Journal of Chromatography, 505 (1990) 19-35
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 244

Review

Some aspects of capillary supercritical fluid chromatography

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CONTENTS

1. COMPARISON OF CHROMATOGRAPHIC PERFORMANCE OF COLUMNS IN SUPERCRITICAL FLUID CHROMATOGRAPHY

In order to compare chromatographic conditions in different types of columns, it is very convenient to use dimensionless parameters, and this is considered in the following sub-sections.

1.1. Capillary columns

1.1.1. Reduced plate-height equation. For open-tubular columns, the relationship between the plate height, H (cm), and the linear velocity of the mobile phase, u (cm s^{-1}), is given by the Golay equation':

$$H = \frac{2D_m}{u} + f(k)\frac{d_c^2 u}{D_m} + g(k)\frac{d_f^2 u}{D_s} \quad (1)$$

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TABLE I

DEFINITIONS OF REDUCED (DIMENSIONLESS) PARAMETERS

Parameter	*Capillary columns*[a]	*Packed columns*[a]
Reduced plate height	$h = \frac{H}{d_c}$	$h = \frac{H}{d_p}$
Reduced velocity	$v = \frac{u\, d_c}{D_m}$	$v = \frac{u\, d_p}{D_m}$
Dimensionless film thickness	$\delta_f = \frac{d_f}{d_c} \cdot \frac{D_m}{D_s}$	–
Capacity factor	$k = K \cdot \frac{V_s}{V_m}$	$k = K \cdot \frac{V_s}{V_m}$

[a] d_c, column diameter (cm); d_p, particle size (cm); V_s,V_m, volumes of stationary and mobile phase, respectively.

where D_m and D_s are the diffusion coefficient of the solute in the mobile and the stationary phase, respectively ($cm^2\ s^{-1}$), d_f is the thickness of the stationary-phase film (cm) and k is the capacity factor (dimensionless retention time):

$$f(k) = \frac{1 + 6k + 11k^2}{96(1+k)^2} \tag{2}$$

$$g(k) = \frac{2k}{3(1+k)^2} \tag{3}$$

By using the dimensionless parameters as defined in Table 1, the Golay equation can be rewritten in a simple reduced form[2]. The dimensionless plate-height equation is

$$h = \frac{2}{v} + f(k)v + g(k)\ \delta_f^2\ v \tag{4}$$

where

$$\delta_f^2 = \frac{d_f^2}{d_c^2} \cdot \frac{D_m}{D_s} = \frac{\tau_s}{\tau_m}$$

is the ratio of the diffusion times in the stationary and the mobile phase.

1.1.2. Speed of analysis. Based on reduced parameters (Table 1), a very interesting equation for the speed of analysis in all forms of column chromatography can be derived[2]. Starting from the retention time equation:

$$t_R = t_0(1+k) = \frac{L}{u}(1 + k) \tag{5}$$

where t_R, t_0 are the retention time of a solute and an unretained component, respectively. Substituting $L = NH$ and the reduced parameters as defined in Table 1 it follows that

$$t_R = N \cdot \frac{h}{v} \cdot \frac{d_c^2}{D_m} (1+k) \quad (6)$$

Under identical conditions (N, h, v and k), the analysis time is proportional to d_c^2/D_m (or d_p^2/D_m for packed columns). The order of magnitude of D_m in supercritical fluid chromatography (SFC) is about 10^{-4} cm^2 s^{-1} compared with 10^{-5} and 10^{-1} cm^2 s^{-1} for liquid and gaseous mobile phases, respectively. From eqn. 6 and the respective diffusion coefficients, it can be concluded that in order to obtain equal speed of analysis in gas chromatography (GC), SFC and liquid chromatography (LC), the diameters of the columns should be chosen in the ratio of 1.0:0.03:0.01.

Table 2 gives a comparison of d_c^2/D_m and thus the relationship between the analysis times for the three forms of capillary fluid chromatography. A dimensionless film thickness of 0.3 is used for all columns. The very narrow capillary columns essential in open-tubular LC possess a very limited sample capacity. This, together with the requirement for extremely small detector volumes poses enormous technological problems. Therefore, it can be concluded that capillary SFC, because of the larger column diameters involved, is more within the scope of current technology than is capillary LC. An additional advantage of SFC is the fact that if the correct mobile phase is selected, sensitive GC detectors with low effective dead volumes may be used (e.g., flame ionization, mass spectrometric or nitrogen-phosphorous-specific detectors).

TABLE 2

COMPARISON OF RETENTION TIMES IN OPEN-TUBULAR CHROMATOGRAPHIC METHODS (δ_f = 0.3)

Method	d_c *(μm)*	d_f *(μm)*	D_m *(cm² s⁻¹)*	d_c^2/D_m *(s)*	*Relative time*
GC	*250*	*0.25*	10^{-1}	*0.006*	1
SFC	*50*	*0.79*	10^{-4}	*0.250*	*40*
	8	*0.13*	—	*0.006*	1
LC	10	0.71	10^{-5}	0.100	16
	5	0.35	—	0.025	4

For the exact calculation of retention times by means of eqn. 6 (at a given value of N), values for h and v have to be included (see Table 3). For fairly high values of k, v = 45 and h = 4.5 are reasonable values for columns with thin films ($\delta_f \approx 0.3$) as used in SFC. A further increase of v in order to increase the speed of analysis does not make sense, because at high velocities h is proportional to v and hence h/v remains constant.

