Journal of Chromatography A, 1218 (2011) 2058–2064

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Kinetic behaviour in supercritical fluid chromatography with modified mobile phase for 5 μm particle size and varied flow rates

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ARTICLE INFO

Article history:
Received 29 July 2010
Received in revised form 9 December 2010
Accepted 14 December 2010
Available online 21 December 2010

Key words:
Supercritical fluid chromatography
Efficiency
Particle size
High flow rate
Temperature gradient
Kinetic plots

ABSTRACT

After much development of stationary phase chemistry, in recent years the focus of many studies in HPLC has shifted to increase the efficiency and analysis speed. Ultra high pressure liquid chromatography (UHPLC) using sub-2 μm particles, and high temperature liquid chromatography (HTLC), using temperatures above 100 °C have received much attention. These new approaches allow the use of flow rates higher than those classically used in HPLC, reducing the analysis duration. Due to the low viscosity of supercritical fluids, high velocities, i.e. high flow rates, can be achieved with classical pumping systems typically used in supercritical fluid chromatography (SFC). The effects of the flow rate increase with CO_2/methanol mobile phase was studied on the inlet pressure, t_0, the retention factor of the compounds, and on the efficiency. Simple comparisons of efficiencies obtained at varied temperature between SFC and HPLC, with a packed column containing 5 μm particles, show the greater kinetic performances achieved with the CO_2/methanol fluid, and underline specific behaviours of SFC, occurring for high flow rates and sub-ambient temperature. Some values (N/t_0) are also compared to UHPLC data, showing that good performance can be achieved in SFC without applying drastic analytical conditions. Finally, simple kinetic plots (t_0 vs N) at constant column length are used to select combinations of temperature and flow rate necessary to achieve a required theoretical plate number.

1. Introduction

Recently many papers have focused on the use of sub-2 μm particles in ultra high performance liquid chromatography (UHPLC) because of the very high efficiency achieved with these materials [1–17]. Obviously, the very high pressures required to use these particles have led to work with specially designed pumps, able to apply pressures of several hundred up to one thousand bars. There have been concurrent developments in the use of kinetic plots, which allow one to compare efficiency for all types of chromatographic systems, including column dimensions, particle size, temperature and flow rates. Some studies have emphasized the importance of frictional forces at high flow rates with small particles, requiring the use of small diameter columns to provide efficient heat transfer in order to avoid efficiency loss [16,17]. Moreover, high temperature liquid chromatography (HTLC) employs elevated temperatures to reduce the C term and to reduce solvent viscosity, allowing higher flow rates to be achieved with small particles without excessive pressure drop.

It was logical that these small particles should be tested in supercritical fluid chromatography (SFC), because the lower viscosity of these fluids is favourable to that use. Higher flow rates are routinely used in SFC, greatly reducing the analyses time [18–20]. Due to higher diffusion coefficients, the C term of the Van Deemter curve is smaller in SFC than in HPLC, and the optimum efficiency is obtained around 3–5 mL/min using 4.6 mm i.d. columns packed with 5 μm particles [18,19]. Moreover, despite the back pressure required at the column outlet (above 80 bar) and these high flow rates, the inlet pressures are not higher than those observed in HPLC with similar columns at 1 mL/min.

The dependence of retention and efficiency on temperature, pressure and flow rate is more complex in SFC than in HPLC. Solute retention depends strongly on mobile phase density, which in turn depends on the temperature and pressure. Due to the compressibility of the mobile phase, the mobile phase density and linear velocity vary along the column, and the average density increases with the flow rate when the outlet pressure is held constant.

The effects of these variations on the observed retention and efficiency have been discussed in several different studies [21–25]. Because of these more complex behaviours, recent approaches to the use of kinetic theory to predict overall performance in HPLC are not so easily transferred to SFC. For example, the utility of widely adopted Poppe plot (N/t_0 vs N) is based partly on the assumption

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doi:10.1016/j.chroma.2010.12.057

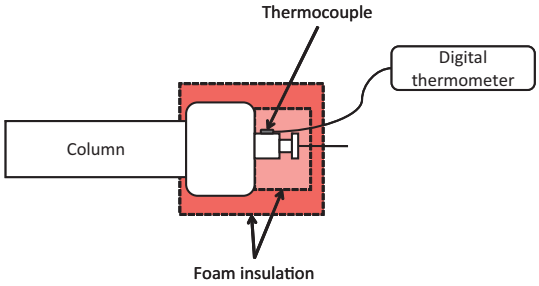

Fig. 1. Schema of the temperature measurement at the column extremities.

