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Application of the isopycnic kinetic plot method for elucidating the potential of sub-2 μm and core–shell particles in SFC

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ABSTRACT

In this work the isopycnic method to construct kinetic plots for SFC was used to investigate the performance limits of an SFC system when using sub-2 μm fully porous particles and sub-3 μm superficially porous (core–shell) particles. This isopycnic kinetic plot method for SFC was developed and tested earlier for SFC separations on native silica with pure CO_2 as mobile phase. In the current work, octadecyl based reversed phase columns were used in combination with a mobile phase that contains 10% methanol as modifier in order to study the applicability of the described methodology to assess the kinetic performance limits of experimental setups in which SFC is used and will, according to all probability, be evolving. SFC and HPLC van Deemter and kinetic plots are constructed for columns packed with fully porous particles with various diameters and for a column packed with core–shell particles. The influence of the experimental kinetic performance limits of the particle diameter and morphology in SFC is shown to be the same as in HPLC. Additionally, kinetic plot predictions were constructed for separations on 1 μm and 0.5 μm particles using the data measured on the 5 μm, 3.5 μm and 1.8 μm fully porous particles. By doing this the potential applicability of 1 μm particles on the contemporary SFC and HPLC systems was demonstrated together with the irrelevance of the use of 0.5 μm particles in SFC.

1. Introduction

It is a general assumption that SFC can be used with longer columns and smaller particles than HPLC without the need for high pressure CO_2 pumps [1–4]. This is due to the lower viscosity of the mobile phase in SFC compared to the one in HPLC resulting in a smaller pressure drop over the column. The boundary conditions of applicable column lengths and particle sizes on contemporary instruments are, however, only rarely investigated and therefore exploited. This is partially due to the fact that these limitations depend on the viscosities of the mobile phases used. However, the most important reason for this lack of knowledge is that, until recently, there was no possibility to measure the kinetic performance limits (KPL) of SFC separations in an accurate way. Comparing the true potential of chromatographic systems with different pressure characteristics (mobile phase viscosity, column length, particle size and column morphology) can only be done by the means of constructing kinetic plots because the kinetic plot method not only uses the separation performance as a measure of comparison, but also directly incorporates the column pressure drop information [5–7].

Due to the compressible character of the mobile phases in SFC, the construction of kinetic plots in SFC is more challenging than in HPLC. Measuring the SFC performance with fixed back pressure results in varying retention factors (k) [8,9]. As a consequence, the effect of flow rate on performance becomes obscured by the accompanying changes in retention and diffusion coefficients and therefore the performance can essentially not be measured as a function of flow rate while keeping the outlet pressure a constant. Although this fact is generally accepted, attempts have been made to construct H–u plots [10–12] or kinetic plots [13] by measuring the performance in SFC keeping the outlet pressure a constant. A remedy was proposed by Poe et al. and Xu et al. as they described an isopycnic method of measuring plate heights (H) as a function of changing flow rate while keeping the average density constant [14,15]. In this methodology the back pressure applied after the detector is lowered with increasing flow rate such as to keep the average mobile phase density a constant during construction of the H–u plot. Recently the correctness of the isopycnic construction of kinetic plots in a fast way for applications in SFC was corroborated [8]. A comparison between the kinetic performance limits in SFC and HPLC was made but the SFC separations were then performed on bare silica columns with only 1% modifier and at 50 °C in order to work under the most challenging conditions. The isopycnic kinetic plot method needs evaluation under more realistic experimental parameters since

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SFC applications are typically performed using organic modifier amounts between 10% and 40% [16]. Also, there is a growing interest in the use of reversed phase columns in SFC [17–20] and therefore it is logical to evaluate the possibility to construct isopycnic kinetic plots in SFC on C18 columns.

Columns packed with sub-2 μm fully porous particles and sub-3 μm superficially porous particles are widely used in liquid chromatography in order to develop fast separations without the loss of resolution [21–25]. As a consequence of the high pressure drops that are present when working with sub-2 μm particles in LC, ultra high pressure LC (UHPLC) instruments were developed that can deliver up to 1300 bar. A drawback of working with those very high pressure drops is the need to work with columns with small internal diameters (around 2 mm) in order to avoid efficiency losses caused by the presence of thermal gradients inside the columns due to viscous heating [26–32]. This means that it is not possible to perform efficient UHPLC separations on columns broader than 2.1 mm limiting the use of small particles for semi-preparative UHPLC separations. As supercritical fluids are less viscous than liquids, SFC separations are performed with lower pressure drops over the column than HPLC separations and for this reason columns packed with small particles can be used in SFC on instruments with conventional pressure limitations [1,12,33]. More recently, the possibilities of using superficially porous particles for SFC separations were investigated [19,34–36]. It is, however, currently unclear where the performance limits of contemporary SFC systems are when applying sub-2 μm and core–shell particles as there are only few, if any, reports found in the literature that compare kinetic plots for SFC separations on different particle sizes and porosity and no kinetic plot comparisons were made between UHPLC and SFC separations on this particles. In the light of envisaging future 1 μm and sub-micron sized particle design, the boundary conditions of the use of current state of the art particles require more unequivocal determination. As a consequence of this, it is important to keep in mind that when smaller particles are used, working at the kinetic performance limit of an SFC system would be accompanied by fairly high pressure drops over the column. As was thoroughly investigated recently, this pressure drops can result in rather high axial and radial temperature inhomogeneities due to the cooling of the compressible mobile phase as it decompresses in the column which in their turn can result in efficiency losses when the experimental parameters are not well chosen [37–45].