For an excellent discussion on the concept of reduced film thickness and its implications for values of h and v the reader is referred to ref. 2.

TABLE 3

COMPARISON OF OPEN-TUBULAR AND PACKED COLUMNS (DIMENSIONLESS PARAMETERS) UNDER OPTIMUM AND DAILY PRACTICAL CONDITIONS

Type of column	φ_0	h	h_{min}	E	E_{min}	v	v_{opt}
Capillary	32	4.5	0.8	650	20	45	5
Drawn (packed) capillary	150	3	2	1350	600	10	5
Packed	1000	3	2	9000	4000	10	2.5

1.1.3. Speed and pressure drop. For non-compressible fluids (liquids) and for conditions of relatively low pressure drops in GC and SFC, the pressure drop is described by the Darcy equation:

$$\Delta P = B_0 \eta L u \tag{7}$$

The permeability B_0 for open-tubular columns equals $32/d_c^2$, or expressed in general terms φ_0/d_c^2, where φ_0 is the column resistance factor. Rewriting with the dimensionless parameters h and v:

$$\Delta P = \frac{32}{d_c^2} \cdot \eta N h v D_m \tag{8}$$

or with

$$v = \frac{ud}{D_m} = \frac{L}{to} \cdot \frac{d}{D_m} = \frac{N h d^2}{t_0 D_m}$$

$$\Delta P = \frac{\varphi_0 N^2}{t_0} \frac{h^2 E \eta N^2 \eta}{t_0} \tag{9}$$

$h^2\varphi_0 = E$ is defined as the separation impedance. From eqn. 9, it follows that

$$\Delta P t_0 = \text{constant} \tag{10}$$

For a given fluid state, the analysis time (proportional to t_0) can be reduced proportionally to d_c^2 (eqn. 6) at the price of a proportionally increasing pressure drop. For a given plate number N, the constant depends on the fluid state (η). For high pressure drops (packed columns) with high plate numbers in SFC, η decreases along the column length. In open-tubular columns this effect can generally be neglected and eqn. 10 can be applied.

Table 4 gives values for $N/\Delta P$ (plates per bar pressure drop) for two column diameters in open-tubular SFC (after eqn. 8). For the calculation of the data in this table, values of h = 4.5 and v = 45 were used.

In SFC, in contrast to GC and LC, retention is very dependent on the pressure

(density) of the mobile phase. A large pressure drop will result in a density gradient along the column. The effect is similar to a negative temperature gradient along the axis of a GC column. The migration of the components will slow down along the column and (in extreme cases) will eventually stop. This problem can be solved partly by pressure (density) programming. A disadvantage of pressure programming, however, is the increasing linear velocity of the mobile phase and thus a reduction in the number of plates attainable.

From Table 4 it can be concluded that the pressure drop is relatively small in open-tubular SFC; even with 8-μm columns about 10 000 plates can be achieved with a pressure drop of 10 bar (column inlet 210 bar, outlet 200 bar).

1.2. *Packed columns*

1.2.1. Reduced plate-height equation. For packed columns, no exact analytical ***H-u*** or ***h-v*** equation analogous to the Golay equation for capillary columns exists. The form of the equation for packed columns is adequately described by the reduced plate-height equation given by Knox:

$$h = Av^{1/3} + \frac{B}{v} + Cv \qquad (11)$$

The dimensionless quantities ***h*** or v for packed columns are defined in Table 1.

Typical values[3] for the constants in eqn. 11 are A = 1-2, ***B*** = 2 and C = 0.05-0.5. Typical values for ***h*** and v in practice are 3 and 10, respectively.

1.2.2. ***Speed of analysis.*** Eqn. 6 describes the speed of analysis for packed columns, if d_c is replaced by the particle size d_p and if the appropriate values for ***h*** and v as given above are used. Contemporary packed columns contain particles of size 10, 5 or even 3 μm. From eqn. 6, it can be concluded that open-tubular fluid chromatography cannot compete with packed columns with respect to speed of analysis unless very small column diameters are used. This, however, is not within the scope of today's technology.

1.2.3. ***Speed andpressure drop.*** Eqns. 7-10 are applicable to packed columns by inserting d_p instead of d_c and using the values for ***h, v*** and ***E*** from Table 3. It should be borne in mind, however, that these equations are strictly valid only for incompressible fluids. For GC and SFC this limits the validity to situations of low pressure drop and hence low plate numbers. In SFC both the linear velocity and the viscosity η are

TABLE 4

PLATES PER BAR PRESSURE DROP IN OPEN-TUBULAR COLUMNS (h = 4.5; v = 45) AND PACKED COLUMNS (h = 3; v = 10) $\eta = 10^{-3}$ g cm^{-1} s^{-1}; $D_m = 10^{-4}$ cm^2 s^{-1}.

Conditions	*Capillary columns*		*Packed columns*	
	d_c (μm)	*Plates per bar pressure drop*	d_p (pm)	*Plates per bar pressure drop*
Supercritical CO_2, 40°C, 200 bar	50	38 600	10	333
	8	1000	5	83

dependent on the pressure drop. In high-pressure GC, pressure-drop correction factors have to be included in the plate-height equations. One of the results is that the speed of analysis is no longer proportional to d_c^2 or d_p^2 and eventually (at high-pressure drops) it becomes proportional to d_c or d_p (refs. 4 and 5).