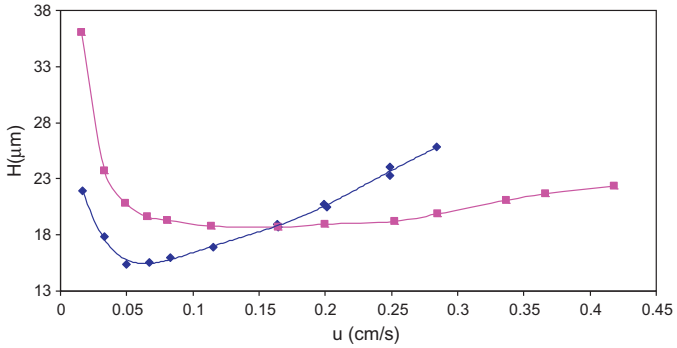

Fig. 2. Variation of the height equivalent plate theoretical (HEPT) vs the mobile phase linear speed (u) in HPLC. Column: Strategy ODS 2 (250 mm × 4.6 mm; 5 µm); mobile phase: methanol/water (75/25, v/v). Temperature: 20 °C (blue diamond); 40 °C (pink square). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

that retention volumes are only weakly dependent on flow rate, which is clearly not the general case in SFC.

In recognition of this situation, we believe that it is too early to predict overall chromatographic performance for a wide range of operating conditions in SFC. Rather, a limited study, including a careful examination of the effects of flow rate and temperature on basic chromatographic phenomena, and application of simple plots to describe performance, in term of plate number, is necessary prior to checking the efficiency of SFC with smaller particle sizes. In this paper we report on such a study using a single column with classical particle size (5 µm) in order to provide guidance in planning future experiments with smaller particle sizes.

2. Material and methods

2.1. Apparatus

The Berger Minigram was used (TharSFC,Waters, Milford, MA, USA). It included a pump cooled at 5 °C by Peltier effect for the CO_2 component of the mobile phase. The column was set in a forced air oven (JetStream, BIO SERV, Thiais, France). The mixed mobile phase was set at the required temperature by circulating in a 50 cm capillary located in the oven before the injection valve (Rheodyne, Cocati, USA). This valve was located outside the column oven. The detector (Knauer, Berlin, Germany) contained a high pressure cell. All data were recorded by the ProNTo software, and retention time and theoretical plate number were calculated by this software. The t_0 was measured by the first negative deflection of the signal due to the passage in the cell of the unretained dilution solvent.

Solvents for HPLC (water and MeOH) were provided by Carlo Erba. CO_2 was provided by Messer (Industrial quality). All experiments in this study, except for the temperature measurements described below, were performed using a silica column from Interchim (Montluçon, France): Strategy ODS 2 (250 mm × 4.6 mm, 5 µm).

The temperature measurements were done in a separate set of experiments on a different SFC instrument described briefly elsewhere [26] using 5% methanol in CO_2 and an Interchim 5 µm RP-C18 column, 250 mm × 4.6 mm i.d. (Fig. 1). For these measurements the injector was removed and the mobile phase was passed through a 50 cm long tempering coil placed in the column oven before the column. Digital thermometers (Fischer Scientific) and small button-style thermocouples with a flat round surface 5 mm in diameter were used to measure the temperature. The probes were attached to the small column endfittings with metal foil tape. The probe and endfittings were covered with polyurethane foam insulation so that only the main body of the column was exposed to the circulating air in the oven.

2.2. Preliminary experiments in SFC

Because many parameters can induce efficiency changes or erroneous measurements, different parameters were studied before performing the studies: solute nature, mobile phase composition, dilution solvent nature, injected volume, detector response time, solute concentration, detection wavelength.

Two compounds were first selected to ensure a sufficiently large retention factor: naphthalene and hexadecylbenzene. The retention factor obtained for hexadecylbenzene at 25 °C and 5% MeOH in the CO_2 was around 3. In HPLC, this value seemed sufficient to avoid the extra void volume effect. Several dilution solvents were studied: MeOH; ACN; MeOH/CH_2Cl_2; ACN/CH_2Cl_2; MeOH/ACN/CH_2Cl_2. The retention factor for both compounds were unaffected by the solvent dilution nature, but the theoretical plate number were greater for mixtures including methylene chloride. Because of its lower retention factor (0.5), the efficiencies (N) measured on naphthalene were lower and more variable than those obtained with the hexadecylbenzene. It led us to select for SFC experiments the alkylbenzene as probe, and the ternary dilution solvent. The wavelength was set at 210 nm because of the greater compound response, the response time of the detector at 0.2 s, the concentration of the injection solution at 1000 ppm, the injected volume at 2 µL, with a pre and post volume of methanol equal at 5 µL (sandwich injection).

3. Results and discussion

3.1. SFC vs HPLC

3.1.1. Flow, pressure and velocity

To be sure that our measurements could be reliable, we first measured the theoretical plate number with our 5 µm column in HPLC with a MeOH/water (75/25, v/v) mobile phase, with naphthalene, often used in HPLC, at 20 and 40 °C. The flow rates ranged from 0.1 to 1.7 mL/min at 20 °C and from 0.1 to 2.5 mL/min at 40 °C, corresponding to pressures ranging from 20 to 285 bars.

