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EFFECTS OF DENSITY AND TEMPERATURE ON EFFICIENCY IN CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY

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SUMMARY

Mobile phase diffusion coefficients found in the literature were used to predict the effect of density on efficiency in capillary supercritical fluid chromatography for several solutes over a typical density programming range. The effect of temperature on efficiency was similarly predicted for naphthalene over a wide temperature range at constant density. Efficiency data for the normal hydrocarbons (C_{10}, C_{24} and C_{36}) at 50 and 100°C with constant capacity ratios were obtained. Linear velocity changes during density programming significantly affected the chromatographic efficiency. Results verified that efficiency was increased by using low densities and high temperatures at constant linear velocity. Based on these results, density programming at elevated temperatures, and simultaneous density–temperature programming modes were investigated.

INTRODUCTION

Capillary supercritical fluid chromatography (SFC) is becoming an important and useful technique for the analysis of non-volatile[1–5] and thermally labile[6–10] compounds. Using small diameter columns (*e.g.*, 50 μm I.D.), efficiencies on the order of 4500 plates m^{-1} (at $k = 3$ and at a linear velocity of 2 cm s^{-1}) can be achieved[11]. Increasing the stationary phase film thickness up to 1.0 μm on a 50-μm I.D. column does not reduce the efficiency significantly[12]. These efficiency data were obtained at 40°C and 72 atm (0.22 g ml^{-1}) with a carbon dioxide mobile phase.

It was of interest to determine the effect of higher density on capillary SFC efficiency since frequently used density programming methods increase the density to around 0.8 g ml^{-1}. Randall[13] predicted that the efficiency for pyrene at 0.8 g ml^{-1} and 40°C with a 50-μm I.D. column would be about 2400 plates m^{-1} with a linear velocity of 2 cm s^{-1}.

In this study, mobile phase diffusion coefficients found in the literature were used to predict efficiencies for benzene, naphthalene, and caffeine at 0.28–0.79 g ml^{-1} (40°C), and also for naphthalene at 60–120°C (0.6 g ml^{-1}), in carbon dioxide with a 50-μm I.D. column. The normal hydrocarbons (C_{10}, C_{24}, and C_{36}) were used to measure the efficiency of a 50-μm I.D. column, coated with SE-54, over density ranges representing typical density programs for 50 and 100°C with a carbon dioxide mobile

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phase. The effect of density and temperature on the mobile phase linear velocity ($\bar{u}$), and the effect of changes in $\bar{u}$ during programmed runs on efficiency were investigated. Based on the results of these studies, density programming at various temperatures, and simultaneous density–temperature programming modes were investigated.

EXPERIMENTAL

Theoretical plate height values (h) were calculated from a modified form of the Golay equation[14], with the contribution to plate height from the stationary phase disregarded:

$$h = \frac{2D_m}{\bar{u}} + \frac{(1 + 6k + 11k^2)\, d_c^2\, \bar{u}}{96\,(1 + k)^2\, D_m} \tag{1}$$

where h is in cm, D_m is the mobile phase diffusion coefficient (cm^2 s^{-1}), $\bar{u}$ is the average mobile phase linear velocity (cm s^{-1}), d_c is the internal column diameter (cm), and k is the capacity ratio.

Fused-silica capillary tubing (28 m × 50 μm I.D.) (Hewlett-Packard, Avondale, PA) was coated with SE-54 (0.25 μm film thickness) without prior surface modification or deactivation, and was used for evaluation of the programming modes. The SE-54 was dissolved in *n*-pentane, statically coated[15], and crosslinked with azo-*tert.*-butane[16]. The column was conditioned using supercritical carbon dioxide at high density (0.8 g ml^{-1}) and temperature (150°C). A 16.5-m length of the same tubing was coated with SE-54 (0.25 μm d_f) after deactivation with polymethylhydrosiloxane[17] and crosslinking and conditioning as above for use in the hydrocarbon efficiency measurements. An uncoated, untreated 4-m length of the same 50-μm I.D. tubing was used to measure the linear velocities at different temperatures and densities.

