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Effect of temperature and density on the performance of micropacked columns in supercritical fluid chromatography

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Abstract

The effects on the efficiency of micropacked columns in supercritical fluid chromatography (SFC) of density at fixed temperature and of temperature at fixed density were investigated. The variation of the retention and resolution achievable for a given pair of solutes with the mobile phase density under different isothermal conditions was also studied. The linear velocity was varied using several flow restrictors of different lengths and inner diameters. The column type investigated was characterized by an inside diameter of <1 mm and the use of large particles (by HPLC standards), resulting in a particle-to-column diameter ratio of 0.1–0.3. Owing to the better permeability derived from both the large particle diameters and the high value of the interparticle porosity, the use of these columns may be an interesting approach in SFC.

1. Introduction

Supercritical fluid chromatography (SFC) is becoming increasingly important for the analysis of thermally labile and non-volatile compounds. Capillary, packed and one type of micropacked columns having particle-to-column inner diameter ratios far lower than 0.1 have been used for SFC and several papers have reported the advantages and disadvantages of these classes of columns [1–14]. In recent years, capillary SFC has been widely used because of its high efficiency, its relatively low degree of activity and its high permeability. However, the low sample capacity of capillary columns may be an inconvenience for the analysis of trace compounds. Further, capillary SFC demands linear flow-rates higher than the optimum in order to achieve a reasonable speed of analysis.

The use of packed columns is favoured when higher sample volumes are demanded (*i.e.*, for the analysis of compounds occurring at low concentrations). Also, packed columns are superior to open-tubular columns in terms of analysis time.

However, some of the packed columns currently used in SFC may occasionally exhibit a high degree of activity, mainly due to disturbing silanol–sample interactions [5]. Working with packed columns may also have an important disadvantage related to the high values obtained for the optimum linear velocity of the mobile phase, and the cost of the eluent, the volume flow capability of the SFC pumping system and the upper flow limit of the detector being used must be carefully considered.

A large difference between capillary and

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packed columns exists in the permeability and hence in the pressure drop required over the column. The pressure drop across packed columns can be great, causing significant differences in fluid densities in different parts of the column. In this respect, most studies conclude that at pressure drops greater than 20 bar, considerable eluting power can be lost across the column, resulting in lower chromatographic efficiency [4,5,15–18]. A few reports, however, contradict this observation [19–22], but it is generally admitted that the study of the consequences of a high column pressure drop in terms of resolution and selectivity demands further investigation in SFC.

In previous papers [23,24] we studied the possibilities in SFC of micropacked columns loaded with liquid stationary phases typically used in GC, immobilized on large particles (by HPLC standards). This type of micropacked column exhibits a particle-to-column inner diameter ratio (d_p/d_c) of 0.1–0.3 whereas a second type of micropacked column used by others in SFC [11–14] is packed with very fine-grained materials resulting in d_p/d_c values far lower than 0.1. Undoubtedly, a high number of theoretical plates can be achieved at the price of a significant pressure drop even for smaller column lengths. The latter type of column is often called packed capillary, although some workers suggest restricting this name to columns with particle-to-column inner diameter ratios of 0.2–0.5 and inner diameters of less than 0.5 mm. The same workers proposed an excellent classification of chromatographic columns which should be considered for distinguishing unambiguously different classes of columns used in SFC [25,26].

With regard to the first-mentioned type of micropacked columns ($0.1 < d_p/d_c < 0.3$), the large particle diameter and the high value of the interparticle porosity allow better permeabilities to be obtained.

A theoretical study of several parameters affecting the permeability of a micropacked column, and its efficiency in gas chromatography, has been reported previously [27] and several applications of this type of column have already appeared [28–31]. Concerning its use in SFC, the initial results obtained in our laboratory [23,24] were highly encouraging, as they showed that micropacked columns are capable of producing acceptable specific efficiencies (*i.e.*, efficiencies per metre of column length), resolution and analysis times, the pressure drop across the column being similar to that of capillary columns and lower than that of typical packed columns having 5- or 10-μm diameter packings. In fact, the low pressure drop observed over micropacked columns could be an essential factor concerning the use of this type of column in SFC.