Fig. 2 shows the experimental HPLC data from the current work, that it adheres to the classical behaviour in HPLC. The increase in temperature (from 20 °C to 40 °C) reduces the Van Deemter C term, and the curve becomes flatter for high flow rates. The optimum HETP at 20 °C is around 15 µm, which corresponds to a reduced plate height value of 3, a classical value for HPLC. However, the minimum HEPT at 40 °C is a little higher (around 18 µm). This behaviour, i.e. an increase in the minimum HEPT for an temperature increase, could be strange but is reported with retention factor of compounds lower than 8 [12]. In our case, the retention factor of naphthalene at 20 °C was equal to 3 and equal to 2.5 at 40 °C.

Moreover, the pressure variation was perfectly described by straight lines (Fig. 3), ensuring the proper pump delivery, the slope of the one measured at 20 °C being higher due to the higher fluid viscosity (η) at 20 °C.

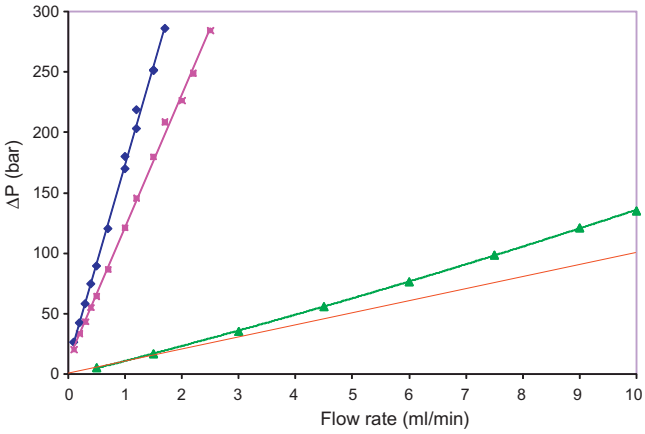

Fig. 3. Variation of the pressure drop vs the flow rate. HPLC: temperature: 20 °C (blue diamond); 40 °C (pink square), same mobile phase as in Fig. 2. SFC: green triangle: same column as in Fig. 2; mobile phase: CO_2/MeOH (95/5, v/v), temperature = 20 °C; Oulet pressure: 15 MPa. Red line; slope of SFC plot at low flow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Following the Darcy law:

$$\Delta P = \frac{K\eta L u}{d_p^2} \quad (1)$$

with K: flow resistance coefficient; L: column length; u: mobile phase linear speed; d_p: particle diameter.

The ratio between the two slopes reflects the ratio in viscosities at the two temperatures, which is equal to 1.48 in HPLC. Over the studied range of flow rates, the t_0 values were identical for the two temperatures (Fig. not shown), consistent with expected behaviour.

As expected, the pressure drop obtained in SFC at 20 °C is dramatically lower than that obtained in HPLC, due to the lower mobile phase viscosity. The ratio of the two slopes HPLC/SFC at 20 °C is around 12, so that high flow rates can be easily reached in SFC, despite the backpressure required to keep the fluid in a supercritical state. One can also note that the curve is not perfectly a straight line (in red on Fig. 3), because the fluid density, which depends on the pressure, increases with the flow rate increase. Consequently, the fluid viscosity increases as the flow rate, and the pressure variation is not strictly linear.

Fig. 4 displays the u_0 values for the different flow rates (F) on the same column with the two techniques (HPLC and SFC), up to $u = 0.5$ cm s^{-1}. The flow rates indicated are those provided by the pumping system, whereas the linear speed (u) are measured by the non retained peak of the dilution solvent (t_0). As expected, due to the lower viscosity of the supercritical fluid, the linear speed of the mobile phase reaches higher values, around 1.5 cm s^{-1} at 10 mL min^{-1} in SFC (not shown in Fig. 4) than those encountered both in HPLC and in UHPLC [14], whereas the inlet pressure in SFC stays lower than 400 bar.

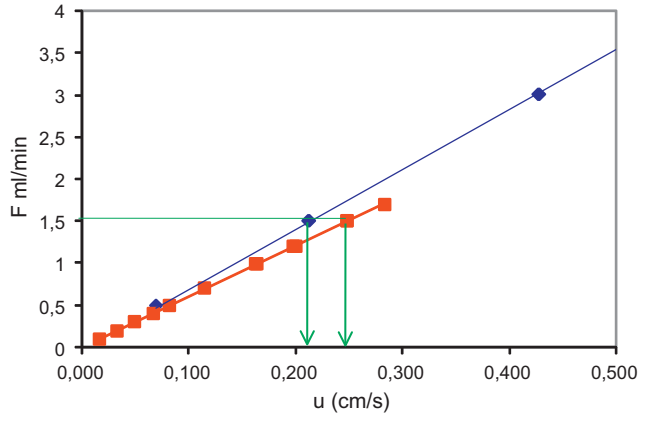

Fig. 4. Variation of u_0 vs F at 20 °C in SFC (blue diamond) and HPLC (red square). Other conditions as in previous Fig. 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

