Performance limits and kinetic optimization of parallel and serially connected multi-column systems spanning a wide range of efficiencies for liquid chromatography

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ABSTRACT

Using a set of experimentally determined liquid chromatography column performance data, it has been investigated how a range of efficiencies can best be covered when using a multi-column system. Two main variants are considered: a serially-connected variant (realizing different column lengths by connecting a different number of column segments in series) and a parallel-connected variant (realizing different column lengths by simply switching between columns with a different length arranged in parallel). Both variants are compared for their ability to keep the average analysis time along a given range of efficiencies as close as possible to the intrinsic Knox & Saleem-speed limit. It was found that the serial connection mode offers a better compromise between average speed and amount of required silica (total required column length) than the parallel connection mode for all efficiency ranges running from 5000–10,000 plates up to 75,000–150,000 plates. Considering an ultra-high performance liquid chromatography (UHPLC) operation at 1200 bar, the best possible serial connection system can get within about within 15–25% of the Knox & Saleem-speed limit, whereas a three-column parallel system can only get to within 40–50% of the speed limit, while needing 50–100% more total column length. In absolute terms, the serially-connected system with individually optimized segment lengths should be able to cover a range of 5000–75,000 theoretical plates (dynamic range of 25) in an average analysis time of 14.3 min when using a 1200 bar instrument. At 400 bar, this would be 37.9 min, showing that the construction of wide-efficiency range systems would be one of the application areas where the advantages of UHPLC-conditions would be most fully realized.

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1. Introduction

Recent developments in high-performance liquid chromatography (HPLC) have mainly been focused on obtaining better separations in faster analysis times. For this purpose, a large number of new support structures and operation modes have become available on the market in the last decade. The size of the stationary phase supports has drastically been reduced to less than 2 μm to allow for a fast mass transfer of the analytes between the mobile and the stationary phase. To cope with the high backpressure that emanates from these small support structures, a lot of effort has been put in the development of instrumentation that can deliver inlet pressures as high as 1200 bar [1–3]. An alternative packing type that has been designed to obtain high separation efficiencies are superficially porous particles with a solid silica core surrounded by a thin porous shell. Because the diffusion of the analytes is restricted to the thin porous shell, a very fast mass transfer becomes possible leading to high column efficiencies. Recently, superficially porous particles with sizes of 2.6 and 2.7 μm have been commercialized [4–6]. Because of their larger size, they give rise to lower backpressures compared to sub-2 μm particles and can therefore be operated at more conventional inlet pressures [7].

Whereas these new technologies can indeed lead to impressive increases in separation speed, it is often forgotten that the most straightforward way to increase the separation speed consists of optimizing the column length. This means selecting the column length such that it exactly delivers the required minimal efficiency needed for a sample under consideration, and nothing more, as any additional efficiency only consumes analysis time without further improving the separation [8]. Since most analytical facilities (R&D labs, routine labs, method development labs, . . .) deal with samples that have a high variability in required efficiency, this implies that they would greatly benefit from a generic column system, i.e., one with an automatically adaptable column length that covers a wide range of efficiencies. Such a generic column system would also

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be highly advantageous in method development. During method development a large number of scouting runs first needs to be performed to select the experimental conditions (mobile phase pH, organic modifier, column selectivity, temperature, . . .) and gradient parameters that allow to maximally spread out the sample across the elution window. As these scouting runs only require a low efficiency, they can be performed on short columns in short analysis times. Subsequently, long columns with high efficiencies can be used to fine-tune the separation of the existing critical pairs [9].

Defining the dynamic efficiency range of a chromatographic system as the range of efficiencies that can be reached by changing the flow rate between the optimal and the maximum flow rate, most columns have a dynamic efficiency range that is usually rather small (a factor of 2–3 only). For example, a 5 cm column with a particle size of 1.7 μm, operated at a maximum pressure of 1000 bar typically has a dynamic range of 8000–14,000 plates, for a 15 cm column with a particle size of 3.5 μm operated at 400 bar, this is between 10,000 and 20,000 plates and for a 10 cm superficially porous column with a particle size of 2.7 μm operated at 600 bar, the dynamic range is between 10,000 and 22,000 plates. Apart from having only a limited dynamic efficiency range, most columns moreover only have a relatively narrow range over which the analysis time can be varied (assuming a fixed analyte retention factor), since the analysis time can only be varied by tuning the flow rate. In a system with automatically variable length on the other hand, a second, much more powerful adjustable parameter would be available for analysis time minimization, i.e., the column length. The powerful optimization possibilities of the latter can for example readily be understood from the fact that halving the column length does not simply lead to a twofold but to a fourfold reduction of the analysis time. This is due to the fact that the analysis time not only halves because of the reduced column length but is reduced by a second factor of two because of the higher flow rate that can be applied when keeping the same total pressure.