Also, micropacked columns could be more advantageous than open-tubular columns for specific applications demanding the analysis of fairly complex samples for compounds present at low concentrations. Additional advantages of micropacked columns are that interfacing with ionization detectors or MS detectors is made easier owing to the low flow-rates required for micropacked columns. On the other hand, it should be emphasized that an interesting advantage of the use of micropacked columns is that different supports impregnated with different percentages of any stationary phase may be considered in order to optimize a specific separation [25,27].

In view of the above, the use of micropacked columns should be considered as a useful approach in SFC in addition to the packed and capillary columns currently in use. To date, however, the evaluation of column performance in SFC has mainly involved capillary and packed columns [21,32–34].

The aim of this work was to investigate the effect of various experimental parameters on the chromatographic efficiency, resolution and retention achieved for a given pair of solutes with an SFC micropacked column.

2. Experimental

Chromatographic measurements were made with a Carlo Erba (Milan, Italy) Model SFC 3000 supercritical fluid chromatograph equipped with a flame ionization detector.

Carbon dioxide of supercritical grade was pumped by using an SFC 300 pump (Carlo Erba). Samples were injected on to the micropacked column through a time-controlled rotating valve (Vici) having a 1-μl internal loop. The sampled volume was 1 μl and a flow split ratio of 1:50 was established for each injection.

A test mixture of n-C_{20}, n-C_{22} and n-C_{24} alkanes containing 5 μg/μl of each component in pentane and a 0.5 m × 0.53 mm I.D. micropacked column containing 3% of SE-54 in Volaspher A-2 (desilanized) were used. Volaspher A-2 is a siliceous synthetic support from Merck which has a narrow size distribution and an average particle size between 100 and 125 μm. It is a stable, spherical support with a uniform porous structure. The chromatographic column was made from a deactivated stainless-steel tube following a procedure described previously [27]. The stationary phase was cross-linked by adding dicumyl peroxide (0.5 mg per 100 mg) and increasing the column temperature at 5°C/min, under a nitrogen flow, from 100 to 160°C. The final temperature was maintained for 3 h.

The linear velocity of the mobile phase was varied by using different linear restrictors of 8 μm I.D. (length 7.5, 10 and 13 cm) and 10 μm I.D. (length 30, 40 and 50 cm), which were made from fused-silica tubing.

Each experiment was performed under isopycnic and isothermal conditions. The density of the mobile phase was varied (at a constant temperature of 100°C) from 0.25 to 0.45 g/ml. Further experimentation was carried out at densities of the mobile phase of (a) 0.25, (b) 0.35 and (c) 0.45 g/ml by considering in each instance different temperatures (from 60 to 160°C). The injection port and the detector were maintained throughout at 40 and 375°C, respectively. In all instances, at least two replicates of each injection were made.

3. Results and discussion

The value of the specific permeability coefficient (B^0) for the micropacked column used was determined experimentally to be $8.6 \cdot 10^6$ cm^{-2}, and a value close to 0.03 bar was established for the pressure drop per metre of column length.

Experimental data obtained for H (height equivalent to a theoretical plate) and u (mobile phase linear velocity) were fitted to the Van Deemter equation (Eq. 1) by using the BMDP statistical package [35] (BMDP-1R program, linear regression).

$$H = A + \frac{B}{u} + Cu \quad (1)$$

This equation can be expanded to

$$H = 2\lambda d_p + \frac{2\gamma D_M}{u} + \left[\frac{8kd_f^2}{\pi^2(1+k)^2 D_L} + \frac{1+6k+11k^2}{96(1+k)^2} \cdot \frac{d_p^2}{D_M}\right]u \quad (2)$$

where λ is the eddy diffusion coefficient, γ (assumed to be 1) is the tortuosity factor, d_p is the average particle size of the column packing, D_M is the diffusion coefficient of the solute in the gas phase, D_L is the diffusion coefficient of the solute in the liquid phase, d_f is the average thickness of the film of stationary liquid phase on the support and k is the capacity factor.