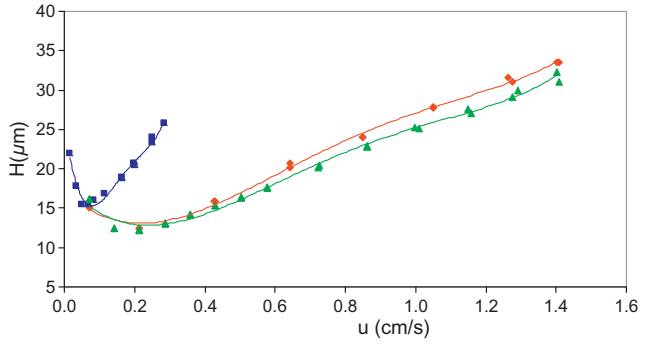

Fig. 5. Variation of the height equivalent plate theoretical (HEPT) vs the mobile phase linear speed (u) in HPLC and SFC at 20 °C. Same column as in Fig. 2. Blue square: HPLC same conditions that in Fig. 2; red diamond: SFC same conditions as in Fig. 3. Green triangle: SFC with pure CO_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The other interesting point in Fig. 4 is that at identical flow rates the linear velocity is lower in SFC than HPLC, corresponding to longer t_0 values in SFC. While the cause of this discrepancy is not totally clear, it is not surprising. In SFC the volumetric flow rate in the column can differ substantially from the nominal pumping rate due to several factors. Expansion of the mobile phase in the column due to higher temperature and lower average pressure than at the pump, or strong adsorption of mobile phase components onto the stationary phase, which is known to occur in SFC [26–30], would lead to a decrease in t_0, which is opposite to the observed behaviour. We believe that the discrepancy is probably due to the compression of the modified CO_2 mobile phase in the reciprocating pump, resulting in a decrease in the effective flow rate. In any case, the linearity of the curves in Fig. 4 leads us to be confident of the pumping quality of the apparatus.

3.1.2. Velocity and plate count

Fig. 5 displays the comparison of HETP variations vs u in HPLC and SFC at 20 °C (on the same column, same apparatus, and similar eluotropic strength, i.e. CO_2/MeOH 95/5, v/v; P_{outlet} = 15 MPa for SFC conditions). A classical behaviour is observed, with an increase in the optimum mobile phase linear speed (u) (0.2 cm s^{-1} in SFC; 0.05 cm s^{-1} in HPLC), a slight decrease of H optimum, 12.5 μm, i.e. a reduced plate height (h) equal to 2.5, and obviously, a flatter curve for higher flow rates, the value of 26 μm being reached for a flow rate equal to 1.7 mL/min in HPLC, and for a flow rate of 6 mL/min in SFC, y.

One can notice that the ratio between the two optimal mobile phase linear speeds (0.2/0.05) is almost identical to the one of the mobile phase linear speed 1.2/0.3 obtained for the value of H equal to 26 μm. This ratio is controlled by the ratio of the diffusion coefficient between the liquid and the supercritical mobile phase, around 4 in these conditions [18].

A curve is also plotted for pure CO_2 at the same temperature and pressure conditions. Few differences appear between pure and modified CO_2, the curve being slightly flatter for pure CO_2. Based on this observation, we have assumed that viscosity of CO_2/MeOH (95/5) could be estimated from viscosity of pure CO_2 [31,32].

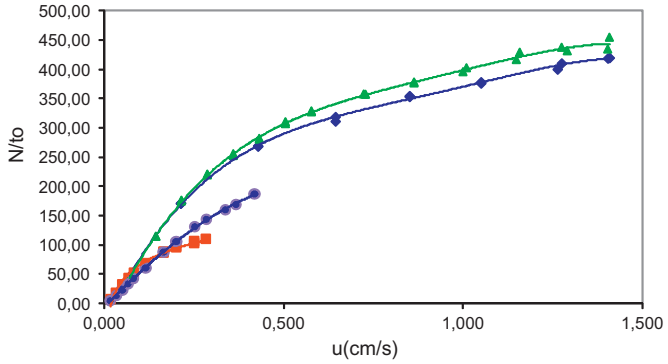

Fig. 6. Variation of N/t_0 in HPLC at 20 °C (red square: 20 °C; blue circle: 40 °C) and SFC with pure CO_2 (green triangle) and CO_2/MeOH (95/5, v/v) (blue diamond). Other conditions as in Fig. 2 for HPLC and Fig. 3 for SFC. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

