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Use of a thermally insulated column for improved speed, efficiency and resolution in packed-column supercritical fluid chromatography

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Abstract

The use of a thermally insulated column for significant improvements in speed, efficiency and resolution in packed-column supercritical fluid chromatography at intermediate mobile phase densities is demonstrated. Elution of a mixture of normal alkanes, containing 12 to 18 carbon atoms from a 150×2.0-mm I.D. column packed with 5- μ m porous octylsilica particles, was performed with carbon dioxide mobile phase at 50°C at reduced densities of 1.0 and 1.5. For elution of *n*-octadecane at a reduced density of 1.0, a two- to sixteen-fold increase in the apparent number of theoretical plates was observed for an insulated column compared to the uninsulated column at pressure drops of 12 and 29 bar, respectively, along with decreased retention times and improved resolution. The improved efficiency obtained with an insulated column at the lower density is due to the effective elimination of radial temperature gradients associated with expansion and cooling of the mobile phase. The insulated column provided no measurable improvement at the higher density. © 1997 Elsevier Science B.V.

Keywords: Column efficiency; Thermally insulated column; Columns; Alkanes

1. Introduction

The deleterious effect of large pressure drops on efficiency in supercritical fluid chromatography (SFC) has been the subject of numerous reports. Significant losses in efficiency have been reported when operating packed-columns at large pressure drops with CO₂ at column inlet densities below 0.7 g/cm³ and temperatures below 100°C [1–7]. In contrast, reduced plate heights less than 2 have also been reported [8], and long columns operated under large pressure drops [9] have yielded over 200 000

plates with no apparent loss in efficiency due to pressure drop.

Recent studies in this laboratory on the elution of the unretained solute methane with supercritical carbon dioxide have shown that, under conditions referred to above, significant axial and radial temperature gradients may develop in the column [10,11]. Results from these studies suggested that the use of a thermally insulated column might provide significant improvements in efficiency for the elution of retained solutes by effectively eliminating the radial temperature gradients. The details of the studies on methane, which preceded this work, are being prepared for publication. The initial results for elution of retained solutes from an insulated column,

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which provided significant improvements in speed, efficiency and resolution, are the subject of this brief report.

2. Experimental

2.1. Apparatus

The SFC system was custom built in this laboratory. The major components consisted of a Varian 2740 gas chromatograph with flame ionization detector (Varian Associates, Sunnyvale, CA, USA), an Isco 260D syringe pump (Isco, Inc., Lincoln, NE, USA), and a Valco CI4W injector (Valco Instruments, Houston, TX, USA) with a 500-nl sample loop and a helium actuator. A flow splitter at the outlet consisted of a Valco low-dead-volume tee. One port of the splitter tee was connected to the column outlet with a short piece of 1/16-inch O.D.×0.007-inch I.D. stainless steel tubing, and the second port to a 35-cm long×30- μ m I.D. fused-silica capillary restrictor leading to the detector. The third port of the splitter tee was connected to a system of 1/16-inch×0.010-inch I.D. stainless steel tubing and connectors which included a high pressure nitrogen source, a secondary outlet restrictor of 0.7 m of 0.007-inch I.D. stainless steel tube, and a pressure transducer. This arrangement provided for direct control of the outlet pressure and accommodated mobile phase flow-rates which exceeded the rather small flow capacity of the restrictor leading to the detector. A 150-ml stainless steel sample reservoir was connected to the sample port of the injector with stainless steel tubing for injection of samples dissolved in carbon dioxide. This SFC system was designed specifically for studies of retention and efficiency in SFC, and is described in detail in a companion paper in this issue [12,13].