Different curves were obtained (Table 1) by fitting to Eq. 1 experimental data collected either at fixed temperature under various isopycnic conditions or at fixed density under different isothermal conditions. For each curve, a minimum of fifteen data points were considered and acceptable values of the standard errors of estimation (s) and the coefficients of determination (R^2) were achieved in all instances.

Experimentally, values of A not significantly different from zero were found, in agreement with data previously reported by several workers for packed columns [36,37]. This is why only B and C terms were finally considered [23].

Fig. 1 includes several plots showing the variation of H with u for the micropacked column described under Experimental. These curves were obtained at four densities ranging from 0.25 to 0.45 g/ml. In all instances the column temperature was maintained at 100°C. The statistical analysis performed on the data by using the BMDP-1R program showed that all curves de-

Table 1
Values of B and C parameters of Van Deemter equation obtained by fitting experimental data at different densities and temperatures

Density (g/ml)	Temperature (°C)	$B(\times 10^{-4})$	C	$R^{2\,a}$	s^b
0.25	100	10.46	0.177	0.977	0.0073
0.25	120	8.00	0.174	0.986	0.0058
0.25	140	7.92	0.158	0.988	0.0051
0.25	160	7.56	0.146	0.991	0.0038
0.35	80	5.90	0.231	0.981	0.0067
0.35	100	5.74	0.178	0.967	0.0074
0.35	120	5.55	0.178	0.983	0.0059
0.35	140	4.40	0.181	0.983	0.0053
0.40	100	4.32	0.205	0.996	0.0030
0.45	60	5.33	0.252	0.981	0.0062
0.45	80	3.89	0.247	0.998	0.0017
0.45	100	3.21	0.257	0.986	0.0043

Column, 0.5 m × 0.53 mm I.D.; 3% SE-54 in Volaspher A-2 (desilanized), 100–125 μm.
[a] Coefficient of determination.
[b] Standard error of the estimation.

picted in Fig. 1 are significantly different (confidence level 95%).

As can be seen, the decrease in column efficiency observed under isopycnic conditions when the linear velocity is increased is more evident at the highest densities. Accordingly, an appreciable decrease in the micropacked column efficiency occurs when the density is increased from 0.25 to 0.45 g/ml and the mobile phase velocities are higher than the optimum. However, values of H corresponding to the optimum velocity of the mobile phase (*i.e.*, $H_{u\,opt}$) decrease when the density is increased, as shown in Table 2, which also includes the confidence limits for $H_{u\,opt}$ corresponding to a confidence level of 95%.

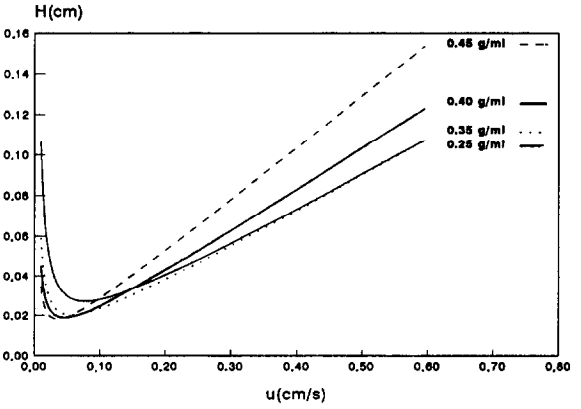

Fig. 1. Effect of the mobile phase density on efficiency: Van Deemter plots, obtained by fitting experimental data, at 100°C and different densities of the mobile phase for a micropacked column. Solute: n-C_{20}.