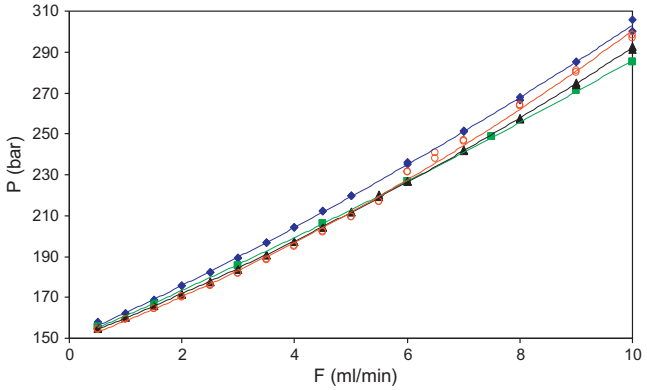

Fig. 7. Inlet pressure variation vs F at different temperatures in SFC. (10 °C) Blue diamond; (20 °C) green square; (40 °C) black triangle; (60 °C) red open circle. Mobile phase: CO_2/MeOH (95/5, v/v); outlet pressure = 15 MPa. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

By using the following equation:

$$\Delta P = \frac{\eta L u}{K_0} \quad (2)$$

with $\eta = 9.3 \times 10^{-5}$ Pa s; $L = 0.25$ m; $u_{opt} = 0.213 \times 10^{-2}$ m/s and $\Delta P = 1.7 \times 10^6$ Pa, the column permeability K_0 was estimated equal to 2.9×10^{-14} m^2.

Using the next equation:

$$E_0 = \frac{H_{min}^2}{K_0} \quad (3)$$

with H_{min} at 20 °C equal to 12.5 μm, the impedance value (E_0) at the optimum of the Van Deemter curve was equal to 5300. This value is in the same range as those obtained with some columns filled with 2 μm particles using water/acetonitrile 40/60 mobile phase at 30 °C [1,7]. Lowest values are equal to 3000–4000 for column displaying a h value equal to 2 and a column permeability K_0 between 700 and 1000 [33]. In our case, K_0 was equal to 850.

Fig. 6 displays the variations in N/t_0 vs u in HPLC. This parameter describes the compromise between high efficiency and analysis time. Because the fluid density of the mobile phase in HPLC (in our conditions, i.e. $\Delta P < 300$ bar) does not vary with the flow rate (the compressibility of liquid does not vary under these pressures), t_0 depends only on the flow rate, and at identical flow rates the change in N/t_0 is due to the modification of the theoretical plate number (N).

At velocities lower than the crossing of the curves in Fig. 2, the N/t_0 values are greater at 20 °C, then above $u = 0.17$ cm s^{-1} (1 mL min^{-1}) the N/t_0 value obtained at 40 °C becomes greater. Also for HPLC, the maximum values reached at the maximum pressure (around 300 bar in this study) are around 100 at 20 °C and 200 at 40 °C. These values correspond to classical values in HPLC [14]. At 40 °C, this value is mainly due to the greater flow rate allowed by the reduced viscosity of the mobile phase (2.5 mL/min), and because of the weak loss of theoretical plate number.

For SFC, the values of N/t_0 in Fig. 6 are slightly higher for pure CO_2 due to the better efficiency related to a higher diffusion coefficient. However, the small difference confirms that the viscosity values of the two supercritical mobile phases used in this study are similar.

In the common part of linear speed (up to 0.3 cm/s), N/t_0 values for SFC are up to twice those for HPLC, due to a greater value of the theoretical plate number. On this point, the efficiency in SFC is close to that obtained in HPLC at 40 °C in our studies, indicating that SFC could be an alternative to HTLC. For higher flow rates, allowed because of the lower pressure drop in SFC (see Fig. 3), the values N/t_0 continue to increase, to reach a value around 400/450 at 10 mL/min. This value is roughly than half of the value obtained in UPLC [1], with pressure equal to 1000 bar and column length of 5 cm. Other studies reported a value around 850 with 2 μm particles at 350 bar, 2.5 mL/min, and a column length equal to 4 cm [34].

3.2. Effect of temperature in SFC

3.2.1. Operating conditions: flow, pressure and temperature

Studies were performed at 10, 20, 40 and 60 °C using 5% methanol in CO_2 as the mobile phase, and with the column filled with 5 μm particles. Outlet pressure was kept constant at 150 bar (15 MPa).

Pressure is an important practical parameter that can place limits on maximum flow rate, especially in HPLC. Large pressure drops can also lead to significant efficiency losses in SFC [21–25]. Fig. 7 shows the relationship between flow rate and inlet pressure for the four temperatures in this study. A close examination of these curves reveals that up to 5 or 6 mL/min, the pressure increase is lower for high temperature, due to lower fluid viscosity at higher temperature. As explained previously, none of these curves is a perfect straight line. However, for higher flow rates, the pressure increases more for the higher temperatures than for the lower. The P vs F curves at 40 and mainly at 60 °C are not linear but somewhat exponential. Because the pressure is related both to flow rate and viscosity of the fluid, one can suppose that viscosity of mobile phase increases, according to Eq. (2). In UHPLC, pressure drop variation was also observed because of the friction of the mobile phase at high flow rates. In this case the friction increased the mobile phase temperature [2,13,15–17], which should reduce the relative pressure drop [8]. Consequently, to explain relative pressure drop increase in SFC, one can suppose that a cooling effect occurs, leading to an increase in viscosity of the mobile phase. This cooling effect is due to the fluid decompression, mainly in the end of the column, and it is able to decrease temperature by several degrees [35]. The cooling effect is illustrated quite dramatically in Fig. 8. At the highest flow rate, a temperature drop of approximately 3 °C is observed at an operating temperature of 60 °C. The temperature drop is higher at 60 °C probably due to opposite phenomenon: the cooling effect due to depressurisation, and a heating effect due to friction between the mobile phase and the particle (at high flow rate). Those two opposite effects act on the final temperature drop measured. A decrease in temperature from the entrance to the oulet could suggest that the cooling effect is stronger than the heating one. At 60 °C, the fluid density is lower than at 20 °C, and both the heating and the cooling effects are smaller. However, whereas the cooling effect is more or less close at 60 and 20 °C, due to the high internal pressure used in this study, the heating one could be strongly reduced at 60 °C, due