The column was 150-mm long×2.0-mm I.D.×6.4 mm O.D., with Parker external end fittings, packed with 5- μ m Spherisorb C₈ (Phase Separations, Norwalk, CT, USA). For the uninsulated case, the column was installed in the usual manner in the oven. For the insulated case, the main section of the column (between the end fittings) was first wrapped with a thin layer of fiberglass insulation to an overall diameter equal to that of the end fittings (about 15

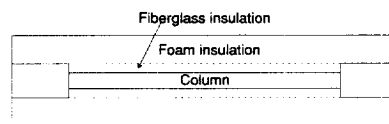


Fig. 1. Diagram of column and insulation.

mm). The entire assembly, including the end fittings, was then covered with polyurethane foam pipe insulation (32 mm O.D., 11-mm wall thickness) of the type used for refrigeration lines, as shown in Fig. 1.

2.2. Chemicals

SFC grade carbon dioxide (no helium headspace) was obtained from Scott Specialty Gases, (Troy, MI, USA). Normal alkanes were obtained from Alltech Associates (Deerfield, IL, USA) as a neat mixture containing equal masses of *n*-dodecane, *n*-tetradecane, *n*-hexadecane, and *n*-octadecane. The sample mixture was prepared by introducing 100 μ l of the alkane mixture into the sample reservoir. The reservoir was then charged with methane at 1 bar (in addition to the air, which was not removed), followed by CO₂ at 80 bar (room temperature).

3. Results and discussion

Chromatograms for this study were obtained at two values of the temporally averaged mobile phase reduced density [14,15], $\langle \rho_R \rangle_t$, where the reduced density is the density of CO₂ divided by its critical density, 0.468 g/cm³. At reduced densities and temperatures around unity, CO₂ is highly compressible, so that large pressure drops result in large decreases in density along the column. The loss of efficiency at large pressure drops is generally attributed to the large density gradients that exist under these conditions.

Fig. 2 shows three chromatograms obtained with the same column and the same sample solution. The relative peak areas varied somewhat due to some apparent difficulty in getting the *n*-alkanes to dissolve homogeneously in the CO₂. Fig. 2A and B represent elution of the sample mixture at $\langle \rho_R \rangle_t = 1.0$, calculated based on assumed isothermal conditions.

