[16]; in addition, in the region of higher linear velocities of the eluent, H increases with increasing u_{mob}. Because of the favourable transport properties of supercritical mobile phases, this increase in H is less pronounced in SFC than in HPLC, especially when columns packed with small particles[17] or small-diameter open-tubular capillary columns[18] are used. To compensate for the decrease in efficiency caused by an increased eluent viscosity, it is advisable to decrease the linear velocity of the eluent during the density programme. This can easily be done be holding constant the mass flow of eluent that leaves the system[16].

In chromatographic practice, it is not the width of single substance peaks that is of primary interest, but the resolution of several peaks. For one pair of peaks the resolution R is given by

$$R = \frac{t_{R,2} - t_{R,1}}{1/2(t_{B,1} + t_{B,2})} \tag{15}$$

where $t_{R,1}$ and $t_{R,2}$ are the retention times of the substances 1 and 2, respectively (with $t_{R,2} > t_{R,1}$), and $t_{B,1}$ and $t_{B,2}$ are the baseline widths of the peaks. The baseline width t_B is given by the baseline distance of the tangents at the points of inflection of the peak; for a Gaussian peak, t_B is equal to $4\sigma_t$.

Here, the influence on the resolution of the factors that lead to peak com-

pression will be considered qualitatively. As has been stated above, the peak compression effect is more pronounced the higher are the gradients of ρ and u_{mob} along the column. However, the same mechanisms that cause a single sample zone to become narrower also result in a greater increase in the velocity of a whole sample zone that is nearer to the column inlet than a sample zone farther down in the column. Therefore, the distance between the sample zones and hence the selectivity of the column are decreased by high density and eluent velocity gradients. Usually, the distance between two sample zones will be larger than each sample zone width. Therefore, the effect of the decrease in distance (that is, in selectivity) will be more pronounced than the peak compression effect, which leads to increased efficiency, the net result being a decrease in resolution.

To achieve optimal resolution in density-programmed SFC, both the relative density drop along the column and the relative decrease in u_{mob} should be kept small. A small density drop is favoured by low eluent flow-rates and the use of open-tubular capillary columns or columns packed with larger particles. Large particles, on the other hand, yield columns with low efficiencies. Hence an optimal value exists for the particle size in packed columns, as has been reported for one application[8].

From eqn. 7, it can be deduced that the relative decrease in the eluent velocity becomes smaller with a decreased density-programming rate at a constant mass flow out of the system or with an increased mass flow $\dot{m}_0$ at constant $d\rho/dt$. Also for $\dot{m}_0$ there may be an optimal value above which the resolution decreases again because of the increase in the density drop connected with higher flow-rates. Hence it results from theoretical considerations that high eluent flow-rates (without a considerable density drop) and a slow increase in the density should lead to improved resolution. These relationships have also been found experimentally; Klesper and Hartmann[9] reported that the resolution of styrene oligomers in pressure-programmed SFC was the better the more slowly the pressure (and thereby the density) was increased. They used relatively high pressure drops along the column and observed an increased resolution with decrease in the eluent flow-rate.

Graham and Rogers[8] used columns packed with larger particles, where the pressure drop along the column was not much affected by the eluent flow-rate. Under these conditions, the resolution was increased by lowering the ratio of the pressure programming rate to the eluent flow-rate. They proposed that this increase in resolution might be caused in part by the peak compression effect. From the theoretical approach given here, it seems more reasonable to assume that the resolution was increased because of the decrease in the relative drop in u_{mob} along the column, which is connected with a decrease in $d\rho/dt$ and/or an increase in $\dot{m}_0$.

EXPERIMENTAL

Substances

In order to determine the accuracy of the retention times calculated with the numerical integration method, experiments with test mixtures were carried out. The test substances were dodecyl phenyl ether, tetradecyl phenyl ether, hexadecyl phenyl ether, octadecyl phenyl ether, phenyl myristate, phenyl palmitate and phenyl stearate, dissolved in heptane. The volume of solution injected was 1 μl, containing about $3 \cdot 10^{-9}$ mol of each test substance.

The mobile phase used was supercritical carbon dioxide. The column (12.5 cm × 5 mm I.D.) was packed with Spherisorb ODS 2 (Phase Separations) with a particle diameter of 5 μm.

Apparatus

The fluid chromatograph used has been described in detail elsewhere[5,19]. It consists of commercial HPLC equipment and some laboratory-made parts and is suitable for operation at temperatures up to 100°C and pressures up to 200 bar.

The mobile phase was delivered by a double-plunger pump, the heads of which were cooled to 0°C. By means of a built-in pressure feedback unit the pump could be used as a manostat. In order to make density programming possible, it was coupled with a CBM 8032 SK microcomputer[4,5]. A conditioning system, the injector and the column were placed in an air thermostat. A high-pressure UV detector with a thermostated flow cell was used. The mobile phase was expanded by a reducing valve after the detector, giving an adjustable constant flow-rate (±1%) that was determined with a soap-bubble flow meter.