In this contribution the SFC isopycnic method is applied for the construction of SFC based kinetic plots for smaller particle sizes and for core–shell type of particles. The influence of particle size and morphology on the kinetic performance limit is investigated for SFC separations on C18 columns using mobile phase compositions which are representative of current applications. Extensive comparison with HPLC and extrapolations to future particle dimension are performed and applicability of the various column formats is investigated for analytical analyses.

2. Experimental

2.1. Apparatus

SFC measurements were performed on a Jasco SFC system (Jasco Corporation, Tokyo, Japan) equipped with following modules: Jasco *PU-2080-plus* HPLC pump, Jasco *PU-2080-CO_2-plus* CO_2 delivery pump, *AS-2059-SF-plus* auto sampler for SFC, *UV-2070-plus* UV–vis detector with high pressure flow cell and *BP-2080-plus* automatic back pressure regulator (BPR). For all SFC measurements, the columns were placed in a polaratherm series 9000 (Selerity Technologies Inc., Salt Lake City, USA) oven set at 40 °C, with preheater at the same temperature and post-column cooler temperature set at 20 °C. Instrument control and data treatment were performed with the ChromNav software (version 1.14.01).

The HPLC measurements were performed on an Agilent 1100 system (Agilent Technologies, Waldbronn, Germany) equipped with a DAD detector. HPLC measurements were performed at room temperature. Chemstation software (version B.03.01) was used for data treatment and instrument operation.

2.2. Chemicals

N48 grade CO_2 was purchased from Air Liquide (Liege, Belgium). Methanol and acetonitrile (both HPLC grade) were purchased from Biosolve (Valkenswaard, The Netherlands). Milli-Q water was prepared in house by a Water Purification Instrument of Millipore (Overijse, Belgium). Uracil, naphtalene, phenantrene, pyrene and benzo(a)pyrene were purchased from Sigma-Aldrich (Bornem, Belgium).

2.3. Chromatography

Agilent Zorbax SB-C18 columns (Agilent Technologies, Brussels, Belgium) of 250, 150 and 100 mm length packed with 5, 3.5 and 1.8 μm particles respectively were used for the experiments with the fully porous packing material. A 100 mm Phenomenex Kinetex XB-C18 column (Phenomenex, Utrecht, The Netherlands) packed with 2.6 μm particles was used to evaluated the performance of core–shell material in SFC.

2.3.1. SFC experiments

For the SFC experiments, the mobile phases consisted of CO_2/MeOH (90:10). The column oven temperature was set at 40 °C and the average pressure in the column was kept at 200 bar (see methodology section). A target amount of modifier between 10% and 40% was chosen in order to work at conditions that are close to the ones used in practical SFC applications. In the presented results 10% of methanol was used because the desired retention factor of 5 for the last eluting compound could be reached with this amount of modifier. Samples consisting of 100 μg/mL naphthalene, phenanthrene, pyrene and benzo(a)pyrene were dissolved in methanol. The injection volume was 2.4 μL for all SFC separations.

2.3.2. HPLC experiments

The mobile phase used on the columns packed with 5 μm, 3.5 μm and 1.8 μm fully porous particles was ACN/H_20 (85:15). For the core–shell column a lower amount of acetonitrile was used: ACN/H_2O (79:21). The composition of the mobile phase was chosen such that the retention factor k of the last eluting component would be the same as in the SFC experiments ($k_{B(a)P}$=5). The HPLC measurements were performed at room temperature.

The same four PAHs as for SFC were dissolved at 100 μg/mL in ACN/H_2O (85:15). Uracil was added at 20 μg/mL in order to determine the dead time t_0. The injection volume was 2 μL for the separations on the column packed with 5 μm particles and 1 μL for the separations on all the other columns. Detection was performed via UV detection at 254 nm for both the SFC and the HPLC analyses.

3. Methodology

In this work, all SFC van Deemter and kinetic plots were constructed using the isopycnic method described in previous work making sure that the retention factor k is a constant when

varying the flow rate [8]. The average column pressure was set at 200 bar by careful selection of the back pressure values for every SFC analysis. Since measurements were recorded up to high flow rates (creating a significant extra-column pressure drop), the pressure drops in the connection tubing before and after the column were also measured as a function of flow rate in preliminary experiments. All displayed data points are the average of three consecutive runs.