The chromatographic system consisted of an ISCO syringe pump (Model 314), modified for pressure control; a Hewlett-Packard 5710A gas chromatograph oven, modified for computer-based temperature control and equipped with a conventional flame ionization detector; an Apple II+ microcomputer; and a Houston Instruments strip chart recorder. SFC-grade carbon dioxide (Scott Specialty Gases, Plumsteadville, PA, U.S.A.) was filtered and distilled before use. The injection system was a 0.2-μl internal volume injector (Valco, Houston, TX, U.S.A.) used in conjunction with a splitter, designed for SFC (Scientific Glass Engineering, Austin, TX, U.S.A.)[8]. The pressure restriction system was either a short piece of 5-μm I.D. fused silica, or a laboratory-made tapered restrictor[2] connected to the analytical column with a zero dead volume butt connector (Scientific Glass Engineering).

RESULTS AND DISCUSSION

The scarcity of diffusion coefficient data in the literature prohibits a thorough estimation of efficiency over the range of operating conditions feasible in capillary SFC. However, there are sufficient data to allow the study of the magnitude of efficiency changes during density or pressure programming.

It was desirable to normalize on the same k value in the theoretical predictions of the effects of density on efficiency. To estimate the k of solutes in a programmed run, the density was increased for isoconfertic chromatograms of a solute until the isoconfertic peak width closely matched that of a programmed run. These data indicated that density or pressure programming in capillary SFC usually results in a capacity ratio (k) of about 2 for most solutes throughout the program. Therefore, a capacity ratio of 2 was assumed in all theoretical calculations of the effects of density in this study.

The equation for the optimum mobile phase linear velocity ($\bar{u}_{opt}$), under these conditions (50 μm I.D., $k = 2$), becomes

$$\bar{u}_{opt} = 1100 \times D_m \tag{2}$$

Changes in D_m would affect $\bar{u}_{opt}$; but, under these conditions, the minimum plate height (h_{min}) would be constant at 0.036 mm. For $k = 1$, h_{min} would be 0.031 mm, and for $k = 5$, h_{min} would be 0.042 mm.

It has been noted[19] that in the interest of producing good efficiency in a reasonable time, linear velocities of 10 $\bar{u}_{opt}$ are most practical in capillary SFC. For the 50-μm I.D. columns assumed here, 10 $\bar{u}_{opt}$ is about 2 cm s^{-1} at 0.22 g ml^{-1} (a typical initial density in programmed runs). At 5–10 $\bar{u}_{opt}$, where longitudinal diffusion is negligible, eqn. 1 shows that a decrease in D_m would cause an increase in plate height.

Mobile phase diffusion coefficient values (D_m) were taken from data of Feist and Schneider[20] for benzene, naphthalene, and caffeine in carbon dioxide at 40°C. The D_m values used, and the corresponding densities are listed in Table I.

A comparison of efficiencies at different densities based on theoretically predicted values requires taking two factors into account. The first factor is the effect of density alone; as the density increases, the D_m of a solute decreases and the plate height increases. At the higher density, a more retained solute is needed to maintain the same k value as at the lower density. Hence, the second factor is the effect of lower solute diffusivity for more retained solutes eluted at high densities, thus increasing the plate height. The consideration of both of these factors in the calculation of efficiency at different densities would represent the real chromatographic situation in a density program where less retained solutes are eluted at lower densities and more retained solutes are eluted at higher densities, both types of solutes having about the same capacity ratio. In the following discussion, the effects of increasing density and decreasing D_m are considered separately and then combined. Studying

TABLE I

DIFFUSION COEFFICIENT VALUES AT VARIOUS DENSITIES

Data are taken from Feist and Schneider[20].

Solute	*D_m (cm^2 s^{-1} × 10^4)*		
	0.28 g ml^{-1}	*0.45 g ml^{-1}*	*0.79 g ml^{-1}*
Benzene	3.3	2.7	1.5
Naphthalene	2.5	2.0	1.1
Caffeine	1.7	1.4	0.8

either factor separately does not represent a real situation, but does provide a basis to understand the effects of the combined factors.

The effect of density on efficiency was studied using D_m values for naphthalene at 0.28, 0.45, and 0.79 g ml^{-1}. Calculated efficiencies at these densities and for $\bar{u}$ = 2 and 1 cm s^{-1} are listed in Table II. These data indicate that over half the efficiency is lost, at either linear velocity, due to the increase in density from 0.28 to 0.79 g ml^{-1}. However, at 1 cm s^{-1} the efficiency at 0.79 g ml^{-1} is still very good (6600 plates m^{-1}).