A chromatographic system with an automatically variable length can conceptually be constructed by starting from a "conventional" coupled column system [10–17] and subsequently automating the process of attaching or detaching one or more segments. Coupled column systems have in the past been proposed many times, and have been shown to lead to very high efficiencies [10–17]. However, changing the number of segments always occurred manually, at the expense of labour time and many ruined connection pieces. To automate the process of column length variation, two general types of connection can basically be conceived of: the parallel and the serial connection (Fig. 1a and b). The parallel connection (Fig. 1a) switches between the different columns attached to the system and allows them to be used separately and hence independent of each other. The serial connection (Fig. 1b) couples different column segments in series. In this set-up, the performance of each segment will be influenced by the pressure and efficiency in the preceding segment(s). Both set-ups make use of rotor-stator valves and a multiple set of columns. The connection mode will depend on the groove pattern in the rotor of the valves. The serially-connected variant corresponds to the "automated column coupler" (ACC) proposed in [9,18].

The present study has been set-up to calculate and compare the theoretical speed limits of parallel and serially-connected multi-column systems for the solution of separation problems with variable difficulty, i.e., covering a wide range of required efficiencies. First, kinetic optimization procedures for parallel and serially connected multi-column systems are established, such that they can be used to optimize the particle size and column lengths needed to produce a given range of efficiencies in the shortest possible over-all time. Subsequently, the established procedures have been used to design the best possible multi-column systems covering different wide efficiency ranges (5000–50,000, 10,000–100,000, etc.).
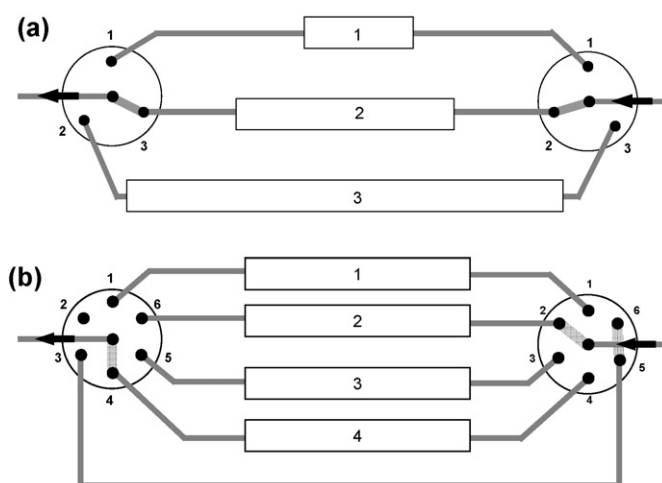

Fig. 1. Representation of variable column length systems using two rotor-stator valves and a dedicated groove pattern, showing (a) a parallel column system capable of switching between $L = 5$ (1), $L = 10$ (2) and $L = 20$ cm (3) and (b) a serial column system capable of switching between $L = 5$, $L = 10$, $L = 15$ and $L = 20$ cm. In the serial column configuration, each segment (1–4) has a length of 5 cm.

This has been done for two pressures (a conventional pressure of 400 bar and an ultra-high pressure of 1200 bar) and using typical experimental data for well-packed fully porous columns. Finally, the differences in dynamic efficiency range and concomitant speed between superficially porous particles with a size of 2.7 μm and fully porous particles with a size of 1.8 μm have been investigated.

2. Experimental

2.1. Chemicals and columns

Propylparaben and thiourea were obtained from Sigma Aldrich (Steinheim, Germany). Acetonitrile (ACN) (HPLC grade) was also purchased from Sigma–Aldrich and HPLC grade water was prepared in the laboratory using a Milli-Q gradient water purification system (Milipore, Billerica, MA, USA).