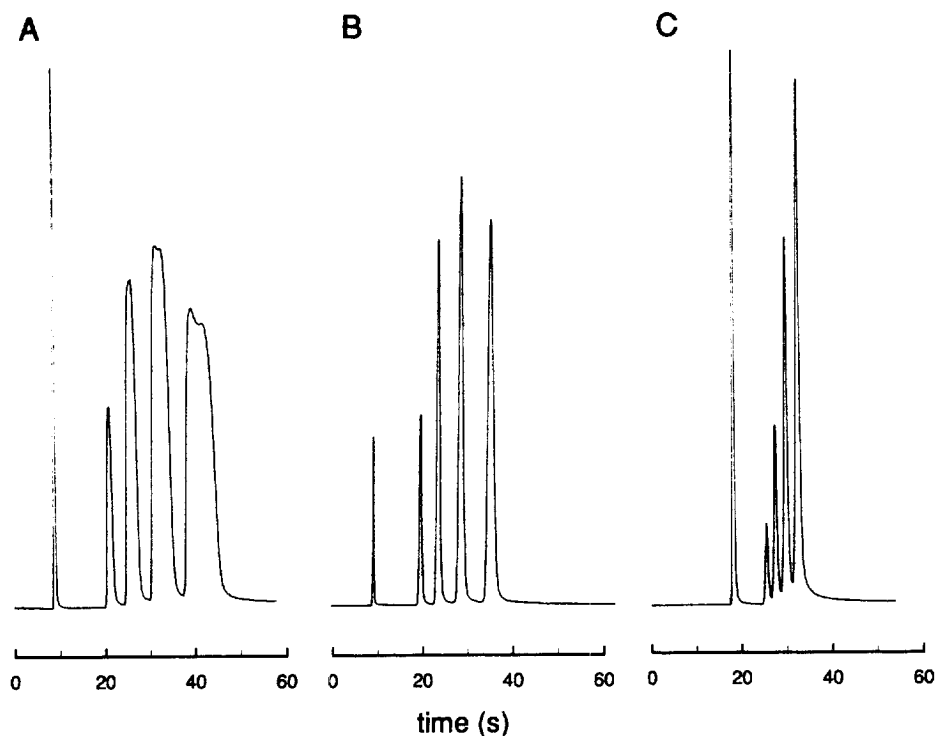


Fig. 2. SFC separation of *n*-alkanes with uninsulated and insulated columns at two densities with a large pressure drop. Column: 150×2.0-mm I.D. 5- μ m octylsilica, CO₂ mobile phase at 50°C. Solutes in order of elution: methane, *n*-dodecane, *n*-tetradecane, *n*-hexadecane, *n*-octadecane. A (uninsulated) and B (insulated): $\langle\rho_R\rangle_1=1.0$, $P_{in}=120.2$ bar, $P_{out}=91.1$ bar, linear velocity=1.63 cm/s. C (uninsulated): $\langle\rho_R\rangle_1=1.5$, $P_{in}=160.4$ bar, $P_{out}=140.6$ bar, linear velocity=0.81 cm/s. Case C also represents results obtained for an insulated column under otherwise identical conditions. P_{in} and P_{out} are pressures at the column inlet and outlet.

All conditions were identical except that in Fig. 2A the column was bare (uninsulated case), and in Fig. 2B the column was insulated (insulated case). The improvement in efficiency for the insulated column is very clear. Due to the presence of longitudinal gradients in the column, the chromatograms yield apparent values of the number of theoretical plates or

plate height, but these are nonetheless useful for describing the overall efficiency of the separation. Calculated values for the apparent number of theoretical plates (based on peak width at half height) for octadecane and its resolution from hexadecane are listed in Table 1. The reduced velocities (linear velocity times the particle size divided by the solute

Table 1
Retention and efficiency data for separations at $\langle\rho_R\rangle_1=1.0$

	Retention time for <i>n</i> -C ₁₈ H ₃₈ (s)	Apparent retention factor	Apparent number of theoretical plates for <i>n</i> -C ₁₈ H ₃₈	Resolution for <i>n</i> -C ₁₆ H ₃₄ and <i>n</i> -C ₁₈ H ₃₈
<i>Data for Fig. 2, pressure drop=29.1 bar</i>				
Uninsulated column (2A)	43	3.8	220	1.3
Insulated column (2B)	35.5	2.7	3500	3.0
<i>Data for Fig. 3, pressure drop=12.4 bar</i>				
Uninsulated column (3A)	108	4.4	4500	4.4
Insulated column (3B)	85	3.1	9100	4.8

diffusion coefficient) achieved in the experiment at $\langle\rho_R\rangle_t=1.0$ (Fig. 2A and B) were approximately 1.4, 4.7, 5.2, 5.7 and 6.2 for methane, *n*-dodecane, *n*-tetradecane, *n*-hexadecane and *n*-octadecane, respectively, based on diffusion coefficients calculated from a modified Wilke-Chang equation [16]. These velocities represent reasonable operating conditions for uniform columns (i.e., columns with no density or temperature gradients). The apparent reduced plate height for *n*-octadecane on the insulated column is about 8.6, compared to 136 on the uninsulated column at the same reduced velocity of 6.2.