TABLE II

CALCULATED EFFECT OF DENSITY ON EFFICIENCY FOR NAPHTHALENE

$\bar{u}$ (cm s^{-1})	Density (g ml^{-1})	h (mm)	Multiple of h_{min}	Plates m^{-1}
2	0.28	0.134	3.7	7500
	0.45	0.167	4.6	6000
	0.79	0.301	8.4	3300
1	0.28	0.071	2.0	14 000
	0.45	0.086	2.4	11 600
	0.79	0.152	4.2	6600

The effect of more retained solutes on efficiency was studied using D_m values for benzene and caffeine at 0.79 g ml^{-1} (the D_m of pyrene was measured by Randall[13] as $8 \cdot 10^{-5}$ cm^2 s^{-1} at 0.8 g ml^{-1}). Calculated efficiencies are listed in Table III. This density represents a final density frequently attained in carbon dioxide capillary SFC. The effect of more retained solutes is seen in the 50% reduction in plates m^{-1} for caffeine compared to benzene at either linear velocity. The efficiency for benzene is still good, but the 50% reduction in efficiency lowers the plates m^{-1} to 2400 at 2 cm s^{-1} for caffeine.

TABLE III

CALCULATED EFFECT OF MORE-RETAINED SOLUTES ON EFFICIENCY AT HIGH DENSITY

$\bar{u}$ (cm s^{-1})	Density (g ml^{-1})	Solute	h (mm)	Multiple of h_{min}	Plates m^{-1}
2	0.79	Benzene	0.221	6.1	4500
		Caffeine	0.413	11.5	2400
1	0.79	Benzene	0.113	3.1	8800
		Caffeine	0.208	5.8	4800

The combination of the two factors, an increase in density and a decrease in D_m resulting from more retained solutes, is shown by calculated efficiencies for benzene at 0.28 g ml^{-1}, naphthalene at 0.45 g ml^{-1}, and caffeine at 0.79 g ml^{-1} (Table IV). These data are representative of a real chromatographic situation found under den-

TABLE IV

CALCULATED EFFECT OF DENSITY AND MORE-RETAINED SOLUTES ON EFFICIENCY

$\bar{u}$ (cm s^{-1})	*Density (g ml^{-1})*	*Solute*	*h (mm)*	*Multiple of h_{min}*	*Plates m^{-1}*
2	0.28	Benzene	0.103	2.9	9700
	0.45	Naphthalene	0.167	4.6	6000
	0.79	Caffeine	0.413	11.5	2400
1	0.28	Benzene	0.057	1.6	17 500
	0.45	Naphthalene	0.086	2.4	11 600
	0.79	Caffeine	0.208	5.8	4800

sity or pressure programming in capillary SFC with a carbon dioxide mobile phase. With a linear velocity of 2 cm s^{-1}, the efficiencies during a density programmed run would decrease by nearly 75% from 9700 to 2400 plates m^{-1}. A similar relative decrease would be found at a linear velocity of 1 cm s^{-1}, but the efficiency would decrease to only 4800 plates m^{-1} at the higher density.

We have assumed a *k* value of 2 in the discussion above, and used published D_m values. A plot of plates m^{-1} *vs.* *k* for a wide range of D_m values (Fig. 1) shows what the efficiencies would be for solutes with very low diffusivities. A solute with a D_m value of $5 \cdot 10^{-5}$ cm^2 s^{-1} would be eluted with about 1000 plates m^{-1} for *k* greater than 2; if the D_m were $3 \cdot 10^{-5}$ cm^2 s^{-1}, the efficiency would drop to about 700–800 plates m^{-1}. From these theoretical predictions, it is evident that significant losses in efficiency can occur at high density in capillary SFC.

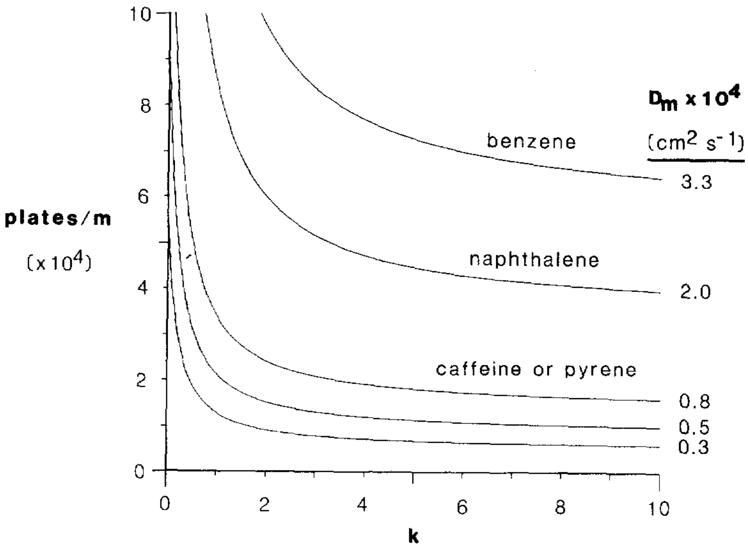

Fig. 1. Efficiency *vs.* retention in capillary supercritical fluid chromatography for a wide range of diffusivities. Conditions: $\bar{u}$ = 2 cm s^{-1}, 50-μm I.D. column, plates m^{-1}, calculated from eqn. 1.