All "measured" kinetic plots are the result of extrapolating the measured $t_{0,exp}$ and N_{exp} values to the KPL values using the kinetic performance limit (KPL) equations. These basic equations that allow to establish the kinetic plot of a chromatographic system with a pressure drop limit of $\Delta p_{max,sys}$ starting from the efficiency (plate count N_{exp}), column dead time ($t_{0,exp}$), column pressure drop ($\Delta p_{col,exp}$) and extra column pressure (Δp_{ec}) measured at different flow rates (F_v) on a fixed column length are given by [6]

$$t_{0,KPL} = \lambda t_{0,exp} \quad (1)$$

$$N_{KPL} = \lambda N_{exp} \quad (2)$$

$$L_{KPL} = \lambda L_{exp} \quad (3)$$

with λ given by:

$$\lambda(F_v) = \frac{\Delta p_{col,max}}{\Delta p_{col,exp}} = \frac{\Delta p_{sys,max} - \Delta p_{ec}(F_v)}{\Delta p_{sys,exp} - \Delta p_{ec}(F_v)} \quad (4)$$

where $\Delta p_{col,max}$ is the maximum pressure drop that can be applied over the column, $\Delta p_{col,exp}$ is the experimental pressure drop over the column during the measurement, $\Delta p_{sys,max}$ is the maximum pressure drop that can be applied over the chromatographic system (from pump to waste or back pressure regulator), $\Delta p_{sys,exp}$ is the experimental pressure drop over the chromatographic system during the measurement and $\Delta p_{ec}(F_v)$ is the extra column pressure as a function of flow rate F_v.

4. Results and discussion

4.1. Column evaluation

4 PAHs were selected and separated by HPLC and SFC a various flow rates on C18 columns packed with decreasing particle sizes and with superficially porous particles. In order to work under representative SFC conditions the mobile phase contained 10% organic modifier. From the resulting chromatograms, the plate heights as a function of flow rate were obtained. By combination with the measured pressure data and the KPL Eqs. (1)–(4), the corresponding kinetic performance limit plots could be constructed.

Fig. 1 represents chromatograms recorded on the column packed with 1.8 μm particles under SFC and HPLC conditions. The compounds eluted in following order for both separation modes: naphthalene (1), phenantrene (2), pyrene (3) and benzo(a) pyrene (4). It can be seen that a good peak shape and symmetry were obtained for all compounds in SFC and HPLC. By careful selection of the respective mobile phase compositions in both the same retention factor in SFC and HPLC was obtained for the last eluting compound ($k_{benzo(a)pyrene}=5$). The earlier eluting compounds depict a slightly different k in HPLC, as it is impossible to independently change the retention factor of the different compounds under isocratic conditions.

Fig. 2 illustrates the influence of the particle size on the van Deemter curves in HPLC and SFC for benzo(a)pyrene. If the curves for the fully porous particles are considered, it can be seen that for both techniques the plate height decreases and that the optimal linear velocity ($u_{0,opt}$) increases with decreasing particle size because of the decreasing A- and C-terms [32]. The corresponding

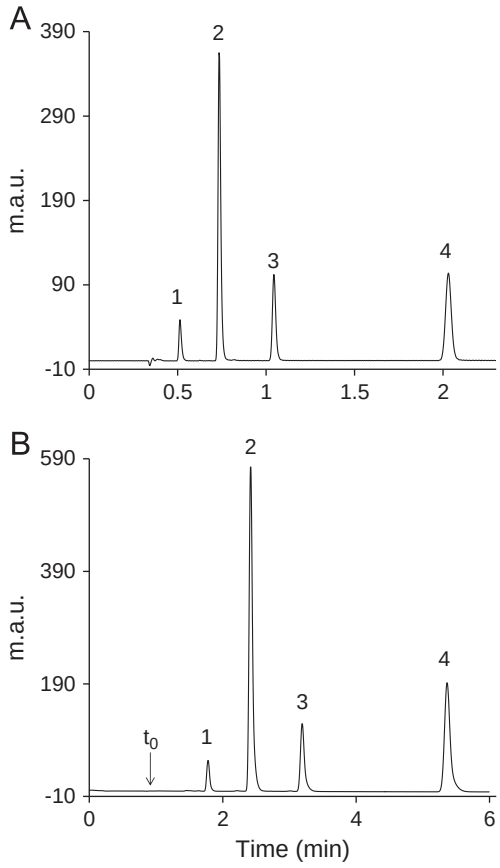

Fig. 1. Example chromatograms at optimal flow rate measured on the column packed with 1.8 μm fully porous particles. (A) SFC separation of the components, $F_v=3$ mL/min, $p_{BPR}=139$ bar, and $p_{av}=200$ bar. (B) HPLC separation of the components, $F_v=1$ mL/min (uracil not present in this chromatogram). Sample consisting of naphthalene (1), phenanthrene (2), pyrene (3) and benz(a)pyrene (4).

C-term is significantly shallower in the SFC experiments compared to HPLC as can be seen in Fig. 2A and B, respectively. The steep slope of this term in HPLC for the 1.8 μm particles is related to the effect of viscous heating and because of the use of a forced-air oven [28,46].

The H_{min} measured on the columns packed with 5 μm and 3.5 μm fully porous particles, was equal to $2d_p$. This was not the case for the column packed with 1.8 fully porous particles. As this was observed in both HPLC and SFC experiments, this is possibly linked to less efficient packing of the 1.8 μm column (4.6 mm) compared to the 5 μm and the 3.5 μm columns.