The pair of chromatograms in Fig. 3 were obtained at $\langle\rho_R\rangle_t=1.0$ and 50°C, but at a smaller pressure drop of 12.4 bar. There is no obvious peak distortion for the uninsulated case (Fig. 3A), but again a significant improvement in efficiency, as well as a decrease in retention time, is obtained by insulating the column (Fig. 3B). A modest increase in resolution is also observed for the last two peaks. The data for *n*-octadecane, given in Table 1, yield an

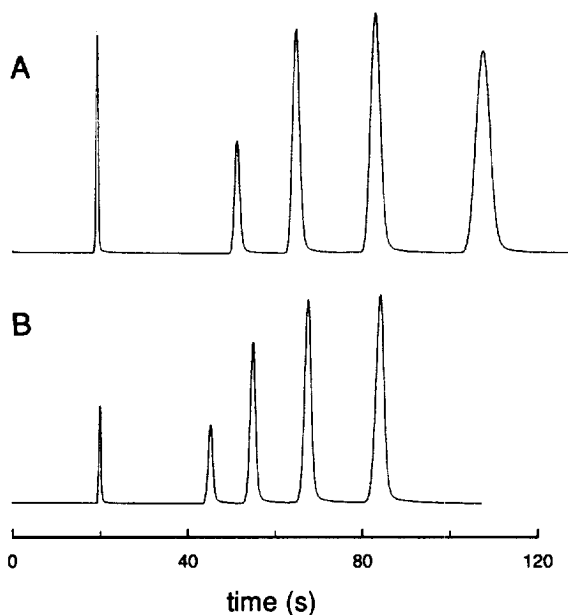


Fig. 3. Separation of *n*-alkanes at $\langle\rho_R\rangle_t=1.0$ with a moderate pressure drop. Column, solutes and conditions same as in Fig. 2, except as noted. A: Uninsulated column, $P_{in}=113.0$ bar, $P_{out}=100.6$ bar, linear velocity=0.77 cm/s. B: Insulated column, $P_{in}=112.8$ bar, $P_{out}=100.4$ bar, linear velocity=0.74 cm/s.

apparent reduced plate height of 3.2 at a reduced velocity of 2.3 on the insulated column, which would be considered acceptable for a uniform column such as is found in HPLC. Thus it appears that in this case we have eliminated most, if not all, of the excess band spreading due to pressure drop.

Recent results obtained in this laboratory for elution of an unretained solute indicate that a primary cause for the loss of efficiency associated with large pressure drops is the formation of radial temperature gradients due to expansion and cooling of the mobile phase [11]. Under the conditions used in Fig. 2B, the temperature of the mobile phase drops by about 8°C from the inlet to the outlet, as measured by thermocouples on the end fittings [11] (see also reference [13]). In the absence of insulation, heat transfer through the column wall and the mobile phase must result in significant radial temperature gradients in the column. The center of the column is coolest, and therefore has a greater density and viscosity than that near the wall. Thus the velocity of the mobile phase at the center of the column is less than that at the wall. Viscous heating in HPLC can generate radial temperature gradients [17,18], resulting in a solute band with a parabolic profile, such that the center of the solute band reaches the outlet first. Considering only the effects of the radial temperature gradient on mobile phase velocity, the solute band in SFC would have a similar profile, but with the center of the band eluting last. Effects of temperature and density on retention factor may result in a different profile. In any case, wrapping the column with insulation would increase the axial temperature gradient, but should effectively eliminate the radial temperature gradients, resulting in a flat band profile. This has apparently been achieved with some success here. Attempts to eliminate the radial temperature gradients in analytical scale HPLC columns resulted in marginal improvements in efficiency [19]. Schoenmakers et al. [20] developed a model for temperature gradients in packed-column SFC, and showed that under typical SFC conditions (pressure drops less than 10 bar) the temperature drop for a column with thermostatted walls should not exceed several tenths of a degree Celsius. They predicted the presence and magnitude of radial temperature gradients, but did not address their impact on efficiency.

One approach to completely eliminate radial temperature gradients would be to use an adiabatic column, in which there is no heat transport across the walls. A column with stainless steel walls covered with insulation is not truly adiabatic due to significant longitudinal conduction of heat along the walls [17,20,21]. As a result, the temperature profile along the column wall does not match that of the core, allowing for the generation of radial temperature gradients. Given the significant improvement in efficiency obtained here with an insulated stainless steel column, it is suggested that even further improvements might be obtained by using a column with a thermally insulating wall. Columns with walls of polyetheretherketone (PEEK) suitable for HPLC are commercially available. Such columns, with additional insulation, should provide something close to an adiabatic column. We have not yet tested these for SFC. (Safety warning: PEEK columns have a much lower temperature limit than stainless steel. Any attempt to use these columns at elevated temperatures and pressures should include appropriate safety precautions).

