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Exploring the speed-resolution limits of supercritical fluid chromatography at ultra-high pressures

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ABSTRACT

The limits of supercritical fluid chromatography have been experimentally explored using inlet pressures at the limits of the current commercial instrumentation (400–600 bar), as well as pressures significantly surpassing this (up to 1050 bar). It was found that efficiencies in the range of 200,000 theoretical plates can be achieved for a void time t_0 of roughly 6 min using superficially porous particles (2.7 and 4.6 μm) while remaining within the pressure limits of current commercial instrumentation and columns. If lower efficiencies are sufficient (<100, 000 plates), smaller particles (e.g. 1.8 μm) provide the best trade-off between analysis time and efficiency. Apparent efficiencies of 83,000 (k' = 2.2) to 76,000 (k' = 6.6) plates could be achieved for void times around 1 min by pushing the pressure limits up to 1050 bar on a column length of 500 mm. As the optimal mobile phase velocity for these small particle columns is even higher, it is required to use narrow-bore columns (2.1 mm ID) to remain within the instrument limits of flow rate. The smaller column volume however puts a strain on the separation efficiency due to extra-column band broadening, resulting in losses up to 50% for weakly retained compounds for column lengths below 250 mm. It is also illustrated that when using sub-2 μm particles, especially for separations where a significant amount of organic modifier is required, the current pressure limits of state-of-the-art instrumentation can sometimes be insufficient. For a gradient running from 4 to 40 *v*% methanol on a 300 mm column at the optimal flow rate the pressure increases from 420 to 810 bar. Operating SFC-columns with a large pressure gradient induces several (undesired) side effects which have been investigated as well. It has been found that, since the viscosity increases strongly with pressure in SFC, the optimal flow rate and the minimal plate height can significantly change when the column length is changed. Whereas e.g. a 3 × 150 mm column (2.7 μm particles) has an optimal flow rate of 1.5 ml/min and minimal plate height of 5.66 μm, a 3 × 1050 mm column has an optimal flow rate of 1.2 ml/min and a minimal plate height of 6.25 μm. Nevertheless, an increase in operating pressure drop in SFC results in a significant gain in kinetic performance.

1. Introduction

Having similar densities as liquids but with viscosities up to 20 times lower (higher diffusion coefficients), supercritical CO_2 is expected to be the ideal (co-)solvent for fast and/or highly efficient separations without mass-transfer limitations or excessive column pressure drops. The higher diffusion coefficient results in a flatter C-term of the van Deemter-curve in supercritical fluid chromatography (SFC), allowing the use of higher flow rates with no significant performance loss.[1] To assess the limits of separation performance of a fully optimized chromatographic system (column length, particle size and flow rate), it is convenient to use the Knox and Saleem (KS) limit-equation, which is given by [2]

$$t_0 = \frac{h_{\min}^2 \cdot \eta \cdot \Phi}{\Delta P} \cdot N^2 \quad (1)$$

where t_0, h_{min}, η, Φ, ΔP and N are, respectively, the column hold-up time, the minimum reduced plate height, the mobile phase viscosity, the flow resistance, the maximal column or instrument pressure drop and the plate count. From Eq. (1), the advantage of SFC over LC is directly apparent, as the much lower viscosity of the mobile phase leads to a reduced column hold-up time to reach the same plate count. A similar observation was made for Hydrophilic Interaction Liquid Chromatography (HILIC) separations where the high organic content of the solvent also results in lower mobile phase

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viscosities than typically encountered in reversed-phase LC (RPLC) and for high temperature RPLC [3,4].

Whereas in supercritical fluid chromatography the maximal column pressure drop is typically around 450 bar (600 bar instrument rating – 150 bar back pressure), this goes up to 1500 bar for LC and was extended beyond 2000 bar in research set-ups [5,6].

Until now, literature reports have been comparing the kinetic performance limits of SFC and LC under conditions of equal instrument pressure, for the sake of making an unbiased comparison [1,7,8]. However, Eq. (1) shows that the higher available pressure drop in LC can compensate for its higher mobile phase viscosity, (partially) leveling the playing field. Eq. (1) represents a fully optimized system. It does not contain the particle diameter (d_p), masking that every point on the KS-limit corresponds to a different particle size. However, rewriting the equation and taking into account that the necessary condition to be on the KS-limit is $\Delta P = \Delta P_{max}$, $h = h_{min}$ and $\nu = \nu_{opt}$, the following equations can be obtained [2]

$$d_p^2 = \frac{h_{\min} \cdot \nu_{opt} \cdot \eta \cdot D_{mol} \cdot \Phi}{\Delta P_{\max}} \cdot N \quad (2)$$

$$d_p^4 = \frac{\nu_{opt}^2 \cdot \eta \cdot D_{mol}^2 \cdot \Phi}{\Delta P_{\max}} \cdot t_0 \quad (3)$$

With D_{mol} and ν the analyte molecular diffusion coefficient and the reduced velocity, defined as

$$\nu = \frac{u_0 \cdot d_p}{D_{mol}} \quad (4)$$

Eq. (2) provides the corresponding optimal value for the particle diameter, illustrating that high efficiencies can be achieved using larger particles, whereas Eq. (3) illustrates that smaller particle diameters result in lower void times (and thus faster separations). Besides the particle diameter, also the packing quality and bed morphology influence the kinetic performance through the value of minimal plate height and the flow resistance of the column.