The mobile phase pressure was measured before and after the column with two strain gauges. The temperature was determined with a thermocouple mounted in the eluent stream before the injector.

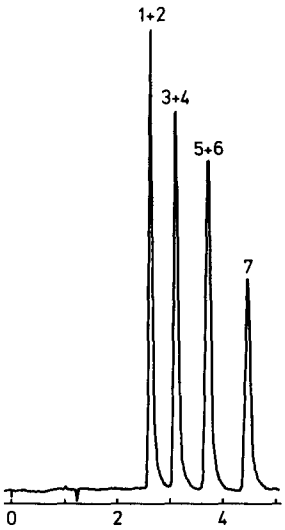
RESULTS AND DISCUSSION

Retention data were first measured at different constant pressures and temperatures in order to determine the density dependence of the capacity ratios of the test substances. Some selected data are presented below.

Fig. 2 shows a chromatogram of the test mixture obtained at a relatively high mobile phase density. Here some of the seven ethers and esters under test exhibit the same retention times and thus only four peaks can be discriminated.

From the chromatogram shown in Fig. 3 the effect of a decrease in pressure at constant temperature becomes obvious. With the same mobile phase flow-rate the retention times of all substances are much higher than in Fig. 2, showing the increase in the capacity ratios with decreasing eluent density. By means of the decrease in pressure, the retention times of the esters are increased more than those of the ethers, being co-eluted at higher pressures; as a result, all components of the test mixture are well separated at the lower density. We shall report our systematic measurements on the density dependence of the capacity ratios in detail elsewhere[14]. The results of the experiments are given in Table I, where the fitting parameters according to eqn. 9 are listed for all test substances at two different temperatures.

It can also be deduced from Fig. 3 that for each homologous series the distances between the peaks and the peak widths increase the longer the retention times become. Hence the analysis takes relatively longer. The analysis time can be decreased, however, by density programming. Fig. 4 shows the effect of a linear density programme on the separation of the test substances. After the injection of the sample the eluent density was first held constant for a delay time t_a of 3.6 min; during t_a the density was low enough to allow the separation of dodecyl phenyl ether and phenyl myristate. The density was then increased linearly at 0.043 g cm^{-3} min^{-1}. All test substances were eluted during the density programme, the peaks being well separated and having almost constant widths.



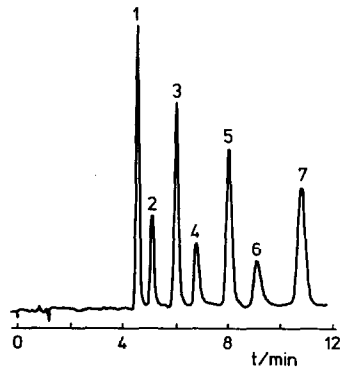


Fig. 2. SFC trace of the test mixture at high constant density of the eluent. Mobile phase, CO_2; $\bar{\rho}$ = 181 bar; T = 39.5°C; $\bar{\rho}$ = 0.82 g cm^{-3}; $\dot{m}_0$ = 1.25 g min^{-1}. Stationary phase, Spherisorb ODS 2. Substances: 1 = dodecyl phenyl ether; 2 = phenyl myristate; 3 = tetradecyl phenyl ether; 4 = phenyl palmitate; 5 = hexadecyl phenyl ether; 6 = phenyl stearate; 7 = octadecyl phenyl ether.

Fig. 3. SFC trace of the test mixture at low constant density of the eluent. Mobile phase, CO_2; $\bar{\rho}$ = 108 bar; T = 39.5°C; $\bar{\rho}$ = 0.68 g cm^{-3}; $\dot{m}_0$ = 1.25 g cm^{-3}. Stationary phase and substances as in Fig. 2.

The retention times of the test substances under various density programming conditions were calculated using the numerical integration method. For all density programmes the increase in mobile phase density at the column inlet and the outlet, respectively, within the time of the density increase was determined experimentally from the measured values of pressure and temperature. For the calculation of the densities, pVT data for carbon dioxide taken from the literature[20] were used.