The HALO Fused Core C$_{18}$ column (50 × 2.1 mm, 2.7 μm) was purchased from Advanced Materials Technologies (Wilmington, DE, USA). The Zorbax Stable Bond C$_{18}$ column (50 × 2.1 mm, 1.8 μm) was purchased from Agilent Technologies (Diegem, Belgium).

2.2. Apparatus

All experiments were performed on an Ultimate 3000 system (Dionex Corporation) equipped with a high pressure gradient pump, an autosampler, a thermostatted forced-air oven (TCC 3000 RS) with a maximum operating temperature of 110 °C and a variable wavelength detector with a flow cell of 45 nL. The maximum pressure of the system was 800 bar. Chromeleon software was used for system operation and data evaluation (Dionex, Munchen, Germany). The tubing used to make the connections between the system and the column was PEEKsil tubing (SGE Analytical Science, Melbourne, Australia) with a diameter of 75 μm and a total length of 800 mm. PEEKsil tubing has a pressure limit of 1034 bar.

2.3. Methodology

Plate height data were experimentally determined for propylparaben (20 μg/mL dissolved in the mobile phase) on each column, using a mobile phase composition of 35/65 vol%/vol% ACN/H$_2$O. Under these conditions, the retention factor of propylparaben was determined to be $k = 6.9 \pm 0.6$. Thiourea was used as unretained

marker. For the fully porous column the flow rate was varied between 0.05 mL/min and 1.2 mL/min, for the superficially porous column the flow rate ranged between 0.05 mL/min and 1.8 mL/min. The columns were operated at 30 °C. The obtained plate height data were fitted to a plate height model (van Deemter) in MatLab (The MathWorks Inc, Natick, MA, USA) to obtain a, b and c constants [19,20]. The same software was used to assess the confidence intervals (95%) of the fitted parameters and the overall quality of the fitting. The permeability of the columns was experimentally determined using Darcy's law [21]. These data were used in the general sheet of the serially connected column optimizer to construct plots of time versus efficiency for both column types. Absorbance values were measured at 254 nm with a sample rate of 50 Hz. The injected sample volume was 1 μL.

3. Optimization procedures

The optimization of single column systems has recently been revisited and applied to state-of-the-art columns by Carr, Stoll and coworkers [20,22]. In the two sections below, the necessary procedures are established to optimize the design (column or segment lengths, number of segments, particle size in each column or segment) of the parallel- and serially-connected system. In all cases, the optimization goal was the mean analysis time (t_{av}) needed over a given efficiency range (t_{av} is averaged over x equally spaced efficiency values lying between the minimal and maximal N-value of the selected efficiency window, with $x = 1 + ((N_{max} - N_{min})/1000)$. To compare the over-all speed of the different considered systems in dimensionless terms, the following dimensionless quantity has been defined:

$$\Delta \tau = \frac{t_{av} - t_{KS,av}}{t_{KS,av}} \times 100\% \tag{1}$$

where $t_{KS,av}$ is the average analysis time needed to cover the same efficiency range when the considered particle type would be operated on its Knox & Saleem (KS)-limit. The KS-limit represents the kinetic performance of an infinite set of columns with a different column length and filled with particles of the same type but with a different size such that each column is operated at its optimal flow rate (such that $h = h_{min}$) when the maximum inlet pressure is applied [23]. As such, the KS-limit line represents the absolute minimal analysis time needed to achieve a given efficiency using a given particle or support type [20,23,24]. As shown in [25], the relation between t_R and N on the KS-limit line can be expressed as:

$$t_R = \frac{N^2 \eta h_{min}^2 \phi}{\Delta P_{max}} (1 + k) \tag{2}$$

In Figs. 2–5, the KS-limit line as defined by Eq. (2) is represented by the gray straight line.

All calculations presented in Sections 3 and 4.1–4.2 are carried out with P_{max} = 400 or 1200 bar, $\eta = 10^{-3}$ Pa s, $D_{mol} = 10^{-9}$ m²/s, $\phi = 800$ (ϕ = column flow resistance), and $a = 1$, $b = 2.5$, $c = 0.1$ (van Deemter-parameters, see Eq. (3) further on). The latter are typical values for a fully porous particle column leading to the well-established $h_{min} = 2$-value [24].