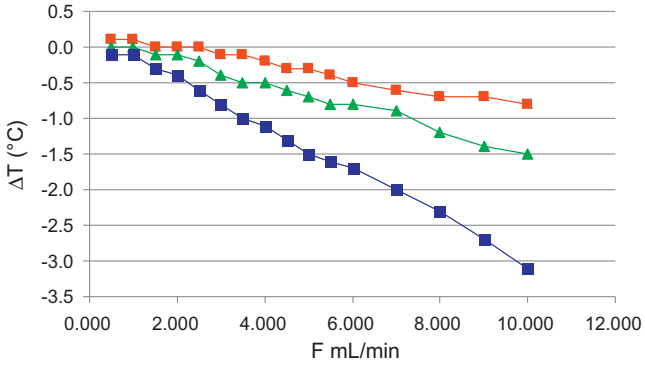

Fig. 8. Temperature difference between the inlet and the outlet of the column. (20 °C) Red square; (40 °C) green triangle; (60 °C) blue square. Conditions as in Fig. 7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

to the lower fluid viscosity. As noted below, the temperature drop also has an impact on the retention factor and on efficiency.

3.2.2. Retention factor

In SFC, the retention factor depends on both temperature and density as predicted by theory and experiment. Fig. 9 displays the retention factor variation with the flow rate for our system at four temperatures (10, 20, 40 and 60 °C). Whatever the temperature, the retention factor (k) decreases with increased flow rate. The increase in the fluid density at higher flow rates increases the eluotropic strength that decreases the retention factor.

At low flow rates, where the average pressure in all cases is near 150 bar, Fig. 9 shows that retention factor initially decreases as the temperature increases from 10 °C to 40 °C, and then increases at 60 °C. The behaviour at lower temperatures is similar to the temperature dependence for a liquid mobile phase, where temperature has little influence on density. The idea that the density of the mobile phase changes little with temperature in this region is supported by the flow and velocity data presented in Fig. 10. For temperatures at or below 40 °C, the observed linear velocity of the mobile phase at low flow rates is nearly independent of temperature, consistent with a nearly constant density over this temperature range. With little change in density, an increase in temperature results in descreased retention. At 60 °C there is a significant increase in the linear velocity above 0.8 cm/s, reflecting a decrease in the mobile phase density, resulting in increased retention. Similar retention behaviour related to pressure changes at different temperatures has been reported [19]. At high flow rates the average pressure in the column is over 210 bar, and

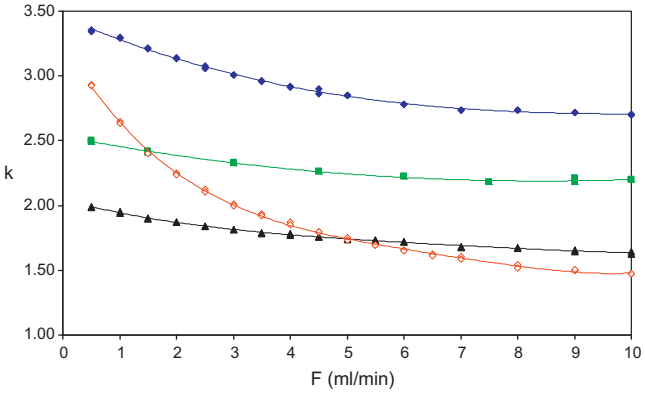

Fig. 9. Variation of the retention factor vs the flow rate at different temperatures. Conditions and symbols as in Fig. 7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

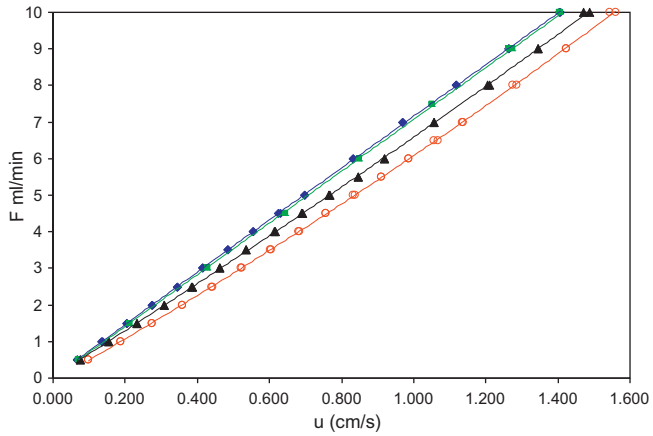

Fig. 10. Variation of u vs F in SFC at different temperatures. Conditions and symbols as in Fig. 7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the retention factor decreases steadily as the temperature in increases.