For these calculations it was assumed that during the time before the density programme the eluent density decreased linearly from the value at the column inlet,

TABLE I

FITTING PARAMETERS FOR THE DENSITY DEPENDENCE OF THE CAPACITY RATIOS k' ACCORDING TO EQN. 9

Substance	*T = 39.5°C*		*T = 54.9°C*	
	a	*b*	*a*	*b*
Dodecyl phenyl ether	0.60	5.36	0.38	5.57
Tetradecyl phenyl ether	0.72	5.74	0.44	5.97
Hexadecyl phenyl ether	0.87	6.13	0.49	6.40
Octadecyl phenyl ether	1.02	6.59	0.55	6.84
Phenyl myristate	0.55	6.02	0.32	6.21
Phenyl palmitate	0.66	6.41	0.37	6.64
Phenyl stearate	0.79	6.78	0.42	7.08

TABLE II

MEASURED AND CALCULATED RETENTION TIMES FOR DIFFERENT DENSITY PROGRAMMES

No.	*T (°C)*	ρ_A *(0)* ($g\ cm^{-3}$)	ρ_A *(L)* ($g\ cm^{-3}$)	ρ_E *(0)* ($g\ cm^{-3}$)	ρ_E *(L)* ($g\ cm^{-3}$)	$\dot{m}_0$ ($g\ min^{-1}$)	t_a *(min)*	t_g *(min)*	*Substance**	t_R *(exp.)* *(min)*	t_R *(calc.)* *(min)*
1	55	0.535	0.519	0.697	0.693	0.58	0.1	14.9	C_{12}	10.53	10.60
									C_{14}	12.91	13.00
									C_{16}	15.42	15.53
									C_{18}	18.40	18.66
2	55	0.540	0.523	0.697	0.692	0.59	0.2	5.8	C_{12}	7.64	7.67
									C_{14}	9.37	9.53
									C_{16}	11.52	11.83
									C_{18}	14.23	14.73
3	55	0.539	0.524	0.697	0.692	0.60	0.2	2.8	C_{12}	6.56	6.62
									C_{14}	8.19	8.37
									C_{16}	10.24	10.53
									C_{18}	12.84	13.33
4	39.6	0.676	0.649	0.838	0.832	1.28	3.6	3.8	C_{12}	4.48	4.48
									M	4.89	4.93
									C_{14}	5.40	5.42
									P	5.81	5.85
									C_{16}	6.32	6.37
									S	6.70	6.77
									C_{18}	7.23	7.28
5	39.6	0.638	0.589	0.838	0.832	1.30	4.2	1.5	C_{12}	4.90	5.07
									M	5.18	5.38
									C_{14}	5.43	5.63
									P	5.68	5.95
									C_{16}	6.11	6.37
									S	6.42	6.72
									C_{18}	6.97	7.25

* C_{12} = dodecyl phenyl ether; C_{14} = tetradecyl phenyl ether; C_{16} = hexadecyl phenyl ether; C_{18} = octadecyl phenyl ether; M = phenyl myristate; P = phenyl palmitate; S = phenyl stearate.

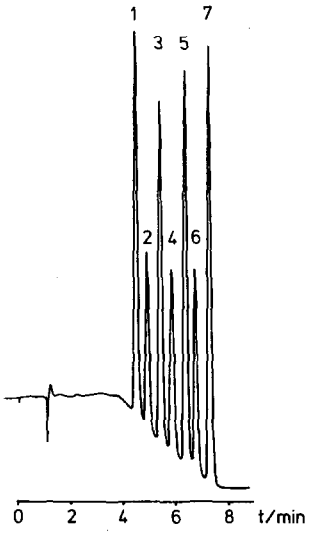


Fig. 4. SFC trace of the test mixture with a linear density programme. Mobile phase, CO_2; T = 39.6°C; $\dot{m}_0$ = 1.29 g cm^{-3}. Gradient conditions: t_a = 3.6 min; $\bar{p}$ = 104.3 bar; $\bar{\rho}_A$ = 0.63 g cm^{-3}; t_g = 3.8 min; $d\rho/dt$ = 0.043 g cm^{-3} min^{-1}. Stationary phase and substances as in Fig. 2.

$\rho_A(0)$, to the value at the column outlet, $\rho_A(L)$. As only linear density programmes have been considered, the density programming rate $d\rho/dt$ was constant; it was calculated according to

$$\frac{d\rho}{dt} = \frac{\rho_E(L) - \rho_A(z)}{t_g} \tag{16}$$

where $\rho_E(L)$ is the eluent density at the column outlet after the programme, $\rho_A(z)$ is the density at position z in the column where the centre of the sample zone is located when the density programme begins, and t_g denotes the duration of the density programme. For the time after the start of the density programme the density drop along the column was no longer taken into account, and the eluent density at the location of the sample zone was calculated from

$$\rho(t,z) = \rho_A(z) + t\frac{d\rho}{dt} \tag{17}$$

where t is the time since the start of the gradient and $d\rho/dt$ is given by eqn. 16. In our experimental arrangement, the other parameters needed for the calculation of retention times were L = 12.5 cm, A = 0.112 cm^2 and V_2 = 4.7 cm^3.