In a first series of optimization exercises, only solutions with commercially available column lengths or particle diameters are allowed (see Figs. 2–7): column lengths of 3, 5, 7.5, 10 and 15 cm and combinations thereof and particle sizes of 1.5, 1.7, 1.8, 1.9, 2.0, 2.2, 2.5, 3.0, 3.5, 5.0 and 10.0 μm. In the final exercise (see Fig. 8), this constraint is removed (although it is still imposed that $L \geq 1$ cm and $d_p \geq 1.5$ μm). All presented analysis times relate to a compound with a retention factor $k = 10$.

3.1. Optimization of parallel-connected column systems

In the parallel column system, each different column length is used separately (Fig. 1a) and can therefore be optimized independently. In this case, the column leading to the fastest separations in a pre-defined efficiency range is the one that has a particle size that is exactly small enough ($d_{p,min}$) to obtain the maximum plate count (N_{max}) of the range when operated at the maximum pressure (P_{max}). The particle size of this column can be derived using a general plate height equation:

$$H = A + \frac{B}{u_0} + C u_0 = H = a \cdot d_p + b \cdot \frac{D_{mol}}{u_0} + c \cdot \frac{d_p^2}{D_{mol}} \cdot u_0 \tag{3}$$

where D_{mol} is the molecular diffusion coefficient (m²/s) and u_0 is the mobile phase velocity that can be related to the optimum mobile phase velocity (u_{opt}) using a constant α, representing the number of times the velocity at the maximum pressure is larger or smaller than u_{opt}:

$$u_0 = \alpha \cdot u_{opt} = \alpha \cdot \sqrt{\frac{B}{C}} = \alpha \cdot \sqrt{\frac{b}{c}} \frac{D_{mol}}{d_p} \tag{4}$$

Substituting Eq. (4) in Eq. (3), the following expression is obtained:

$$H = \left[a + \sqrt{b \cdot c} \left(\frac{1}{\alpha} + \alpha \right) \right] \cdot d_p \tag{5}$$

Using Darcy's law the mobile phase velocity can also be expressed as a function of the column permeability (K_{v0}) or the column flow resistance (ϕ):

$$u_0 = \frac{\Delta P \cdot K_{v0}}{\eta \cdot L} = \frac{\Delta P \cdot d_p^2}{\phi \cdot \eta \cdot L} \tag{6}$$

where ΔP (Pa) is the pressure drop measured over a column with length L (m) when operated at a velocity u_0 and η is the mobile phase viscosity (Pa s). Finally, the plate height H can also be expressed as a function of the plate count N:

$$N = \frac{L}{H} \tag{7}$$

Substituting now Eq. (7) in Eq. (6) and using expression (5) for H, while replacing u_0 by the expression given in Eq. (4), it is found that:

$$d_p = \sqrt{\alpha \left(a \sqrt{\frac{b}{c}} + b \left(\frac{1}{\alpha} + \alpha \right) \right) \phi \eta \frac{D_{mol}}{\Delta P} N} \tag{8}$$

Eq. (8) is the central expression for the optimization of parallel-connected column systems. The two variable parameters in the expression are α, the number of times the velocity at the maximal pressure is larger (or smaller) than u_{opt}, and N, the target efficiency.

3.1.1. Single column system

If a given efficiency range is to be covered using only a single column (in which case the difference between parallel and serially-connected vanishes), there are three main choices for the selection of N in Eq. (8): the optimization can be either based on $N = N_{max}$ or $N = N_{min}$, or on some intermediate value of N ($N = N_{im}$).