The insulated column also shows a significant decrease in retention relative to the uninsulated case. A simple explanation for this is offered here. The pressure at the outlet is the same in both cases, but the temperature is somewhat lower near the outlet for the insulated column, and therefore the density of the mobile phase is higher. This would lead to lower values of the retention factor in the insulated column, and thus shorter retention times, at low temperatures when retention is controlled primarily by density. This may also have a positive impact on efficiency, because the more strongly retained solutes would not undergo such large changes in retention factor near the outlet as they would in the uninsulated column. Changes in solute retention have been identified as a significant source of efficiency loss [4,7].

The apparent retention factor is defined as $(t_r - t_o)/t_o$, where t_r and t_o are the retention times for the solute and the mobile phase, respectively. Assuming that methane is unretained, the apparent retention factors (Table 1) show a significant decrease in retention for the insulated column relative to the uninsulated column at the same pressure. Even at the smaller pressure drop, the decrease in the apparent retention factor is significant. The effect of

pressure drop on retention has been treated for isothermal conditions [3,14], but in these experiments the pressure drop is associated with a significant temperature drop, and a straightforward treatment of the overall effect on the apparent retention factor is not presently available. As stated earlier, the inlet and outlet pressures were set to achieve the desired values of $\langle\rho_R\rangle_t$ assuming isothermal conditions at 50°C. With the significant temperature drops involved, the actual values of $\langle\rho_R\rangle_t$ are not known, but are almost certainly greater than the indicated values.

Loss of efficiency due to large pressure drops is not a significant problem at high mobile phase densities due to the low compressibility of the dense fluid. A chromatogram of the same sample obtained at $\langle\rho_R\rangle_t = 1.5$ with an uninsulated column is shown in Fig. 2C. The pressure drop was adjusted to yield retention times which are similar to the other cases in Fig. 2. There is no apparent efficiency loss due to pressure drop, but the retention factors are small and the resolution is not as good as with the insulated column at the lower density. The same conditions used with an insulated column produced essentially the identical chromatogram. Thus there appears to be no advantage to using an insulated column at high densities. It should be noted, however, that the solutes used in this study are very weakly retained at $\langle\rho_R\rangle_t = 1.5$. The situation could be different for more strongly retained solutes.

Reproducibility of retention times in chromatography is generally a major quality assurance issue. While the chromatogram in Fig. 2B was highly repeatable in all respects when two consecutive injections were made (e.g., retention times for *n*-octadecane were 34.5 and 34.4 s), the long-term reproducibility of retention times has not been examined.

In HPLC the magnitude of the radial temperature gradient due to viscous heating depends on column diameter and the pressure gradient, in addition to other parameters including mobile phase velocity [17,18]. Thus the effect of pressure drop on efficiency would depend not only on mobile phase conditions, but on column geometry as well. The relationship between column geometry and efficiency in packed-column SFC is a topic that has been studied [1,9,22], but now needs to be re-examined

with the effects of thermal conditions as an important consideration.

4. Conclusion

These results indicate that the use of insulated columns may provide a simple, inexpensive solution to the problem of efficiency loss due to pressure drop at low reduced densities and temperatures in packed-column SFC, while also providing improvements in speed and resolution. This approach addresses directly the band spreading due to radial temperature gradients; other contributions to band spreading due to nonuniform conditions, such as changes in density, temperature, and retention factor along the column axis, have not been examined in this study. Further investigations into the nature of the phenomena involved are in progress.

Acknowledgements

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