Table 4 gives values of plates per bar pressure drop for packed columns (φ_0 = 1000) and open-tubular columns (eqn. 8). For drawn (packed) capillary columns, the numbers given have to be multiplied by 1000/150, *i.e.*, the ratio of the column resistance factors of the respective column types (Table 3). For packed capillaries, the ratio is close to 1000/500.

From Table 4, it is clear that for a given allowable pressure drop, *AP,* capillary columns always allow much higher plate numbers to be achieved. This is illustrated in Fig. 1, which shows the analysis of a paraffin wax on a 10 m x 50 μm I.D. column. Apart from the almost Gaussian peak profiles of the main components two tine structures can be observed. The plate numbers that can be generated using packed columns would definitely be insufficient for separating the sample in these three series. At 170 bar and 100°C this column had approximately 60 000 plates for docosane as the test solute (k = 0.75, linear velocity 1.2 cm s^{-1}). The maximum number of plates that can be obtained from any type of column in the three forms of fluid chromatography depends on the maximum allowable pressure drop. In GC and LC this is dependent on instrument design. In SFC retention depends heavily on pressure (or density). The effect of the pressure drop in SFC on retention and plate height is one of the research goals in the near future.

Doubtless in the near future, instrument companies will provide practising chromatographers with the dedicated instrumentation needed to benefit from all the advantages that narrow-bore capillary columns potentially offer. For typical applications, especially high-speed analysis, columns packed with small particles will remain essential in the forthcoming decade.

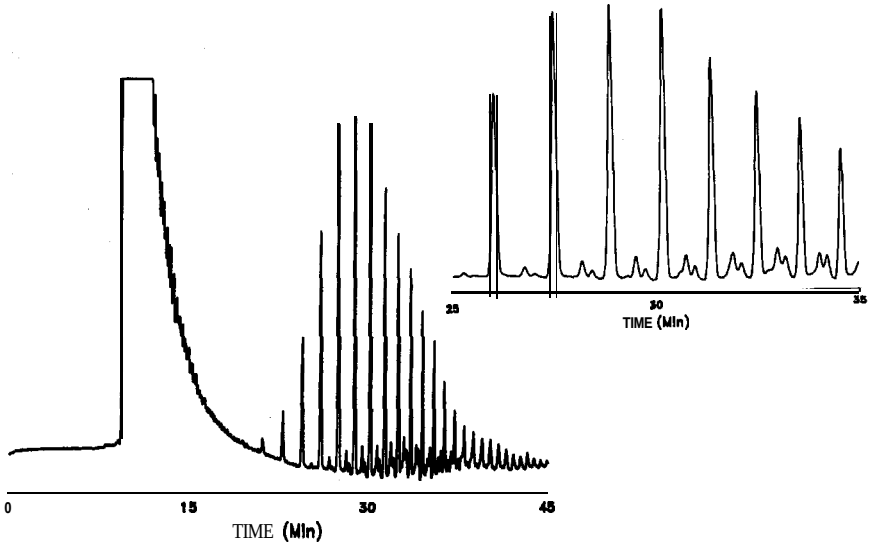

Fig. 1. Analysis of a paraffin wax. Column, 10 m x 50 μm I.D.; stationary-phase, SE-30, 0.25 μm; temperature, 120°C; pressure program, 150 bar (10 min), 5 bar/min to 305 bar.

The choice of the column type for a particular separation is not only governed by the speed of analysis or the maximum plate number. Several other aspects can affect the ultimate choice. One aspect to consider is the volumetric flow-rate through the system. Mass spectrometric detection seems to be better compatible with the lower flow-rates from capillary columns. In contrast, infrared detection, using relatively large-volume detection cells is easier to combine with packed columns. Another aspect influencing the choice of the column is the chemical nature of the solutes. The analysis of polar compounds in packed columns often requires modified mobile phases. If the use of modifier-containing mobile phases is precluded by the detector, the use of capillary columns might be the only alternative. The effect of modifiers added to the mobile phase in packed and capillary SFC will be discussed below. We shall discuss the problems that arise in the on-line combination of capillary SFC with FT-IR.

2. MODIFIERS IN CAPILLARY SFC

2.1. *Effects of modifiers on retention*

To date, very few studies on the effects of modifiers in capillary SFC have been described. In capillary SFC, relatively polar components can be eluted with pure carbon dioxide. Elution of these components in packed-column SFC often requires the addition of a polar modifier to the mobile phase. Fig. 2 shows the analysis of a polar liquid crystal mixture using both packed and capillary columns. Whereas the polar constituents of the sample show up as broad, tailing peaks in packed columns without modifiers (Fig. 2a), these components can be eluted as sharp, symmetrical peaks from the capillary column (Fig. 2b). The peak shapes on the packed column can be improved by using a mobile phase containing a few percent of methanol, as illustrated in Fig. 2c.