Considering first the $N = N_{max}$-case, the fastest solution is always obtained for $\alpha = 1$. This is illustrated in Fig. 2, showing as an example the case wherein the required efficiency varies between 5000 and 50,000 plates. The three full symbol data series correspond to the case of $\alpha = 0.6$; $\alpha = 1$ and $\alpha = 2.3$. As can be noted, the shortest analysis time is indeed obtained for $\alpha = 1$ (corresponding in this case to a column with a particle size of 1.8 μm and a length of 18 cm). This column is exactly long enough to yield 50,000 plates when
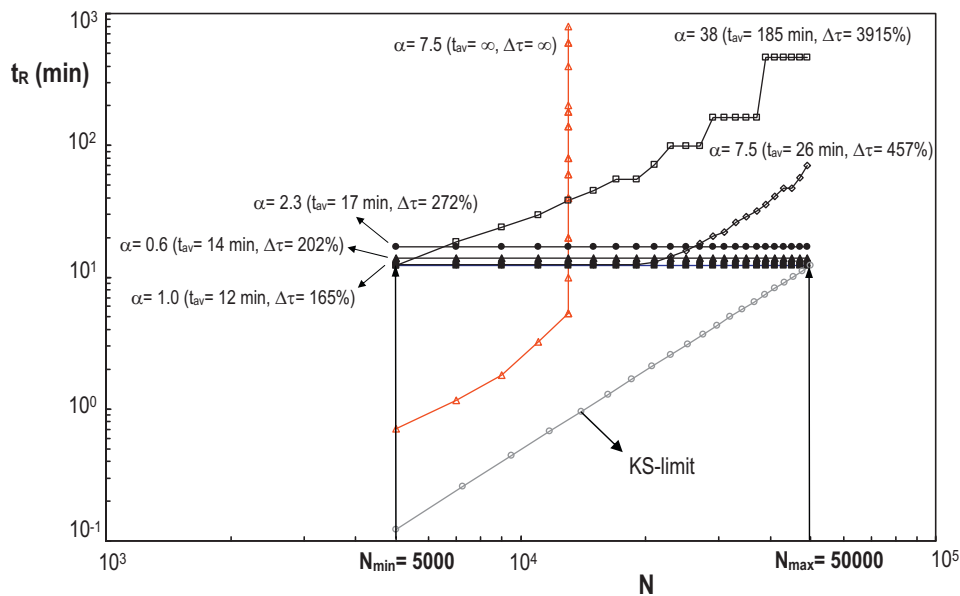
Fig. 2. Single column solutions to cover an efficiency range between 5000 and 50,000 plates: (■) $d_{p,min}$ = 1.8 μm, L = 18 cm, (●) d_p = 3.0 μm, L = 36 cm, (▲) d_p = 1.5 μm, L = 16 cm, (□) d_p = 10 μm, L = 113 cm, N_{min} = 5000 and (◇) d_p = 5.0 μm, L = 50 cm, N_{im} = 21,000, (△) d_p = 2.5 μm, L = 5 cm, N_{min} = 5000. The α-, t_{av}- and $\Delta\tau$-values are shown. The open, gray symbols represent the KS-limit. All other parameter values are given in the text (Section 3).

operated at its optimum velocity when the maximum inlet pressure is applied (1200 bar). In this case, the average analysis time (t_{av}) needed to cover the required efficiency range is 12 min. For columns with a larger particle size (corresponding to an increase of α), the column length needs to be increased in order to obtain the maximum plate count when operated at the maximum pressure (because larger particles have a lower plate count per meter column length). These longer column lengths will therefore lead to longer analysis times. For example, for a column with a particle size of 3.0 μm (α = 2.3), the average analysis time to cover the range of efficiencies is 17 min. Columns with a particle size that is smaller than the optimal value on the other hand need to be operated at a velocity below u_{opt}, i.e., in the kinetically unfavourable

B-term region. As can be noted for the case of α = 0.6 (d_p = 1.5 μm and L = 15 cm), this also leads to an increase of the average analysis time compared to the α = 1-case (14 min versus 12 min.).