The curves measured on the column packed with superficially porous kinetex particles coincided closely to the curves measured on the column packed with 1.8 μm fully porous particles. For HPLC this core–shell curve is situated at lower H values than the curve for fully porous 1.8 μm particles while for SFC, the difference between the curves is smaller. The diffusion coefficients (D_{mol}) of the analytes are lower in HPLC compared to SFC and as a consequence the relative importance of the lower resistance to mass transfer in superficially porous particles is accordingly higher in HPLC compared to SFC [47].

Fig. S1 (in Supplementary material) displays a comparison of the measured plate heights in both the HPLC mode and the SFC mode for benzo(a)pyrene on the column packed with 3.5 μm fully porous particles. The higher diffusivity in supercritical fluids compared to liquids results in a 3 times higher optimum linear velocity in SFC ($u_{0,opt,HPLC} \approx 1.5$ mm/s, $u_{0,opt,SFC} \approx 5$ mm/s). The minimum plate height measured in both techniques is comparable ($H_{min} \approx 7$ μm$=2d_p$).

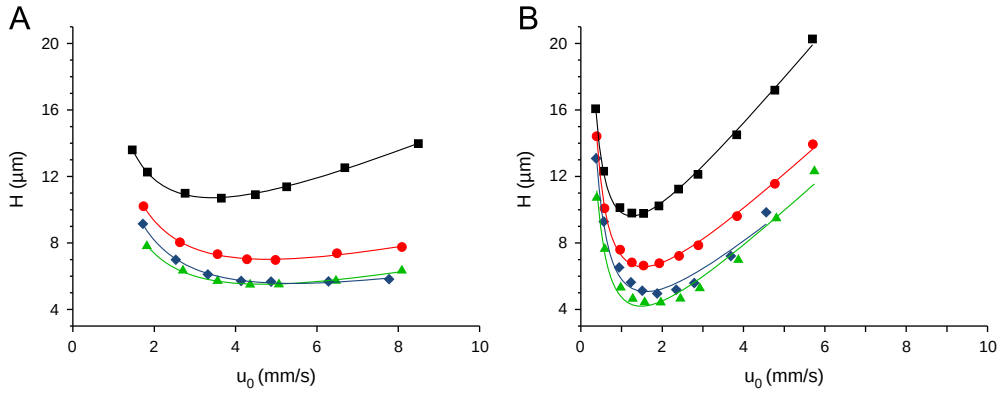

Fig. 2. Comparison of van Deemter curves measured on the different columns for benzo(a)pyrene in SFC (A) and HPLC (B). SFC curves are measured isopycnic with p_{av}=200 bar. Black squares: column with 5.0 μm fully porous particles. Red circles: column with 3.5 μm fully porous particles. Blue diamonds: column packed with 1.8 μm fully porous particles. Green triangles: column packed with 2.6 μm superficially porous particles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

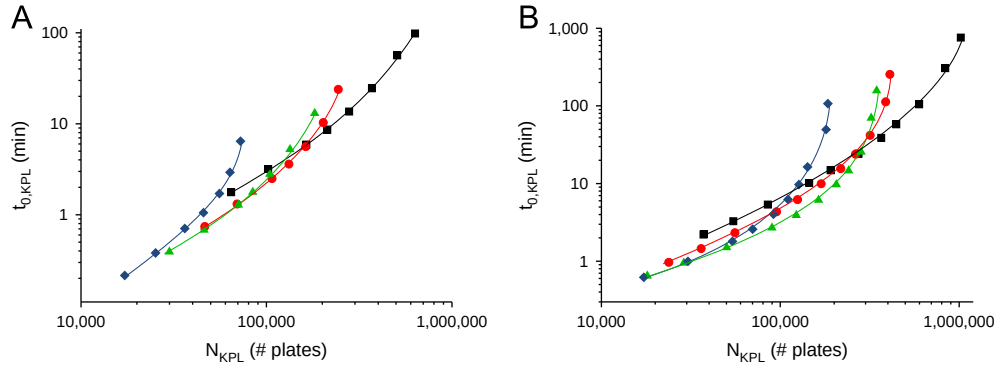

Fig. 3. Comparison of kinetic performance limit curves measured on the different columns for benzo(a)pyrene in SFC (A) and HPLC (B). SFC curves are measured isopycnic with p_{av}=200 bar. Symbols as in Fig. 2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

As the only way to truly assess the performance of columns and systems involves the incorporation of the pressure/permeability information, the kinetic plot method outperforms the van Deemter curve in terms of information it can provide. In previous work, it was shown that the isopycnic way of working can deliver correct kinetic plots for SFC separations with nearly pure CO_2 as mobile phase on bare silica particles with a diameter of 5 μm [8]. Using this isopycnic kinetic plot method for SFC separations, it is now possible to compare the performance limits of columns with different particles (size and morphology).