A possible solution to mitigate the loss of efficiency at higher densities is to increase the operating temperature. At constant density, an increase in temperature results in two positive effects in SFC analysis: first, an increase in mobile phase diffusion coefficients[20], and second, an increase in volatility with a corresponding decrease in retention (k), thus increasing efficiency. Recent results[1,2,7,8] have shown that higher mobile phase temperatures (greater than 40°C) can be very useful in carbon dioxide SFC. The use of temperatures up to 100°C would still be feasible in the analysis of some thermally labile compounds[7,8]. Even higher temperatures (*ca.* 150°C) allow for more efficient elution of high-molecular-weight and polar solutes[2].

The effect of temperature on efficiency at constant density was studied using D_m values found in the literature[20] for naphthalene at a density of 0.6 g ml^{-1}. The temperature range was 60 to 120°C, and a change in k from 1.9 to 1 over this temperature range was assumed to make the predictions more realistic. Calculated efficiency data are listed in Table V. From these data, it is evident that an increase in temperature can significantly increase the chromatographic efficiency of solutes.

TABLE V

CALCULATED EFFECT OF TEMPERATURE ON EFFICIENCY AT CONSTANT DENSITY

Efficiency data are at 2 cm s^{-1}

Temp (°C)	*D_m (cm^2 s^{-1} × 10^4)*	*k*	*h (mm)*	*Plates m^{-1}*
60	1.85	1.9	0.174	5700
90	2.19	1.45	0.130	7700
120	2.58	1.0	0.091	11 000

The linear velocity changes during density programming were not incorporated into the predictions above. In the operating range of capillary SFC, increases in the linear velocity will decrease efficiency. Changes in the linear velocity were studied using constant restrictor conditions while the column temperature and density were varied (Fig. 2). The density ranges corresponded to a pressure range of 72 to 200 atm. These conditions resulted in different $\bar{u}_{\text{init}}$ values for different temperatures. Higher operating temperatures resulted in more rapid increases in $\bar{u}$ with density. These losses in efficiency due to the increases in $\bar{u}$ can be compensated for, to some degree, by starting at a slower initial linear velocity. Efficiency losses are still present, but the analysis begins with more potential separating power. Naturally, the trade-off in starting at slower initial velocities is lengthening of analysis times.

The dependence of $\bar{u}$ on density or pressure varies with the type of mobile phase used. Carbon dioxide exhibits an increase in $\bar{u}$ with pressure, whereas a decrease in $\bar{u}$ with increasing pressure has been observed[21] for n-pentane as a mobile phase. The different behaviour is a consequence of the competing forces of decreased mass flow from higher density and increased pressure drop across the column pressure restrictor. A carbon dioxide mobile phase escapes as a gas with pressure drops of up to 400 atm, while n-pentane escapes as a liquid with pressure drops of less than 50 atm.

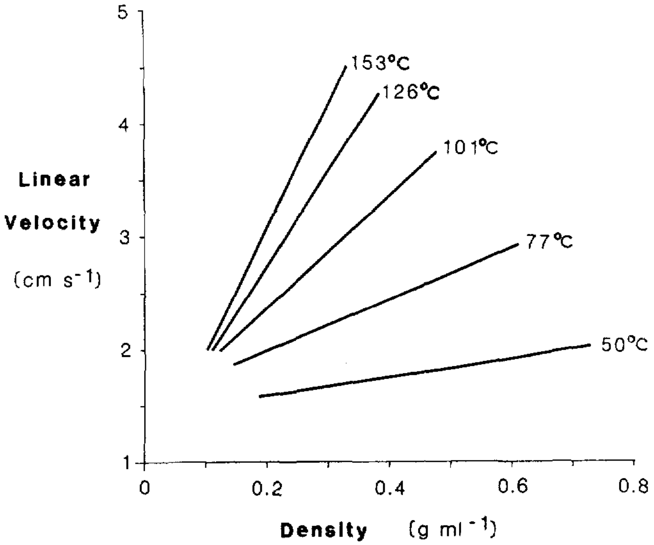

Fig. 2. Linear velocity *vs.* density for different temperatures in capillary supercritical fluid chromatography. Conditions: CO_2 mobile phase, 4 m × 50 μm I.D. bare fused silica, *ca.* 5 cm × 5 μm I.D. fused-silica restrictor at 350°C.