The limited number of reports on mixed mobile phases in capillary SFC have shown that relatively large retention changes, although not as large as in packed columns, can also occur in capillary columns. For example Yonker and Smith[7] observed a 40-fold reduction in the capacity factor of myristophenone on increasing the concentration of the modifier (2-propanol) in CO_2 from 0 to 20 mol-%. Fields *et al.*[8] found an approximately ten-fold reduction in the capacity factor of coronene on adding 9 mol-% of 2-propanol to CO_2. Wright *et al.*[9] used 2.5% (w/w) methanol in CO_2. A direct comparison of their data with the values with pure CO_2 was not possible as these experiments were carried out at different pressures and temperatures. In a recent study[10] using packed columns, we observed a 50-fold reduction in the capacity factor of 2-hydroxyethyl methacrylate on a C_{18} packed column on adding only 0.5% of ethanol to the CO_2. The mechanisms that underlie these observations are not yet fully understood.

We recently identified three different ways in which modifiers may influence retention in packed-column SFC[10]: an increase in the mobile-phase polarity, which can give rise to the occurrence of specific interactions between the solute and the modifier in the mobile phase; an increase in the mobile-phase density; and deactivation of active sites on the surface of the packing material.

Whereas the first two effects represent mobile-phase modifications, the third is related to stationary-phase effects. In our classification of the effects of modifiers, we have neglected stationary-phase swelling and increased solvation of the stationary phase by the organic modifier. Recent work by Yonker and Smith" indicates that

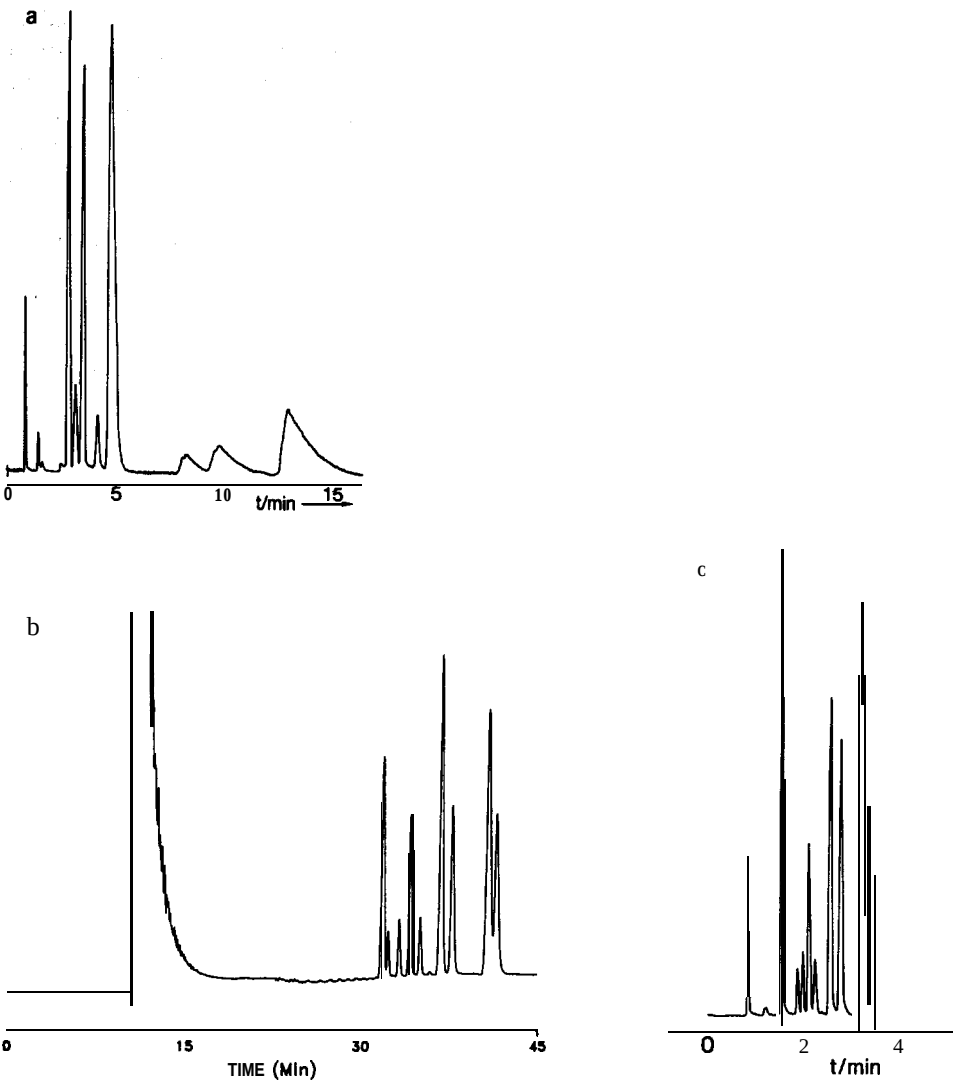

Fig. 2. Analysis of a polar liquid-crystal mixture using packed and capillary columns. (a) C_{18} packed column, 150 x 4.6 mm I.D., particle size 5 μm; P_{in} = 190 bar; ΔP = 16 bar; temperature 45°C; detection, UV at 254 nm. Reprinted from ref. 6 with permission. (b) Capillary column, 10 m x 50 μm I.D.; temperature, 80°C; stationary-phase, SE-30, 0.25 μm; pressure program, 140 bar (10 min), 5 bar/min to 305 bar. (c) C_{18} packed column, 150 x 4.6 mm I.D., particle size 5 μm; P_{in} = 188 bar; ΔP = 20 bar; temperature 50°C; mobile phase, methanol-CO, (5:95, v/v); detection, UV at 210 nm.