In Fig. 3 the KPL curves (as $t_{0,KPL}$ as a function of N_{KPL}) were constructed for the last eluting compound, benzo(a)pyrene, for SFC (Fig. 3A) and HPLC (Fig. 3B). All curves are made using Eqs. (1)–(4) using a $\Delta p_{sys,max}$ value for HPLC equal to the limit of the columns (400 bar for the columns packed with 3.5 μm and 5 μm fully porous particles; 600 bar for the columns packed with 1.8 μm fully porous and 2.6 μm superficially porous particles). The SFC curves were constructed at a p_{av} of 200 bar using a $\Delta p_{sys,max}$ of 200 bar. This $\Delta p_{sys,max}$ results from the maximum pressure that can be delivered by the CO_2 pump (i.e. 300 bar) and the applied back pressure that is required in SFC. A back pressure of 100 bar was selected resulting in the $\Delta p_{sys,max}$ value of 200 bar. Note that the maximum pressure of 300 bar is not a fundamental upper limit for SFC, but that it is the practical limit of the instrumentation which was used in this study.

For both SFC and HPLC, the same (expected) results are seen. Due to the dependency of the pressure drop over the column with the inverse of the square of the particle diameter, small particles can be used for fast separations and the larger particles can be used to reach very high efficiencies as is consistent with the findings of Gritti and Guiochon who measured Poppe plots for different particle sizes in HPLC (Fig. 11 in [23]). For SFC, comparable behavior could be expected and Fig. 3A shows the isopycnic kinetic plots measured on the columns with different particles in SFC. The same behavior is visible as in HPLC: small particles are suited for fast analyses and larger particles can deliver very high efficiencies when long analysis times are used. This results are consistent with the recent findings of Gritti and Guiochon who constructed theoretical Poppe plots for different particle sizes in HPLC and SFC using a pressure drop over the column of 200 bar [48]. Since the 2.6 μm superficially porous particles generate around the same plate heights as the fully porous 1.8 μm particles, but deliver less pressure drop over the column due to a higher column permeability, the curve of the column packed with core–shell particles is in the vicinity of the 3.5 μm curve in both Fig. 3A and B. The gain in kinetic performance of the kinetex column compared to the columns packed with fully porous particles is larger in HPLC than in SFC, which is again due to the lower diffusion coefficients of benzo(a)pyrene in the liquid mobile phase compared to the one in the supercritical fluid mobile phase [47].

Fig. 3 proves for the first time that isopycnic kinetic plots can be used to compare HPLC and SFC separations on different particle sizes and porosity. It also shows that the effect of using small particles for SFC is the same as for HPLC and that the use of core–shell particles in SFC can deliver the same advantages as in HPLC albeit to a lesser extent.

In Fig. S2 (in Supplementary material), the kinetic performance limit of the column packed with the 1.8 μm fully porous particles in SFC is compared with that in HPLC (this corresponds to an overlay of the blue curves represented in Fig. 3). The same conclusions as in

previous work [8] can be drawn: in the high speed region (short analysis time), the SFC system shows a better kinetic performance than the HPLC system meaning that SFC can be the method of choice for high speed separations. On the other hand, the SFC curves cross the HPLC curve at an intersection point that is determined by the selected back pressure column permeability and by the pressure limitation of the instrument. As a result, SFC is not capable of achieving the same kinetic performance as HPLC in the very high efficiency region if the same particle size columns are used. These findings are consistent with the recently theoretically constructed kinetic plots by Gritti and Guiochon [23] that compare the performance limits of HPLC and SFC. The smaller advantage of SFC over HPLC on Fig. S2 compared to Fig. 4 in [23] is a result of the fact that the same 400 bar pressure was assumed for both systems in [23], where Fig. S2 takes into account the required back pressure and is considered for an SFC system with an upper pressure limit of 300 bar and a HPLC system with an upper pressure limit of 600 bar. Also, the viscosity of the SFC mobile phase was calculated to be 'only' 3.67 times lower than the viscosity of the mobile phase used in the HPLC separation. So, although it could be expected that the kinetic performance in SFC is higher in HPLC due to the lower viscosity of supercritical fluids compared to the viscosity of liquids (similar to high temperature HPLC), other factors, such as the required back pressure and the lower maximum operating pressure have to be taken into account. As already mentioned, this results in a substantially lower $\Delta p_{sys,max}$ in SFC compared to HPLC. In addition, the higher diffusion coefficients in SFC conditions ($D_{mol} \propto \eta$) require the column to be operated at higher mobile phases velocities to reach the optimum efficiency. The much flatter C-term under SFC conditions allows, however, improved kinetic performance for fast and low to medium efficiency separations.

4.2. Extrapolations to sub-micron particles

Currently, the fully porous particle sizes that are mostly used are around 1.7–1.8 μm in diameter and they are packed in columns with small internal diameters around 2.1 mm and column lengths between 10 and 15 cm. The trend in particle size reduction in HPLC is expected to continue considering the evolution of UHPLC systems allowing now to be used up to 1300 bar inlet pressure. The work by Jorgenson et al. has illustrated the possibilities of performing liquid separations up to 6000 bars, providing narrow capillary columns are implemented for efficient heat removal [49–51]. Although prototype sub-micron type of particles have been described [52], thus far no such material and corresponding UHPLC systems have been developed allowing to reach plate heights of 2 or smaller.