Subsequently considering the case where the particle optimization via Eq. (8) is based on the minimal required efficiency (N_{min}), the value of α has to be selected such that the particle size is so large that on the one hand the column can produce $N = N_{min}$ when operated at u_{max}, and on the other hand can also achieve $N = N_{max}$ when operated at u_{opt}. For the range considered in Fig. 2, this requires a value of α = 38 (corresponding to a particle size d_p = 10 μm). As can be noted, this solution leads to a much larger t_{av}- and $\Delta\tau$-value than the optimizations based on $N = N_{max}$. Taking a smaller value of α, only part of the efficiency range can be addressed, as the
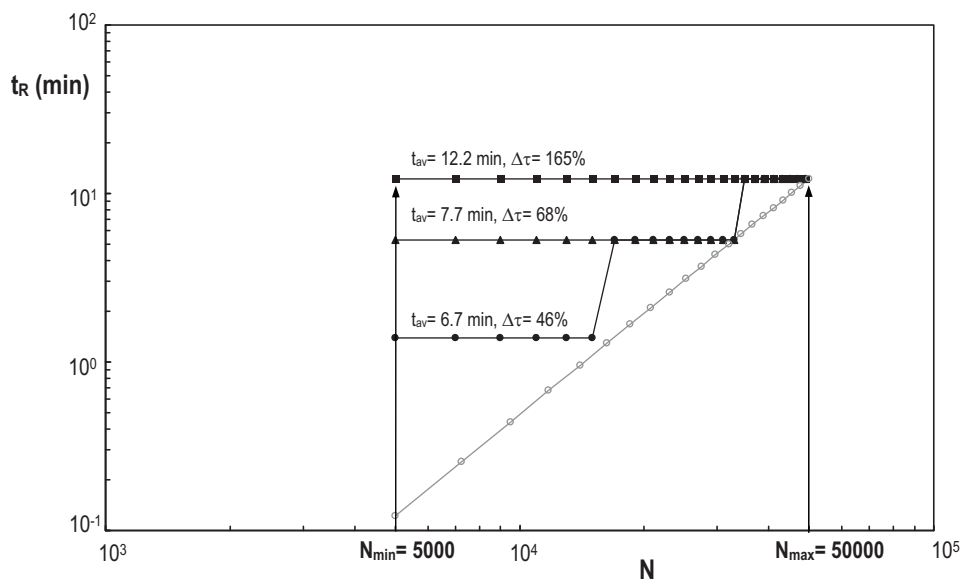

Fig. 3. Single, dual and triple column solutions (parallel connection) to cover an efficiency range of 5000–50,000 plates. (■) Optimal single column solution from Fig. 2 ($d_{p,min}$ = 1.8 μm, L = 18 cm, α = 1). (▲) Optimal dual column solution: d_p = 1.8 μm and L = 18 cm (α = 1) for the longest column, and d_p = 1.5 μm and L = 10 cm (α = 1) for the shortest column. Optimal triple column solution (●): d_p = 1.8 μm and L = 18 cm (α = 1) for the longest column; d_p = 1.5 μm and L = 10 cm (α = 1) for the intermediate column and d_p = 1.5 μm and L = 5 cm (α = 2) for the shortest column. All other parameter values are given in the text (Section 3).
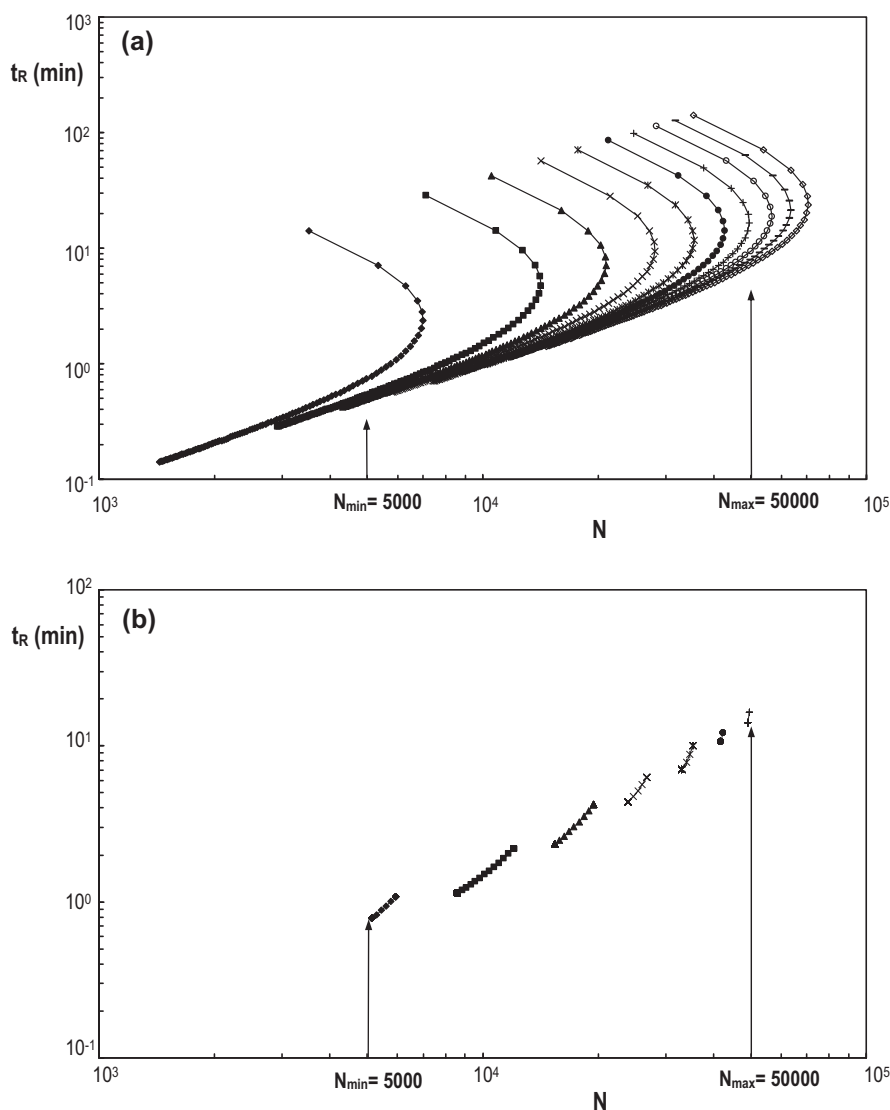
Fig. 4. (a) Unconstrained plots of analysis time versus plate count obtained for a serially coupled column system composed of 10 segments and (b) same plots, but now for a specific efficiency range ($N = 5000–50,000$), under a specific pressure constraint ($P_{max} = 1200$ bar) and after the logical functions added to the MS Excel-sheet have eliminated all the overlapping points. The particle size was set to 2.2 μm and the segment length to 3 cm, all other parameter values are given in the text (Section 3).