solvation of the stationary phase by the organic modifier can occur. Data from Strubinger and Selim[12] seem to indicate that a change in the polarity of the stationary phase can occur due to its enrichment with the polar modifier. The effect of this on retention is still unknown. For a true fundamental understanding of the effects of modifiers, it is of considerable importance to know the relative influence of the

processes described above. For capillary columns with low surface areas and a high degree of deactivation we can neglect the third process. The relative magnitude of the two remaining processes can be estimated from plots of the capacity factor of a solute *versus* the density of the CO_2–modifier mixture for various modifier concentrations. In these plots, the course of the capacity factor *versus* the density of the fluid at a certain modifier concentration reflects the influence of the mobile phase density on retention, whereas the change in the capacity factor with the modifier concentration at constant density reveals the influence of the mobile-phase polarity on solute retention. An increased solvating strength of the mixed mobile phase would result in the retention being lower with the mixed phase than with a pure CO_2 phase. This method of comparing retention in mixed fluids and pure CO_2 requires accurate density values for binary fluids. Experimental density data for CO_2-containing mixtures, however, are scarce.

If experimental data are not available, estimation methods must be employed. These methods are mostly based on reduced temperatures and pressures, which in turn also have to be estimated. Several methods for the estimation of the critical properties of binary fluids have been described[13].Since estimation methods for critical properties of polar fluids can be subject to significant errors, methods for calculating densities will hence inherently be subjected to the same errors. In Fig. 3, a comparison of three methods for estimating critical properties of binary fluids is given. The Lee-Kesler method (data taken from Schoenmakers and Uunk[6]), the Kreglewski-Kay method[14] and the Chueh-Prausnitz method" were employed for estimating the critical pressures of methanol and CO_2 mixtures as a function of the mole fraction of methanol. Fig. 3 clearly indicates the differences in the estimated critical pressures for the three methods.

Yonker and Smith[7] compared the critical loci calculated for methanol–CO_2 according to the Chueh-Prausnitz method with experimental data. For this particular

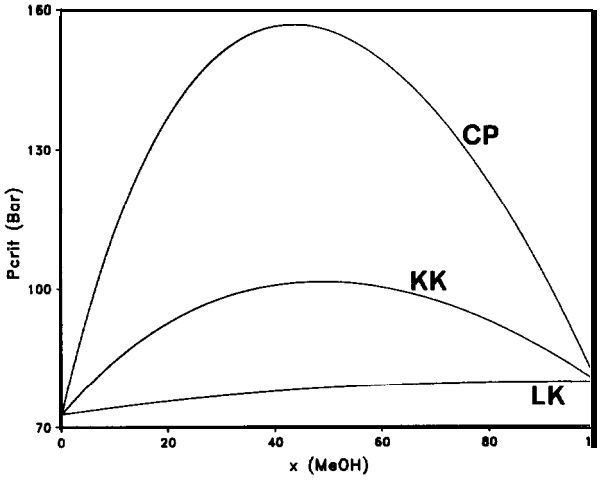

Fig. 3. Calculated critical pressures of CO,-methanol (MeOH) mixtures as a function of the molar percentage of methanol. LK = values estimated according the Lee-Kesler method; KK = estimated according to the Kreglewski-Kay method; CP = estimated according to the Chueh-Prausnitz method. In the Chueh-Prausnitz method, a binary interaction parameter of 0.15 was used[16].

example, a reasonable fit was found between the experimental data and the estimated values.

The approach of comparing retention in mixed mobile phases with varying modifier concentration as a function of the density was adopted by Yonker et *al.*[17] and by Fields *et al.*[8], and Fig. 4 shows the data from the former, the capacity factor of 9-phenanthrol at 127°C being plotted against the density at three different mole fractions of 2-propanol. The densities of the binary fluids were calculated using the Peng-Robinson equation of state.

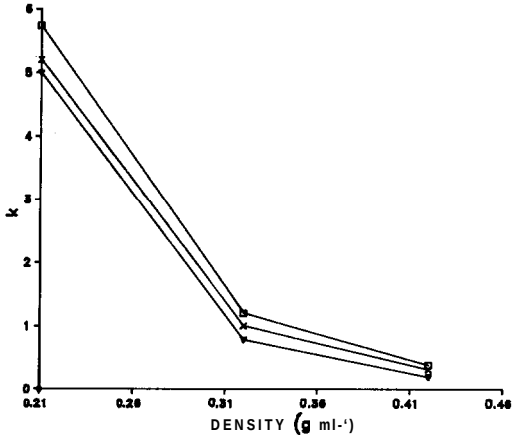

Fig. 4. Capacity factors of 9-phenanthrol versus mobile phase density at various modifier concentrations. Modifier mole fractions: □, 0; x, 0.018; ▽, 0.04. Column, 10 m x 50 μm I.D.; stationary-phase, 5% phenylmethylpolysiloxane; temperature, 127°C. Data taken from ref. 17.