For a given solute, an increase in temperature requires a decrease in density to maintain the same capacity ratio. Efficiency data were measured for the aliphatic hydrocarbons (n-C_{10}, n-C_{24}, and n-C_{36}) at 50 and 100°C where the density was adjusted to give $k = 2$ in all cases. The n-C_{10} and n-C_{24} data were obtained using a short piece (*ca.* 5 cm) of 5-μm I.D. fused-silica tubing as the restrictor. This restrictor resulted in very poor peak shape for n-C_{36}, with much tailing. A laboratory-made, tapered fused-silica restrictor was drawn from 25-μm I.D. tubing, similar to the design of Chester[2], to obtain data for n-C_{36}. In this case, the tip of the restrictor was positioned about 5 mm below the tip of the flame jet. No tailing or peak asymmetry was observed with this arrangement. The results are shown in Table VI. Each restrictor was maintained at constant temperature and was not moved during the course

TABLE VI

MEASURED EFFICIENCIES AT DIFFERENT TEMPERATURES WITH $k = 2$ FOR HYDROCARBON SOLUTES

Solute	*Temp (°C)*	*Density (g ml^{-1})*	*$\bar{u}$ (cm s^{-1})*	*Plates m^{-1}*
C_{10}	50	0.22	2.0	5000
	100	0.10	2.0	7800
C_{24}	50	0.56	2.2	3100
	100	0.40	4.9	2350
C_{36}	50	0.67	1.3	1500*
	100	0.54	3.8	1720*

* Analysis performed with a tapered restrictor.

of the experiments, this resulted in faster linear velocities at the higher temperature. The tapered restrictor gave a linear velocity about 70% as fast as the non-tapered restrictor at 100°C and 0.10 g ml^{-1}.

Several interesting observations can be made about the efficiency data for the hydrocarbons. *n*-Decane is a small, low-molecular-weight solute that exhibits GC type behaviour at the higher temperature. The efficiency is better at higher temperatures, partially due to greater change in volatility, and insignificant linear velocity changes. For the other hydrocarbons, the gains in efficiency from working at higher temperatures are offset by changes in linear velocity, indicating the need to use slower linear velocities for better efficiency, if analysis time can be sacrificed.

A series of hydrogenated triglycerides (trimargarin, tristearin, and triarachidin) were chromatographed at 90°C (Fig. 3) to illustrate the efficiency attainable in capillary SFC. Chester[2] showed analysis of a mixture of mono-, di-, and triglycerides using a 100-μm I.D. column and an operating temperature of 90°C. When analyzed by gas chromatography, these solutes required temperatures near the upper tolerance limits of the stationary phases.

The aliphatic fraction of a Wyoming recluse crude oil was used to evaluate

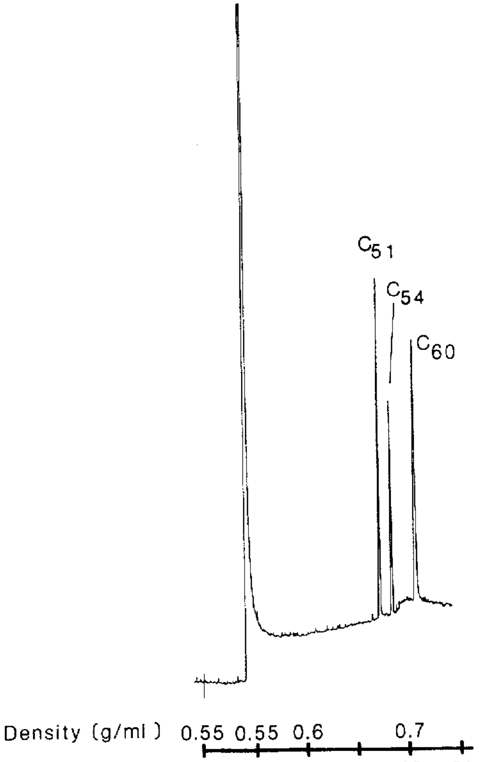

Fig. 3. Capillary supercritical fluid chromatogram of hydrogenated triglyceride standards at 90°C, total side-chain carbon number indicated above peaks. Conditions: 16.5 m × 50 μm I.D.; 0.25 μm d_f (SE-54), polymethylhydrosiloxane-deactivated, with tapered 25-μm I.D. fused-silica restrictor, programmed from 0.55 to 0.70 g ml^{-1} at 0.005 g ml^{-1} min^{-1} after a 10-min isoconfertic period.