As it can be expected that these challenges will be easier to overcome with SFC and in order to have an idea of the applicability of these very small particles on the instruments used in this work, an extrapolation of the data measured on the column packed with 1.8 μm particles was performed to construct kinetic plot predictions for columns packed with 1 μm and 0.5 μm particles. In order to be able to perform these extrapolations, it was assumed that the reduced plate heights reachable with those small particles would be comparable to the values measured on the column packed with 1.8 μm particles used in this study and that the efficiency losses due to frictional heating and adiabatic cooling in HPLC and SFC respectively, are at the same level as for the 1.8 μm column. Note that it will be challenging to achieve these situations in real experiments.

4.2.1. General methodology

For the prediction of the pressure drop over the columns packed with small particles, the measured $\Delta p_{col,exp}$ values per cm of column length on the columns packed with 5 μm, 3.5 μm and 1.8 μm were used. For each of these columns, the $\Delta p_{col,exp}/L$ depicted a linear dependency on the linear velocity of the mobile phase (u_0) consisting of 10% MeOH in CO_2 in SFC and consisting of 15% water in acetonitrile in HPLC:

$$\frac{\Delta p_{col,exp}}{L(cm)} = A(d_p)\, u_0 \tag{5}$$

This $A(d_p)$ was plotted as a function of particle diameter and was fitted such that $A(d_p)$ could be calculated for every desirable particle size. In that way, an extrapolated value for $\Delta p_{col,exp}/L$ for every value of u_0 could be calculated for every particle size.

The van Deemter data were extrapolated via the reduced plate heights and reduced linear velocities. In this way, an extrapolation of the measured H and u_0 values from one particle size to any other size could be performed.

The predicted N_{KPL}, $t_{0,KPL}$ and L_{KPL} values were subsequently calculated using (1)–(4) with the extrapolated van Deemter and pressure drop data.

4.2.2. Confirmation of correctness of general methodology

In order to obtain an idea of the viability of the extrapolation method, Fig. S3 (in Supplementary material) illustrates the extrapolation to 3.5 μm particles using the measured data on the column packed with 5 μm particles (S3A) and to 5 μm using the measured data on the column packed with 3.5 μm particles (S3B) for the SFC measurements. In both cases, the measured curves are also shown and overlap closely to the predicted ones. From this result, it can be concluded that it is possible to use data measured on one particle size in order to predict the kinetic performance on another particle size when both columns are equally well packed. The same extrapolations were performed for the HPLC measurements resulting in the same conclusions (data not shown).

Performing these extrapolations of pressure drop and van Deemter data measured for 5 μm particles to 1.8 μm particles would not result in the same overlap between the measured 1.8 μm data and the predicted data. This results from the fact that the 1.8 μm column could not deliver the same reduced plate heights as the 5 μm column and that the C-term for the HPLC measurements on the 1.8 μm column is steep due to thermal effects (see Fig. 2).

If, however, the predicted van Deemter data are adapted for these higher reduced plate heights and steeper C-term, the prediction of the kinetic performance limit for particles that are 3 times smaller than the original particles, is justified. This can be seen in Fig. S4 (in Supplementary material) where the predicted curve for 1.8 μm particles is the result of an extrapolation of the pressure drop data from the 5 μm and 3.5 μm particles to 1.8 μm particles combined with the measured van Deemter data on the 1.8 μm column. This curve represents a very good overlap with the measured KPL curve on 1.8 μm particles. The result for this extrapolation for HPLC is not shown but was similar.

4.2.3. Predictions for 1 μm and 0.5 μm particles

Fig. 4 represents the result of the extrapolations in SFC (4A) and HPLC (4B) from the measured data on the 1.8 μm column to 1 μm and 0.5 μm particles. The resulting extrapolated curves are compared with the experimental curve on the column packed with 1.8 μm particles. For the same reasons as for the curves in Figs. 3 and S2, the $\Delta p_{sys,max}$ was 200 bar for the SFC curves and 600 bar for the HPLC curves. Note that the curves for 1 μm and 0.5 μm particles were constructed assuming that they could be equally well packed as the 1.8 μm particles and that the influence of the thermal effects is also the same as for the 1.8 μm column. The results are as expected and in general, similar conclusions as