Fig. 4 shows two trends. First, there is a decrease in the capacity factor with increasing density at constant composition. Second, a decrease in ***k*** is observed with increasing mole fraction of the polar modifier at constant density. The first observation reflects the general trend in SFC with pure mobile phases, ***i.e.,*** a decreased retention as the fluid density increases. The decrease in solute retention as the mole fraction of the solvent modifier increases at constant density can be attributed to a qualitative change in the solute-solvent interactions. The polar modifier 2-propanol is believed to interact effectively with the 9-phenanthrol owing to its polar substituent.

Independent evidence of a change in the nature of the solute-solvent interactions can be obtained from spectroscopic measurements. Yonker *et al.*[18] used spectroscopic measurements of solvatochromic shifts to probe the solute-solvent interactions for 2-nitroanisole in CO_2–methanol. Different solvatochromic shifts were observed for different modifier concentrations. From Fig. 4, it appears that the effect of an increase in the mobile-phase density on retention can be much larger than the effect of adding a polar modifier to the fluid. This indicates that comparing the retention in binary fluids with reference values in pure CO_2 at constant pressure instead of constant density can give a distorted picture. On adding a modifier to pure CO_2, the density increases because the critical parameters of the mixed fluid are generally higher than those of pure CO_2. This density increase at constant pressure can have an appreciable

influence on retention. If the density change is taken into account, the effect of adding a modifier will be smaller.

In Fig. 5 the results of Fields *et al.*[8] are shown. The logarithm of the capacity factor of coronene is plotted as a function of the estimated mobile phase density for pure CO_2 and for CO_2–2-propanol mixtures. Fields compared the retention data obtained with CO_2–2-propanol binary fluids with retention data obtained at the same temperature and density with neat CO_2. In contrast to what is expected, ***i.e.,*** the retention being less with mixed phases than with pure CO_2, the plots of log ***k versus*** density for CO_2 cross over the lines representing log ***k versus*** ρ for the mixed phase. This would indicate that retention would be greater for a polar liquid–CO_2 mixture than for a CO_2 mobile phase at the same density and temperature. This observation does not seem to be reasonable and is in contradiction with the results from Yonker ***et al.*** described earlier. Most probably, it implies that the estimated density values of the CO_2–2-propanol mixture are not correct, either due to an inaccurate estimate of the critical properties of the mixture or to an erratic calculation of the density.

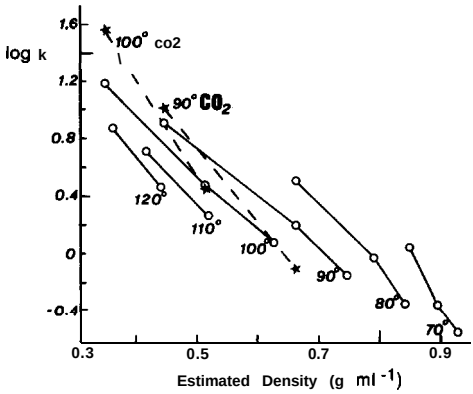

Fig. 5. Log retention of coronene as a function of the estimated density of 8.9 mol-% 2-propanol–CO_2 (solid lines) and density of CO_2 (dashed lines) mobile phases for different temperatures. Capillary column, 10 m x 50 μm I.D.; stationary phase, 100% methylpolysiloxane, 0.25 μm. Redrawn from ref. 8 and reproduced with permission.

2.2. *Preparation of binary mobile phases*

Owing to the absence of polar single-component solvents with acceptable critical properties and safety requirements for SFC, mixed mobile phases are the only alternative when more polar fluids are needed. The preparation of binary fluids is by no means trivial. Here, we shall discuss some methods for the preparation of mixed fluids. We shall limit ourselves to situations where the main solvent is a gas under ambient conditions (e.g., carbon dioxide).

Three methods for preparing mixed fluids for capillary SFC have been described. The simplest is the use of cylinders with premixed mobile phases. These cylinders can either be obtained directly from commercial gas manufacturers or can be prepared in the laboratory. Two reports have been published which describe techniques for the preparation of known compositions of supercritical mobile-phase fluids[19,20]. Both

methods include subsequent steps of evacuating a small gas cylinder, introducing a small (weighed) amount of the (liquid) modifier in the cylinder, adding the appropiate amount of CO_2 and finally mixing the cylinder by agitation. The mole fraction of the modifier in the resulting solution can be determined from the masses of the modifier and CO_2. A distinct disadvantage associated with the use of such premixed fluids, however, is the continuous change in the composition of the residual liquid in the cylinder during usage. As the vapour pressure of CO_2 is much higher than that of the modifier, the gas phase in the cylinder can be assumed to be pure CO_2. Owing to the selective evaporation of the CO_2, the concentration of the modifier in the liquid phase will increase during usage[6].

The second method for preparing mixed fluids for SFC uses mixing of the eluents in the syringe of the pump prior to compression[9,21,22]. The mixtures are prepared by preloading the syringe pump with the proper volume of modifier and then filling the remaining volume with CO_2. Equilibration of the mixture can be accelerated by rapidly increasing and decreasing the pressure of the pump.

The third method is analogous to high-pressure mixing in LC. Here, the fluid flows of two pumps are combined and mixed prior to entering the chromatographic system. Owing to the extremely low volumetric flow-rates in capillary SFC, this method can only be used in combination with high splitting ratios. In contrast to the methods described above, this method is very flexible with regard to the modifier concentration and even allows the use of composition gradients. The applicability of the dual-pump system for gradient elution in open-tubular SFC was first demonstrated by Yonker and Smith[23].