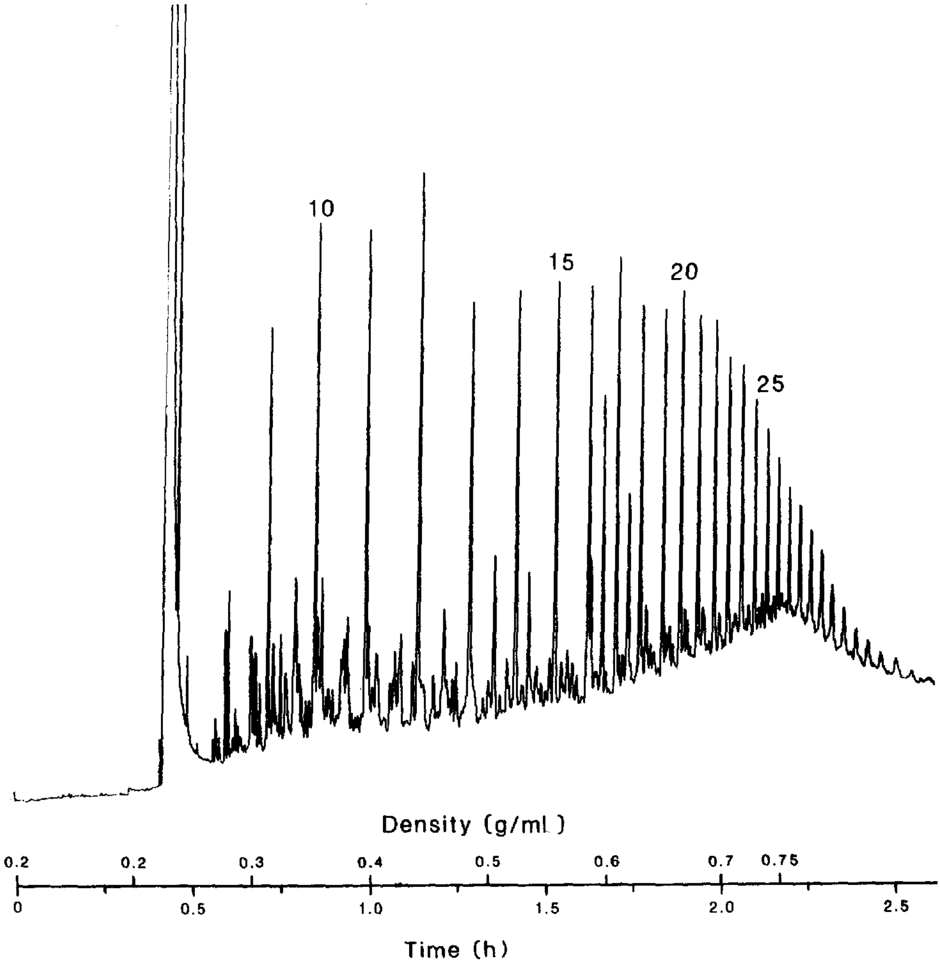

Fig. 4. Supercritical fluid chromatogram of a crude oil with linear density programming. Conditions: 50°C, programmed from 0.2 to 0.75 g ml^{-1} at 0.005 g ml^{-1} min^{-1} after a 20-min isoconfertic period. Peak numbers refer to number of carbons in normal aliphatic chains.

density and simultaneous density–temperature programming. Density programming of carbon dioxide at various temperatures, as noted above, is already proving to be useful, especially for high-molecular-weight, non-volatile, and some polar solutes. Figs. 4 and 5 show linear-density programmed chromatograms at 50 and 100°C, respectively. All components were eluted earlier, and at lower densities, and hence, with increased efficiencies at the higher temperature. The major peaks (*n*-alkanes) would be equally spaced if asymptotic-density programming were used[3]. For thermally stable solutes, these results show that density programming should be performed at as high a temperature as possible to maximize the efficiency of high-molecular-weight solutes.