3.2.3. Efficiency

Fig. 11 displays the HETP variations at the four studied temperatures. As expected, on the basis of the classical behaviours encountered in HPLC, the increase in temperature reduces the C term of the Van Deemter curves, from 10 to 60 °C, up to linear speed of mobile phase around 1 cm/s, i.e. flow rates around 6–7 mL/min. This effect is dramatically strong from 10 to 20 °C. However, for higher flow rates, from 6 or 7 to 10 mL/min, the H value increases significantly at 40 and 60 °C, showing a loss of efficiency at these high flow rates.

The loss in efficiency at high mobile phase velocities at 40 °C and 60 °C can be attributed to the generation of temperature gradients in the column. At these temperatures significant cooling of the mobile phase occurs as the flow rate increases, as noted earlier (Fig. 8). Such temperature variation in a non-isothermal system [36] is able to create two temperature gradients, one longitudinal (between the two column extremities), and one radial (from the center to the column walls) [15,35,36]. A significant radial temperature gradient can lead to significant loss of efficiency, whereas the longitudinal one does not modify efficiency. When using an oven with a circulating air bath to control the column temperature, the cooling effect is reduced in the longitudinal axis, but the radial gradient is rather favoured because the wall are heated by the circulating air (the walls are warmed, but the center is cooled) [37,38].

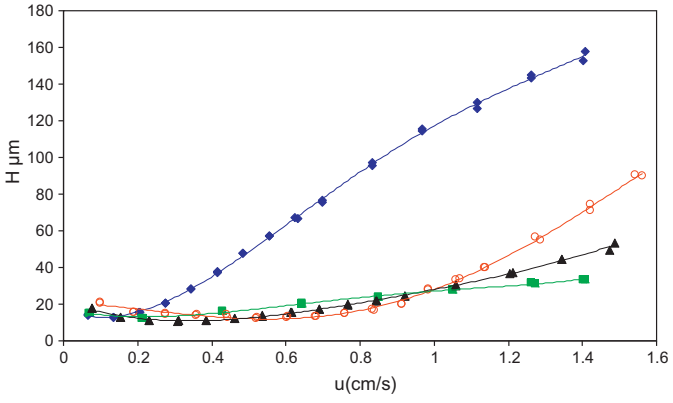

Fig. 11. Variation of H vs F at different temperatures in SFC. Symbols as in Fig. 7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

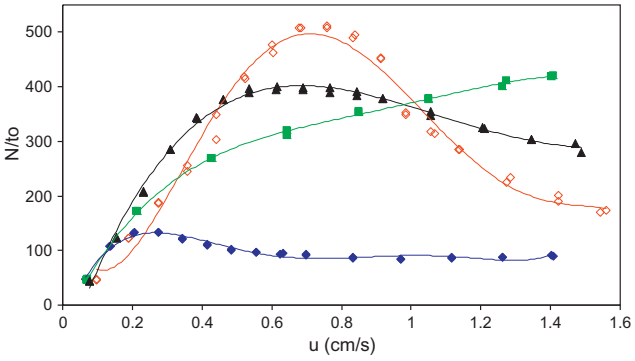

Fig. 12. N/t_0 variation vs u at different temperatures in SFC. Symbols as in Fig. 7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

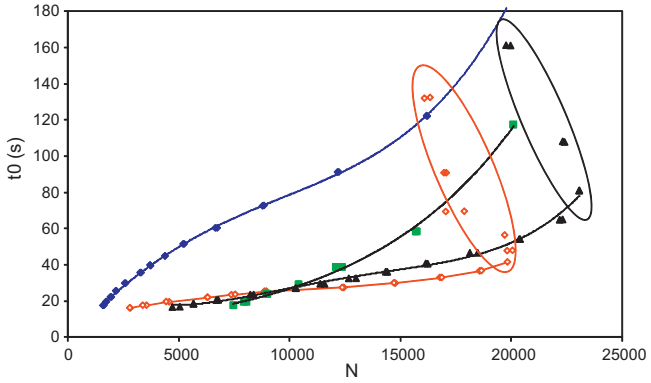

Fig. 13. t_0 variation vs N at different temperatures in SFC. Symbols as in Fig. 7. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

This temperature gradient is opposite to the one observed in UHPLC, which is due to friction. On the other hand, the radial mass transfer is very slow with the type of column used here, and the column can be divided in numerous smaller channels in which compounds are included and subject to different conditions: diffusion coefficient, eluotropic strength [39]. In UHPLC, the friction of the mobile phase increases the temperature in the center of the column, which becomes less viscous, and more eluotropic [15]. These effects cause a progressive increase of the C-branch of the Van Deemter equation [17]. As described, in SFC, the cooling effect, is greater in the column center. This increases the fluid density in the column center, which reduces the diffusion coefficient and the linear speed of the fluid, and also modifies the eluotropic strength of the fluid [40]. Due to the radial temperature gradient, the different phenomenon described above induces solute band dispersion, i.e. loss in efficiency, mainly from 40 °C.