The above-mentioned trend may be explained by considering the effect of the density on the longitudinal diffusion and the resistance to mass transfer (B and C terms, respectively, on the Van Deemter equation). Under isothermal conditions, an increase in mobile phase density leads to higher viscosity values, and thereby a decrease in the solute diffusivity in the mobile phase (D_M)

Table 2
Efficiency of a micropacked column in SFC at constant temperature (100°C) and different mobile phase densities

Density (g/ml)	u_{opt} (cm/s)	$H_{u\,opt}$ (cm)[a]	Intervals[b]
0.25	0.077	0.027	0.024–0.031
0.35	0.057	0.020	0.017–0.023
0.40	0.046	0.019	0.017–0.021
0.45	0.035	0.018	0.014–0.021

[a] Values of H at the optimum velocity of the mobile phase.
[b] Confidence limits for the average value of $H_{u\,opt}$ (confidence level 95%) [38].

should result. Taking into account that the longitudinal diffusion term is proportional to D_M whereas it appears in the denominator of the C term, it is clear that a decrease in D_M may imply an increase in efficiency if the effect of B on H dominates over that of C on efficiency. Nevertheless, experimentation is commonly performed at linear velocities higher than the optimum, thus causing the B/u term to decrease to such an extent that the effect of the C term on efficiency may become clearly dominant. Consequently, lower column efficiencies should be observed at the highest densities (Fig. 1).

Fig. 2 shows the effect of temperature on the micropacked column efficiency at three different densities of the mobile phase, namely 0.25, 0.35 and 0.45 g/ml.

It is clear that the influence of temperature is greater the lower is the mobile phase density. Fig. 2a shows that increasing the temperature at a fixed density results in a higher column efficiency. This happens because the C term of the Van Deemter equation is lowered. Nevertheless, when working at this fixed lower density (0.25 g/ml), significant differences in H values are only found between those analyses performed at higher temperatures (140–160°C) and those carried out at lower temperatures (100–120°C).

If operation is accomplished at a density of 0.35 g/ml (Fig. 2b) and the temperature is varied from 100 to 140°C, no significant differences are observed between the variation of H with u corresponding to each curve. At 80°C, however, the curve obtained is significantly different from those resulting at higher temperatures. Curves obtained at the highest density (0.45 g/ml) at different temperatures are not significantly different (Fig. 2c). In all instances the statistical study was performed by considering a confidence level of 95%.

Fig. 3 illustrates the effect of variations in both density and temperature on retention in a micropacked column. It is clear that increasing the density at a fixed temperature results in faster elution of the solute from the column, whereas the opposite effect (*i.e.*, a retention increase) is observed when working at a fixed density if the temperature is decreased.