The simultaneous density–temperature programming mode involved modifi-

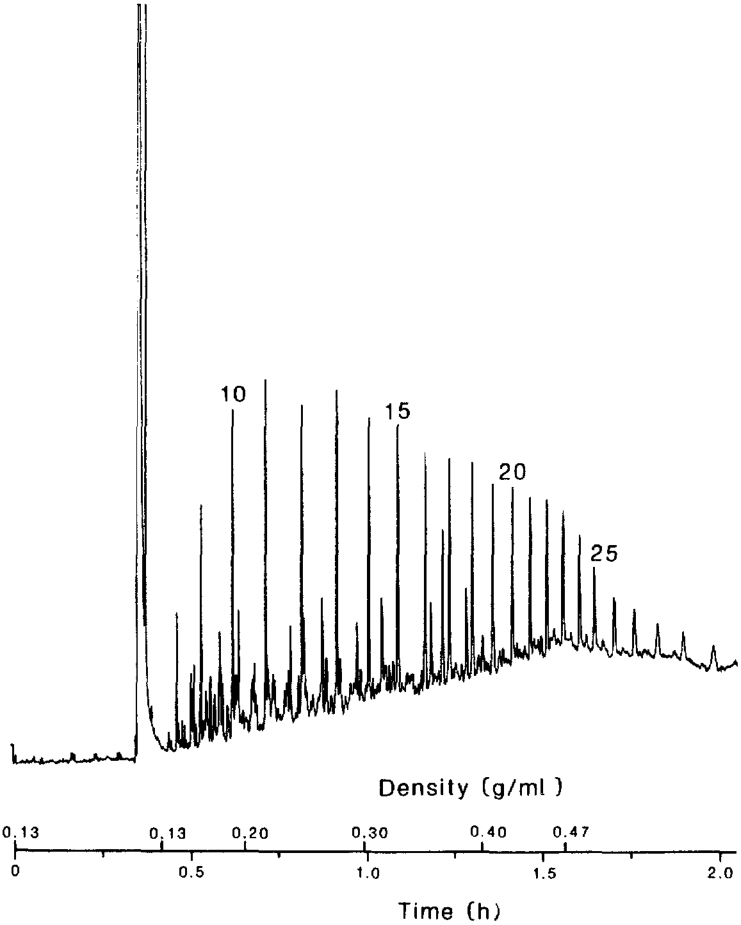

Fig. 5. Supercritical fluid chromatogram of a crude oil with linear density programming. Conditions: 100°C, programmed from 0.13 to 0.47 g ml^{-1} at 0.005 g ml^{-1} min^{-1}, after a 25-min isoconfertic period. Peak numbers refer to number of carbons in normal aliphatic chains.

cation of the chromatographic oven for computer-based temperature control, and manipulation of density data to create pressure *vs.* temperature equations for a range of densities. The desired density was calculated (rounded off to 0.001 g ml^{-1}) from an algorithm and used to retrieve the correct pressure–temperature equation coefficients. The desired temperature was then used in the equation to calculate the pressure. Fig. 6 shows a linear density–linear temperature programmed chromatogram. The density program was identical to that of the isothermal density program at 50°C in Fig. 4, except for a lower final density. A more uniform separation would probably be obtained using asymptotic density–linear temperature programming. Analysis times for the higher-molecular-weight solutes are very close to those in the 100°C linear-density-programmed chromatogram. The simultaneous density–temperature programming with a carbon dioxide mobile phase did not provide any advantages over density