In contrast to LC, extrapolations of kinetic performances in SFC to higher pressure drops (> 600 bar) are limited due to the high dependence of the chromatographic parameters with density and/or pressure [9,10]. And thus it is not straightforward to predict what the gain will be when, for example, the pressure drop is doubled from 450 to 900 bar in SFC. However, from Eq. 2 an increase in pressure drop is expected to result in improved performance in SFC (since the viscosity increases with only 20% when going from an average pressure of 375–525 bar for neat CO_2, based on REFPROP-values [11]) and thus the main goal of presented work is to identify how large this gain will be. In order to do so, the speed-resolution limits for SFC are expanded beyond those of commercially available equipment, for example, >600 bar instrument pressure, using a modified Agilent G4301A-Based SFC system with a pressure rating up to 1050 bar. In addition, both fully as well as superficially porous particles will be considered. Possible effects of using these higher operating pressures in SFC, such as the higher mobile phase density and viscosity and the resulting lower molecular diffusion, will also be examined.

2. Experimental

2.1. Column, tubing and chemicals

Methanol (LC–MS grade) was purchased from Biosolve (Valkenswaard, The Netherlands), CO_2 was purchased from Air Liquide (Paris, France). Test compounds aspirine, phenantrene, pyrene and benzo-k-fluoranthene were purchased from Sigma–Aldrich (Diegem, Belgium). Other test compounds such as testosterone, chlortalidone, altizide, suflamethaxozole, bendroflumethiazide and β-estradiol were kindly provided by Deirdre Cabooter (Laboratory of Pharmaceutical Analysis, KU Leuven, Belgium). The compounds for the C18 stationary phase were dissolved in isopropanol (IPA). The samples for the bare-silica columns were dissolved in ethanol (EtOH). However since this solvent leads to distorted peaks, it was mixed with IPA and Hexane (EtOH/IPA/Hexane-concentration was 20/10/70). The IPA was added to allow mixing of EtOH with Hexane [12].

2.2. Instrumentation and conditions

The SFC-system used in the study was a modified Agilent G4301A-Based SFC system with an extended column pressure range up to 1050 bar in combination with a thermostatted column compartment, autosampler with a 1.2 μL injection loop (used in full loop mode) and a DAD-detector with a 1.7 μL flow cell. For all the experiments the back pressure regulator was set at 150 bar.

Three types of columns were used in the present work: 2.1 mm ID Zorbax HILIC RRHD columns (two 150 mm, two 100 mm, fully porous, 1.8 μm), 3 mm ID HILIC Poroshell columns (superficially porous, 2.7 μm) and 4.6 mm × 250 mm C18 Ascentis Express columns (superficially porous, 4.6 μm). The HILIC columns were kindly provided by Xiaoli Wang (Agilent Technologies, Little Falls, USA) and the Ascentis Express columns by David Bell (Sigma–Aldrich, USA). The pressure rating for the Zorbax HILIC RRHD is 1200 bar, whereas it is 600 bar for the HILIC Poroshell and Ascentis Express columns.

3. Results and discussion

3.1. Pushing the limits

3.1.1. Highly efficient separations using 4.6 μm particles

From Eq. (2), it is clear that highly efficient separations are best achieved in long columns packed with larger particles, in addition to the use of superficially porous particles which have a lower minimal plate height. This was previously illustrated for a separation of triglyceride mixture separation where the resolution could be significantly increased by using long coupled columns, yielding very high efficiencies [14]. As an example, Fig. 1a shows the separation of three polyaromatic compounds (phenantrene, pyrene and benzo-k-fluoranthene) on a 4.6 mm × 250 mm Ascentis Express column (4.6 μm particles) at its optimal flow rate of 3.5 ml/min (other experimental conditions given in the caption). Using this single column 33,700 plates can be achieved with a void time of 0.6 min, close to the performance found in LC-conditions. However, since only an inlet pressure of 234 bar is needed, corresponding to a column pressure drop of only 84 bar, it is clear that this system is still operated far from its maximum kinetic performance, which is achieved at the maximum pressure drop [13]. Therefore six 250 mm columns were coupled in series, with a total length of 150 cm using stainless steel capillaries (with an ID of 120 μm). Coupling columns allows to increase the separation performance with no significant losses due to the extra volumes (if proper tubing ID is chosen and short capillaries are used) and is frequently done in literature [1,14,15].

In this configuration, the optimal flow rate was however found to be equal to 2 ml/min, versus 3.5 ml/min for the single column. The obtained separation is shown in Fig. 1b, yielding a plate count of 185,000 for a void time of 6.8 min, slightly below the expected values of around 200,000 based on the performance of the single column. As expected, due to the dependence of the chromatographic parameters on density and hence pressure, extrapolations towards higher pressure drops or longer column lengths are not straightforward [9,10]. The reasons behind the shifts in optimal