3. HYPHENATED TECHNIQUES

The ultimate aim of a chromatographic analysis is often not only the separation of the sample into its constituents, but also the elucidation of the structure of the components present in the unknown sample. This necessitates coupling of the chromatographic separation technique with identification techniques. Three identification techniques commonly used in combination with SFC are (Fourier transform) infrared spectroscopy (FT-IR), mass spectrometry (MS) and multi-channel UV detection[24,25]. Here we shall give a short discussion of the problems that arise when capillary SFC is being interfaced with IR spectroscopy.

3.1. *SFC–FT-IR*

There are two methods for monitoring the IR absorption of the eluent eluting from a chromatographic column[26–28]: on-line flow-through cells[29] or off-line solvent-elimination techniques[30].

In the flow-cell approach, the effluent passes through a high-pressure light pipe. IR spectra of the effluent are collected in real time while the effluent flows through the cell. In the solvent-elimination approach, the chromatographic effluent is deposited on a surface, the mobile phase is evaporated and the residual sample is examined by FT-IR spectroscopy. Here, the position of SFC between GC and LC becomes apparent. Whereas GC-IR is almost exclusively performed with the flow-cell approach and LC-IR with the solvent-elimination technique, both methods are applied in SFC-IR. Here, we shall give only a short discussion of the on-line flow-cell approach.

The success of the flow-cell approach in the coupling of chromatographic techniques with IR detection depends on the ability to cope with the background absorption of the mobile phase. In GC-FT-IR this is not a problem as normal carrier gases are transparent in the mid-IR region. In LC, the applicability of flow-cell techniques is severely hampered by the high background absorption. A detailed study of the IR transparency of CO_2 was published by Morin *et al.*[31]. Gaseous CO_2 was shown to have large transparent IR regions. However, two important groups of bands obscure the IR spectrum in the regions 3500-3800 and 2200-2500 cm-'. In the supercritical state and the liquid state, the two gaseous bands broaden and additional pairs of bands appear at 2070 and 1944 and at 1387 and 1282 cm-'. The intensity of the latter pair of bands depends on the mobile-phase density. Higher mobile-phase densities lead to a lower transparency in this region. According to Wieboldt *et al.*[32], spectral subtraction in the 1475-1225 cm-' region is possible when using cells with an optical path length shorter than 5 mm.

The problem of background IR absorbance can be eliminated by using an IR-transparent supercritical mobile phase. French and Novotny[33] demonstrated the use of the optically transparent xenon for SFC-FT-IR. Although xenon has convenient critical parameters (T_c = 289.8 K, P_c = 58.0 atm), and diffusion coefficients and a solvent strength comparable to those of CO_2[33,34], its widespread use is hindered by its extremely high price. Whereas the background absorption due to CO_2 can be partially corrected for, the situation is aggravated if modifiers have to be used[35]. In this instance the application of on-line SFC-FT-IR for identification is virtually impossible.

Another problem associated with the use of flow cells in capillary SFC–FT-IR is the extremely low cell volume that can be allowed without affecting the quality of the separation. The design of a flow cell is a compromise between chromatographic and spectrometric requirements. From the chromatographic point of view, the cell volume of the detector must be much smaller than the volume of a chromatographic peak eluting from the column. The maximum allowable cell volume is determined by the maximum acceptable loss of chromatographic resolution.

An equation for the maximum allowable cell volume can be derived starting from the width of a chromatographic peak. For the variance of a peak, σ_c, we can write

$$\sigma_c^2 = \frac{V_r^2 \, H}{L} \tag{12}$$

where σ_c is the standard deviation of the peak (cm^3), *His* the plate height (cm), *L* is the column length (cm) and V_r the retention volume (cm^3). In this equation we can substitute equations for the retention volume of a peak and for the plate height:

$$V_r = V_0 (1 + k) \tag{13}$$

with

$$V_0 = \frac{\pi}{4} \cdot d_c^2 L \tag{14}$$

If we neglect the stationary-phase contribution to chromatographic band broadening and if we assume that the operating velocity is far above the optimum value, the plate-height equation becomes

$$H = \frac{f(k) d_c^2 u}{D_m} \tag{15}$$

Substitution of eqns. 13, 14 and 15 into eqn. 12 and rearrangement yield the following expression for the peak width:

$$\sigma_c = \frac{\pi d_c^3 (1+k)\ f(k)}{4} \left[\frac{u \cdot L}{D_m} \right]^{\frac{1}{2}} \tag{16}$$

For the band broadening, σ_d, that occurs in the detector cell we can write[36]

$$\sigma_d = \frac{V_d}{\sqrt{12}} \tag{17}$$

where V_d is the cell volume of the detector. Here it is assumed that plug flow occurs in the detector cell. The total band width of the peak, σ_{tot}, can be obtained using the rule of the additivity of variances:

$$\sigma_{tot}^2 = \sigma_c^2 + \sigma_d^2 \tag{18}$$

If we accept a 10% loss of theoretical plates we finally arrive at an expression for the maximum allowable cell volume:

$$V_d = \frac{\pi d_c^3 (1+k)}{\sqrt{12}} \left[\frac{f(k) \cdot u \cdot L}{D_m} \right]^{\frac{1}{2}} \tag{19}$$

On substitution of typical values for the various parameters into this equation (Le., $d_c = 50\ \mu m$, $L = 10$ m, $k = 2$, $u = 2 cm\ s^{-1}$, $D_m = 10^{-4}\ cm^2\ s^{-1}$), we obtain a maximum allowable cell volume of cu. 400 nl. A larger cell will unavoidably lead to appreciable band broadening. Hence, from the chromatographic point of view, cell volumes in excess of 400 nl are not permissable.