In Table II the results of the calculations are compared with the retention times found experimentally. The mean relative deviation between the calculated and the measured values of $t_{R,i}$ is only 2%. The calculation method is equally well suited to slow and fast density programmes. We shall report on further experiments with other packed columns and different mobile phase flow-rates elsewhere[5].

Finally, the effects of the density programming rate, the delay time before the

gradient start and the mobile phase flow-rate on resolution were considered quantitatively. For two model substances 1 and 2 the retention times were calculated using the numerical integration method described above. The density dependence of the capacity ratios of these model substances is characterized by the following set of fitting parameters according to eqn. 9: $a_1 = 0.6$, $a_2 = 0.7$, $b_1 = 5$ and $b_2 = 5.5$. For the parameters L, A and V_2 the values given above that resulted from our experimental arrangement were used. The eluent density after the density programme was assumed to be $\rho_E(L) = 0.8$ g cm^{-3}.

In order to calculate values for the resolution according to eqn. 15, the baseline widths of the substance peaks also had to be determined. This was done by using the equation

$$t_B = \frac{4\sqrt{HL}}{u_i(L,t_{R,i})} \tag{18}$$

For the plate height, a constant value of $H = 15$ μm was used. Thus, no effects of the density programming on σ_L were taken into account, an approximation that is supported for packed columns by our experiments[5]. The linear velocities $u_i(L,t_{R,i})$ needed in eqn. 18 were available from the numerical integration procedure.

Table III gives values of the resolution calculated for different delay times t_a and different density programming rates. The resolution is always much higher than would be necessary in practice, but for the intended comparison of the effect of different operating parameters this is of little importance. It can be seen from Table III that for a constant value of t_a the resolution decreases with decreasing t_g, that is, with a faster increase in the density. For a constant t_g the resolution increases with increasing t_a because the sample substances then spend more time in the column when the eluent density is low and therefore the selectivity for the separation of homologues is high. The well known feature of increasing selectivity with decreasing

TABLE III

EFFECT OF DIFFERENT DENSITY PROGRAMMES ON RESOLUTION

$\dot{m}_0$ = g min^{-1}; $\rho_A(0) = 0.4$ g cm^{-3}; $\rho_A(L) = 0.35$ g cm^{-3}; $\rho_E = 0.8$ g cm^{-3}.

t_a (min)	t_g (min)	$t_{R,1}$ (min)	$t_{B,1}$ (min)	$t_{R,2}$ (min)	$t_{B,2}$ (min)	R
0	10	7.63	0.170	8.97	0.173	7.82
0	5	4.97	0.107	5.93	0.173	6.86
0	1	2.80	0.144	3.60	0.173	5.05
10	10	16.70	0.203	18.43	0.190	8.80
10	5	14.33	0.126	15.40	0.173	7.16
10	1	11.96	0.144	13.03	0.173	6.75
20	10	25.63	0.259	27.90	0.212	9.64
20	5	23.63	0.158	24.93	0.130	8.72
20	1	21.26	0.144	22.53	0.173	8.01
Conditions as before the density programme		44.2	2.33	88.9	4.67	11.34
Conditions as after the density programme		3.23	0.144	3.87	0.173	4.04

TABLE IV

EFFECT OF ELUENT FLOW-RATE ON RESOLUTION

t_a = 10 min; t_g = 5 min; $\rho_E(L)$ = 0.8 g cm^{-3}.

$\dot{m}_0$ (g min^{-1})	$\rho_A(0)$ (g cm^{-3})	$\rho_A(L)$ (g cm^{-3})	$t_{R,2}$ (min)	R	$(R/t_{R,2})$ (min^{-1})
0.2	0.40	0.39	27.13	5.62	0.21
1	0.40	0.35	15.40	7.16	0.46
2	0.40	0.30	14.06	8.70	0.62

eluent density in SFC also can be recognized from the results given at the bottom of Table III. There the resolutions were calculated for the low density before the programme and the high density after the programme, the densities being constant with respect to time.

Table IV shows the effect of a change in the eluent flow-rate, $\dot{m}_0$, on the resolution calculated according to eqn. 15. Although it was taken into account that the density drop along the column increases strongly with increasing $\dot{m}_0$ for columns packed with small particles, the calculated resolution still increases with increasing eluent flow-rate. Further, the ratio of the resolution to the analysis time, $R/t_{R,2}$, also increases strongly with increasing $\dot{m}_0$.

CONCLUSIONS

From a simple model, equations for the linear velocities of the eluent and sample zones during a density programme in SFC have been derived that are well suited to the determination of retention times by a numerical integration method. The calculated results also agree well qualitatively with the experimental findings of other workers. As the parameters that are necessary for the calculations are readily available from few measurements, it is feasible to apply the calculation method proposed in this paper in mathematical optimization procedures for density-programmed SFC.

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