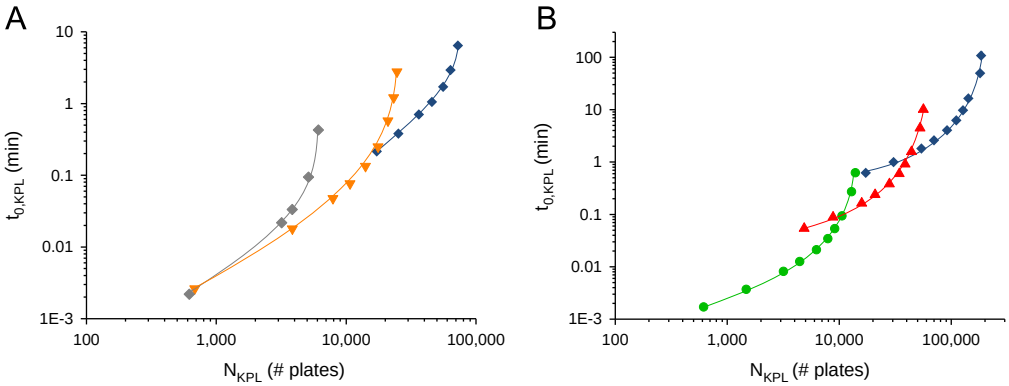

Fig. 4. Comparison of the measured KPL data on the column packed with 1.8 μm particles with predicted KPL data for 1 μm and 0.5 μm particles for benzo(a)pyrene in SFC (A) and HPLC (B). Blue diamonds: measured data on 1.8 μm particles. Orange reversed triangles: predicted data on 1 μm particles in SFC. Gray diamonds: predicted data on 0.5 μm particles in SFC. Red triangles: predicted data on 1 μm particles in HPLC. Green circles: predicted data on 0.5 μm particles in HPLC. All SFC curves are constructed using a $\Delta p_{sys,max}$=200 bar and all HPLC curves are constructed using a $\Delta p_{sys,max}$=600 bar. Curves are drawn for benzo(a)pyrene. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

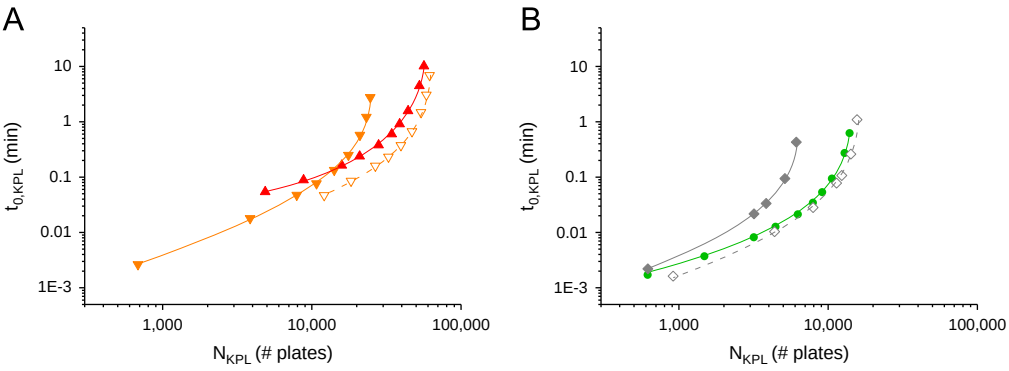

Fig. 5. Comparison between the SFC and HPLC predicted KPL curves for 1 μm (A) and 0.5 μm particles (B). Full symbols are as in Fig. 4. Open orange reversed triangles: SFC predicted KPL curve for 1 μm particles constructed using $\Delta p_{sys,max}$=500 bar. Open gray diamonds: SFC predicted KPL curve for 0.5 μm particles constructed using $\Delta p_{sys,max}$=500 bar. Curves are drawn for benzo(a)pyrene. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in Fig. 3 can be drawn. Especially for the HPLC curves, the anticipated shift to lower $t_{0,KPL}$ and N_{KPL} values is noticed when evolving from larger to smaller particle size columns. This offers the possibility to select an optimum particle size for every combination of N and t_0 that would be desired. In SFC, the kinetic performance limit of the column packed with 0.5 μm particles is systematically lower compared to the 1 μm particle columns. This is a result from the fact that progressing more into the C-term of the 0.5 μm particles would require flow rates that result in extra column pressure drops exceeding the $\Delta p_{sys,max}$ of the SFC system (200 bar).

Fig. 5 is similar to Fig. S2 as it compares the KPL plot for SFC and HPLC for one particle size (1 μm in Fig. 5A and 0.5 μm in Fig. 5B). When the full symbols are considered ($\Delta p_{sys,max}$=200 bar) for SFC (consistent with a pressure limit of the CO_2 pump of 300 bar), the comparison between HPLC and SFC for 1 μm particles delivers the same conclusions as in Fig. S2 from this work and Fig. 9 from [8]. The comparison between SFC and HPLC cannot be generalized because the experimental parameters used in the SFC measurements greatly influence the kinetic performance limits. The choice of the mobile phase composition, temperature and average column pressure determine the viscosity of the SFC mobile phase and thus the pressure drop over the column. In this work, a mobile phase with a rather high viscosity was used in SFC (the viscosity of the SFC mobile phase was only 3.67 times lower than the viscosity of the mobile phase used in HPLC). Taking into account that the linear velocities in the SFC measurements are around 3 times higher than in HPLC, the pressure drop over the column in SFC is not that much lower than in HPLC. When this is combined with a low pressure limit of the CO_2 pump of 300 bar and a desired back pressure of 100 bar, the resulting SFC curve does not reach the same high efficiencies as the HPLC curve. For the 1 μm particles, the SFC curve does cross the HPLC curve when progressing into the C-term region due to the flatter C-term in the SFC van Deemter curve which means that for 1 μm particles SFC is still better than HPLC for fast separations. This behavior is not seen in Fig. 5B as the HPLC kinetic performance limit on the column with 0.5 μm particles is always higher than the SFC KPL. The reason for this is the same as for Fig. 4A: the flow rates that would be necessary to reach the C-term for the SFC separations on the 0.5 μm column result in extra column pressure drops that are higher than 200 bar ($\Delta p_{sys,max}$). The open symbols in Fig. 5 show the KPL of the SFC separations when a system with a pressure limit of 600 bar would have been used (resulting in a $\Delta p_{sys,max}$ of 500 bar). The situation of these curves with respect to the HPLC curves indicate that the KPL of SFC can be higher than that of HPLC if the instruments that are used have the same pressure limit. So in that case, SFC is always a better choice over HPLC (on every particle size) even if a back pressure of 100 bar is applied for the SFC measurements. Note, however, that performing HPLC separations on sub-1 μm particles with a pump that can only deliver 600 bar is also not realistic as the pressure limits of modern UHPLC systems are higher than 1000 bar.