From a spectroscopic viewpoint, three restrictions are placed on the dimensions of the flow cell. For reasons of sensitivity, the path length of the cell should be long. Background absorption of the CO_2, however, limits the path length to a maximum value. Further, the diameter of the light beam is limited to a certain minimum value. For modern instruments, using beam condensing optics, the minimum cross-sectional area of the light beam is cu. 1 mm^2 (ref. 31). In designing flow cells for SFC-FT-IR, a conflicting situation arises between chromatographic and spectroscopic requirements. Starting with a beam cross-sectional area of 1 mm^2, the maximum allowable cell volume is already obtained at a path length of only 0.4 mm. Such a short path length is highly unfavourable with regard to FT-IR sensitivity. An increased sensitivity can only be obtained at the expense of chromatographic resolution by increasing the path length of the cell.

The first report on capillary SFC–FT-IR[37] used a capillary column of 60 m x 0.33 mm I.D. with an 8-μl flow cell that had a path lengh of 10 mm. French and Novotny[33] described 1-μl flow cells with path lengths of 1 mm in conjunction with 150-μm fused-silica capillary columns. Recently, Raynor *et al.*[38] described the use of 50-μm capillary columns in combination with a 0.8-μl light pipe. This cell had a path length of 4 mm. To avoid a significant loss of chromatographic resolution, make-up fluid was added just prior to the flow cell. With this experimental set-up, "library-searchable" spectra could be obtained for minimum amounts ranging from 10 ng for compounds with intense IR absorption to 100 ng for poor absorbers. In the off-line solvent-elimination approach for SFC-FT-IR, most of the problems encountered in the flow-cell method are eliminated. As the mobile phase is evaporated, background absorption is absent. Hence, it is possible to use both neat CO_2 and modified CO_2. Further, the unavoidable loss of resolution in the flow-cell coupling is not a source of concern in the off-line combination, For a detailed discussion of the solvent-elimination technique, the reader is referred to recent reviews[27,28].

4. CONCLUSIONS

The use of equations based on dimensionless parameters allows a direct comparison of the speed of analysis and the maximum obtainable plate numbers in packed-column and open-tubular column SFC. The plate number per bar pressure drop is much higher in capillary columns. The number of plates generated per second, however, favours the use of packed columns. A considerable reduction in the inner diameter of the capillary column is needed to obtain a similar analysis speed in packed and capillary columns. The extremely low sample capacity and the stringent requirements placed on the sample introduction and the detection system currently hamper the use of capillary columns with smaller diameters.

A definite advantage of capillary columns is the high degree of surface deactivation. The low activity of capillary columns allows the elution of relatively polar solutes without the use of solvent modifiers. Although the deactivation effect of modifiers is absent in capillary columns, here also the introduction of a modifier can cause a considerable reduction in the capacity factors of polar solutes. The mechanisms that underlie these effects are not yet fully understood.

The coupling of capillary SFC with FT-IR is a compromise between chromatographic and spectroscopic requirements. The minimum cell volume required for good spectroscopic performance exceeds the maximum value that can be accepted without the loss of chromatographic resolution. For packed columns, the necessity to add modifiers often precludes the on-line coupling of SFC with FT-IR. The ability to elute components covering a wide span of polarities with pure carbon dioxide adds to the attractiveness of capillary SFC-FT-IR.

5. SUMMARY

Based on dimensionless parameters, equations are given which compare speed of analysis, pressure drop and plates per bar pressure drop for capillary GC, SFC with capillary columns and SFC with packed columns. With respect to speed of analysis, contemporary capillary SFC cannot compete with packed-column SFC. A further

decrease in capillary column diameter will be needed to reach this goal. Decreasing the column diameter of capillary columns at the same time decreases the sample capacity and also places extremely stringent requirements on the speed of sample introduction and on the time constants of the detection systems. If the allowable pressure drop is a serious factor, as is expected from theory, open-tubular columns are to be preferred in terms of the maximum obtainable plate number.

The effects of polar organic modifiers in capillary SFC are described and compared with those in packed columns. The introduction of a modifier can cause a considerable reduction of the capacity factors in capillary SFC. Generally, the effects of modifiers in capillary SFC are not as large as those observed in packed columns. The interpretation of retention data is severely hindered by the unavailability of accurate density data for binary supercritical fluids. Three methods for the calculation of critical properties of mixed fluids are compared.

The coupling of SFC with FT-IR detection is discussed. For capillary SFC, a conflicting situation arises in which the cell volume that can be allowed without loss of resolution is much lower than the minimum volume required for good spectroscopy. It is shown that the on-line coupling of capillary SFC with IR spectroscopy will always be a compromise between chromatographic and spectroscopic requirements. For packed columns restrictions placed on the detector-cell volume are less stringent. Here, however, the on-line approach is severely hampered by the necessity to add modifiers to the mobile phase for the analysis of (even mildly) polar solutes.

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