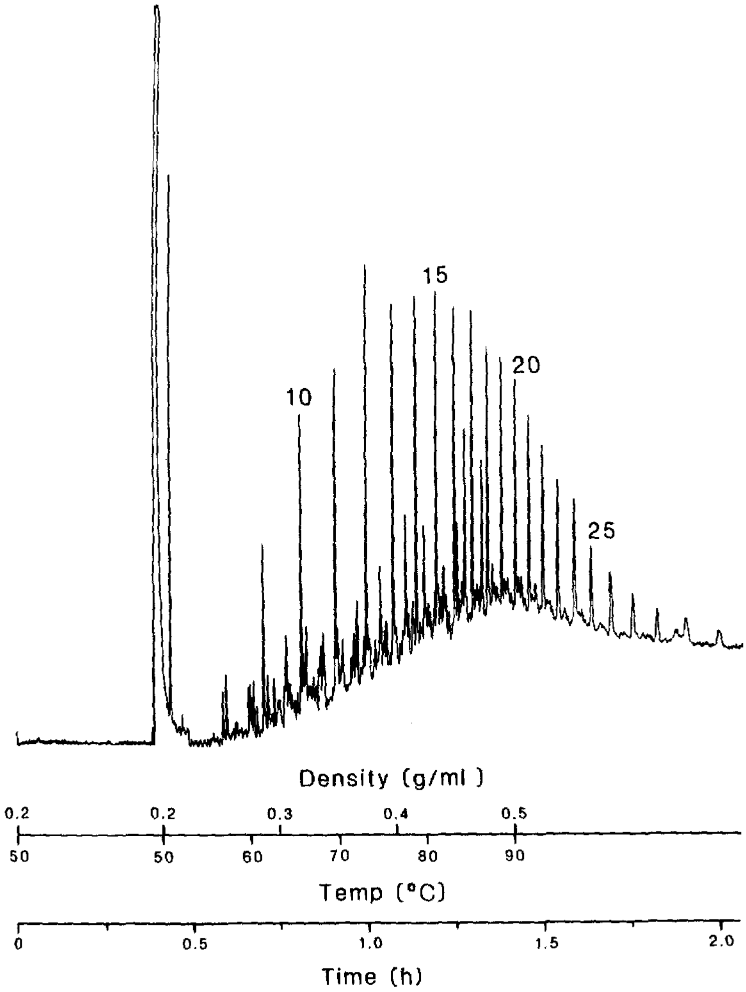

Fig. 6. Supercritical fluid chromatogram of a crude oil with linear density–linear temperature programming. Conditions: programmed from 0.2 to 0.5 g ml^{-1} at 0.005 g ml^{-1} min^{-1} and from 50 to 90°C at 0.7°C min^{-1}, after a 25-min isoconfertic-isothermal period. Peak numbers refer to number of carbons in normal aliphatic chains.

programming at elevated temperatures in terms of efficiency for the example shown here. The simultaneous programming may be very useful for optimizing certain other separations.

ACKNOWLEDGEMENT

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REFERENCES

1 B. W. Wright and R. D. Smith, *Chromatographia*, 18 (1984) 542.
2 T. L. Chester, *J. Chromatogr.*, 299 (1984) 424.
3 J. C. Fjeldsted, W. P. Jackson, P. A. Peaden and M. L. Lee, *J. Chromatogr. Sci.*, 21 (1983) 222.
4 B. E. Richter, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, in press.
5 W. P. Jackson, *Ph.D. Dissertation*, Brigham Young University, Provo, UT, 1985.
6 B. W. Wright, H. R. Udseth and R. D. Smith, *Environ. Sci. Tech.*, in press.
7 B. W. Wright and R. D. Smith, *J. Chromatogr. Sci.*, 23 (1985) 192.
8 B. W. Wright and R. D. Smith, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 8.
9 K. E. Markides and M. L. Lee, *ACS Symp. Ser.*, (1985) in press.
10 J. C. Fjeldsted, R. C. Kong and M. L. Lee, *J. Chromatogr.*, 279 (1983) 449.
11 S. M. Fields, R. C. Kong, J. C. Fjeldsted, M. L. Lee and P. A. Peaden, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1984) 312.
12 S. M. Fields, R. C. Kong, M. L. Lee and P. A. Peaden, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 423.
13 L. G. Randall, in S. Ahuja (Editor), *Ultrahigh Resolution Chromatography, ACS Symp. Series*, 250, American Chemical Society, Washington, DC, 1984, p. 135.
14 M. Golay, in D. H. Desty (Editor), *Gas Chromatography*, Butterworths, London, 1958, p. 36.
15 R. C. Kong, S. M. Fields, W. P. Jackson and M. L. Lee, *J. Chromatogr.*, 289 (1984) 105.
16 B. W. Wright, P. A. Peaden, M. L. Lee and T. J. Stark, *J. Chromatogr.*, 248 (1982) 17.
17 C. L. Woolley, R. C. Kong, B. E. Richter and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Comm.*, 6 (1984) 329.
18 J. C. Fjeldsted, *Ph.D. Dissertation*, Brigham Young University, Provo, UT, 1985.
19 P. A. Peaden and M. L. Lee, *J. Chromatogr.*, 259 (1983) 1.
20 R. Feist and G. M. Schneider, *Sep. Sci. Technol.*, 17 (1982) 261.
21 J. C. Fjeldsted, W. P. Jackson and M. L. Lee, unpublished results.