The scope of this extrapolation work was to examine the possibilities of working with smaller particles than currently commercially available on the instrumentation that was used to measure on the columns packed with 5 μm, 3.5 μm and 1.8 μm

particles. Therefore it must be concluded that working with 0.5 μm particles in SFC is not beneficial over working with these particles in HPLC (see Fig. 5B) or over working with SFC on 1 μm particles (see Fig. 4A). The problem with working with 0.5 μm particles in SFC is also reflected when the optimal linear velocities are considered. The pressure needed to percolate an SFC mobile phase consisting of 10% methanol in CO_2 at optimal linear velocity (17.5 mm/s) through a 1 cm column packed with 0.5 μm particles is 1126 bar and the resulting efficiency would only be 6350 plates (if the same reduced plate height of 3.15 as for the measurements on the 1.8 μm column is considered). This pressure limit is double of what is currently possible with SFC instruments. To mobilize a liquid mobile phase consisting of 15% H_2O in acetonitrile through the same system at optimal linear velocity (6.8 mm/s), the pumps need to deliver only (680 bar) and the measured plate number would be 7300 (when a reduced plate height of 2.75 is assumed). Pumping the same SFC and HPLC mobile phases through a 5 cm column packed with 1 μm particles at optimal linear velocities (8.8 mm/s and 3.4 mm/s respectively) requires 427 bar and 405 bar respectively. The measured plate heights are in those cases 15,900 and 18,200 respectively for SFC and HPLC.

5. Conclusions

Isopycnic van Deemter and kinetic plots were constructed for SFC separations on columns packed with C18 particles of various diameters and with core–shell C18 particles using a mobile phase with 10% methanol as modifier. The average column pressure was set at 200 bar and the measurements were performed in a forced air oven at 40 °C. The same experiments were repeated in HPLC at room temperature using a mobile phase that contained ACN and water (85:15). The influence of decreasing particle size on the SFC van Deemter plots and the kinetic performance was the same as for HPLC and was for the first time experimentally confirmed. Using smaller particles in SFC results in smaller H_{min} values and higher $u_{0,opt}$ values in the van Deemter plots. With the SFC kinetic plots, it is possible to select an optimal particle size for every combination of efficiency and analysis time that is desired in the same way as for HPLC. A comparison between SFC and HPLC kinetic plots was made and the method of choice depends on the desired efficiency and analysis time as the SFC kinetic performance limit curve crosses the curve for HPLC.

This work is a confirmation that the isopycnic approach is valid to compare SFC with HPLC and to compare SFC separations on columns with different pressure characteristics (particle size and morphology) using realistic chromatographic conditions. For every efficiency and analysis time, the optimal mobile phase (supercritical or liquid) and column type can now be selected by constructing kinetic plots and comparing them. As the isopycnic SFC kinetic plots are highly influenced by experimental parameters such as average column pressure and pump pressure limits, a uniform comparison between SFC and HPLC is not possible and it is very important always to evaluate the results of such a comparison within the knowledge of the chosen experimental conditions.

The measured SFC and HPLC van Deemter data and pressure drop data on the columns packed with 5, 3.5 and 1.8 μm particles were used to predict van Deemter data that would be measured on a 1 cm column packed with 1 μm particles or 0.5 μm particles. These predicted van Deemter data were extrapolated to the kinetic performance limits on these particles by using the predicted pressure drop data. Hereby it was assumed that the columns with the very small particles can be equally well packed as the 1.8 μm particles and that the influence of the thermal effects is also the same as for the 1.8 μm column. The final predicted kinetic plots for 1 μm and 0.5 μm particles used on the same instrumentation as in the measurements on the other columns in this work show that working with 0.5 μm particles in SFC requires pressures that are much higher than the pressure limits of current SFC instruments. The use of 0.5 μm particles in HPLC is possible but only very short columns can be used. 1 μm particles show potential for SFC and HPLC as the required pressures to pump the used SFC and HPLC mobile phases through a 5 cm column packed with 1 μm particles at the optimal linear velocities (8.8 mm/s and 3.4 mm/s respectively) would be 427 bar and 405 bar respectively. Those pressures are reachable with current state of the art SFC and HPLC instrumentation.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.08.023.

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