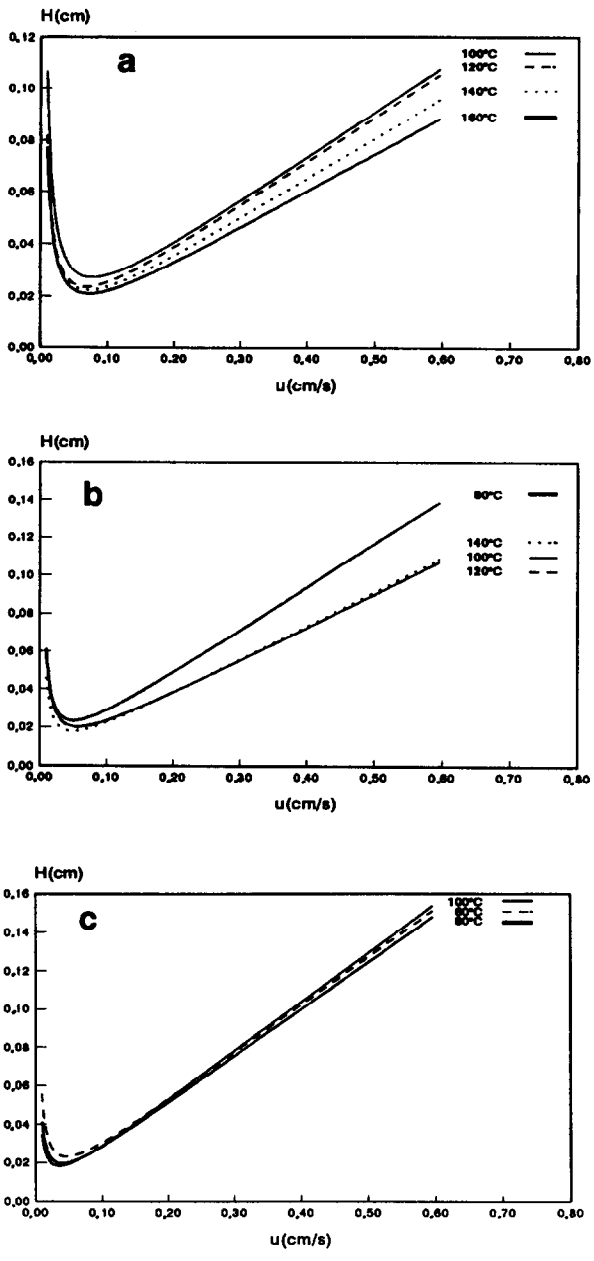

Fig. 2. Effect of temperature on efficiency: Van Deemter plots, obtained by fitting experimental data, at different temperatures, at densities of (a) 0.25, (b) 0.35 and (c) 0.45 g/ml for a micropacked column. Solute: n-C_{20}.

With respect to the resolution achieved for a given pair of solutes in a micropacked column, Fig. 4 shows its variation with density and temperature. To understand the observed vari-

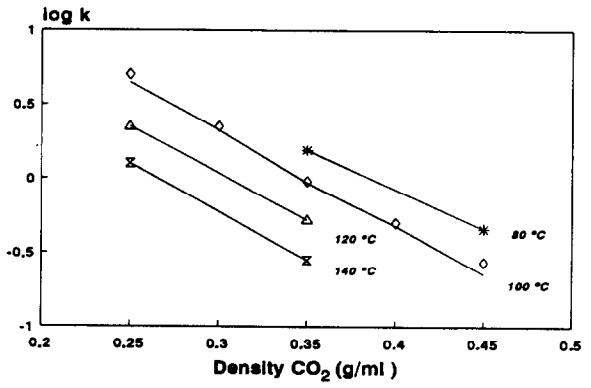

Fig. 3. Variation of the logarithm of the capacity factor (k) with the mobile phase density at different temperatures for a micropacked column. Solute: n-C_{20}.

ation of the resolution, the combined influence of three factors, namely column efficiency (N), relative retention (α) and column capacity factor (k), described by the Purnell resolution equation, should be considered.

Working under isothermal conditions, it is evident that decreasing the density gives higher k values but also, according to Fig. 1, lower H values may be achieved. Thus higher resolutions are finally observed.

If operation is performed at a fixed density, it is also clear that variation of the temperature causes an important variation of the partition coefficient (K_D). Specifically, increasing the temperature (under isopycnic conditions) brings about a diminution of both K_D and k. Conversely, changes in column efficiency at fixed density (Fig. 2) may contribute to obtaining better resolution if the temperature is increased. The influence of k on the resolution achievable must dominate over that of N, as a diminution of the resolution is ultimately observed.

The data obtained reveal that the effect of variations in both density and temperature on the retention and resolution achievable in a micropacked column is similar to the effect reported previously by several workers for capillary and packed columns in SFC [32,39–41].

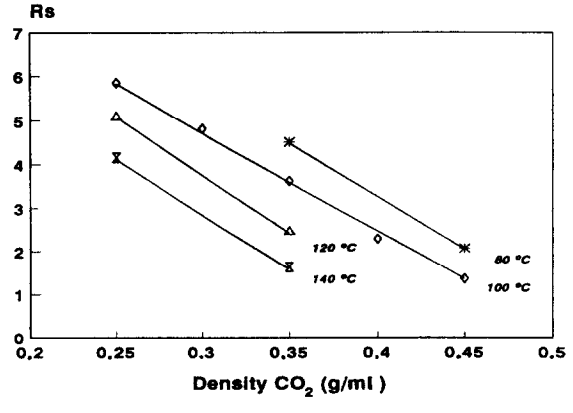

Fig. 4. Dependence of the resolution (R_s) on the mobile phase density at different temperatures for a micropacked column. Solutes: n-C_{20}/n-C_{22}.

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