3.2.4. Theoretical plate and analytical time

Fig. 12 shows the N/t_0 changes at different temperatures. It clearly appears that the lowest values are obtained at 10 °C, due to the lower diffusion coefficients. The increase in temperature favours the efficiency by time unit, up to a linear speed limit (0.7–0.8 cm/s) corresponding to a flow rate close to 5 mL/min at 40 °C and close to 4 mL/min at 60 °C.

At velocities greater than about 0.8 cm/s, the efficiency loss due to radial temperature gradients described earlier causes the N/t_0 value to decrease. The magnitude of this efficiency loss is related to expansion of the mobile phase and corresponding generation of axial and radial temperature gradients, and depends on a number of operating conditions including the pressure drop, temperature, particle size, column diameter, and the outlet pressure [41].

The highest value of N/t_0 in Fig. 12 is equal to 500 for a temperature of 60 °C. For fast and efficient analyses, this temperature should be selected up to u = 1 cm/s (F = 6 or 7 mL/min, at 60 or 40 °C). This performance of SFC under these conditions as measured by N/t_0 is thus about 5 times greater than that obtained in HPLC, but only the half of that obtained in UHPLC with 2 μm particles. However, due to the fluid decompression inducing a radial temperature gradient, a lower temperature (20 °C), should be preferred for very high flow rates (from 7 to 10 mL/min). It should be emphasized that the specific conditions recommended here apply only to separations carried out at an outlet pressure of 150 bar using similar packed column.

Finally, Fig. 13 displays curves which link the efficiency (N) to the t_0 time for one column (25 cm; d_p = 5 μm), at different temperatures. Obviously, in regards to UHPLC data treatment, these curves are not obtained at ΔP_{max} because 1/the column length was constant for all flow rates and 2/the column dimension at higher flow rates (10 mL/min) is 25 cm length and 0.46 cm internal diameter, that obviously provide higher t_0 value than the ones reported in UHPLC when using column of 5 cm length and 0.2 cm internal diameter. However, these curves can be used in the same way, i.e. to determine the combination of temperature and flow rate to reach a required value of N. To maintain maximum flow rate, i.e. minimal t_0 and rapid analyses, 20 °C should be preferred up to 10,000 theoretical plates, 60 °C up to 20,000 and 40 °C for higher values (up to 23,000). The figure also shows (in colored ellipsoids) that, for low flow rates (high t_0 values), strong diffusion of compounds into the mobile phase occurs, reducing the efficiency. It underlines that at 40 and 60 °C, and lower internal pressure (because the flow rate is low), the diffusion of compounds in the mobile phase causes significant longitudinal dispersion (B-term).

4. Conclusion

This analysis of kinetic performance for a column filled with 5 μm particles provides a comparison of the performances reached in HPLC, UHPLC and SFC. The best performances of SFC using 5 μm particles in a 250 mm × 0.46 mm column was about 500 plates/s approximately half of what has been reported in UHPLC with sub-2 μm particles working around 1000 bar. It indicates that UHPLC with short columns can exceed the performance of classical SFC with 5 μm particles for very fast separations. However, using longer packed columns with SFC at a maximum inlet pressure of 400 bar can provide higher total plate counts than have been reported for UHPLC. By the way, the maximum effective plate number that can be achieved is greater in SFC than in UHPLC, because the lower fluid viscosity allows the use of longer column in SFC. It ensures greater separation performances in term of resolution for complex mixtures.

At moderate velocities above the Van Deemter optimum, the plate height with increasing temperature. At higher velocities, above about 1 cm/s in this study, or 6–7 mL/min, the trend reverses, with better efficiency at lower temperatures. The decreased efficiency at the higher temperatures is due to the radial temperature gradient, which is more significant at the higher temperatures.

The primary conclusion of this paper indicates that every change of the analytical condition modifying the pressure in the column, i.e. inducing a change in radial gradient temperature by the depressurization of the mobile phase in the column, could affect the separation efficiency. This study also confirms that simple presentation of t_0 vs N at constant column length have some utility for selection of optimum operating conditions in SFC.

The full benefits of the kinetic plots used in HPLC, to select optimum combinations of column length and particle size are not

realized in SFC due to effects arising from the compressibility of supercritical fluid mobile phases, but some attempts to achieved calculation in this goal will be described in future paper

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