solution runs into a vertical asymptote (red data points). Subsequently taking an intermediate value for N ($N = N_{im}$), and selecting α such that both N_{min} and N_{max} can be reached with the given column length ($\alpha = 7.5$, leading to $d_p = 5$ μm), again t_{av}- and $\Delta\tau$-values are obtained that are larger than those obtained for the best possible case, i.e., d_p-selection based on $N = N_{max}$ and $\alpha = 1$.

The underlying reason for the fact that the best kinetic result is always obtained when d_p is optimized based on the largest N-value of the target range is that the highest efficiencies have the largest weight in the calculation of t_{av} and $\Delta\tau$. Basing the optimization on any of the lower efficiencies, the optimization always leads to a column whose α-value needs to be larger than unity, i.e., away from the KS-limit (where always $\alpha = 1$).

3.1.2. Parallel dual column system

When two columns are available in parallel, the length and particle size of the two columns need to be selected such that they both cover the entire (N_{min}, N_{max})-range in the shortest possible average time. Since the two columns are used separately, Eq. (8) remains valid. Again, it was found that the smallest t_{av} is obtained when the optimization is based on N_{max} (with $\alpha = 1$, or the value

lying closest to one and leading to a commercially available particle size and column length) rather than on any smaller N-value. The optimization of the dual column parallel system therefore simply corresponds to optimizing a first column based on the N_{max}-value of the specified efficiency range, followed by the optimization of a second column that can be used in the lower range of efficiencies. To optimize the second column, some intermediate N-value (N_{im}) needs to be selected, and this value should then be used in Eq. (8), again with $\alpha = 1$, to calculate the optimal particle size and length.

Leaving aside some small variations of α around unity that need to be made in order to obtain a commercially available particle size, the optimization exercise for the parallel dual column system thus simply comes down to finding the value of N_{im} that leads to the smallest overall t_{av}- and $\Delta\tau$-value (see solid triangles in Fig. 3, where the best possible dual column solution is compared with the best possible single column solution for an efficiency range $N = 5000–50,000$).

As can be noted, the best possible solution is obtained for an intermediate value of N lying around $N = 33,000$. This value is slightly larger than the midway-value (which lies at $N = 27,500$) or the average value ($N = 32,500$). The fact that the shortest column
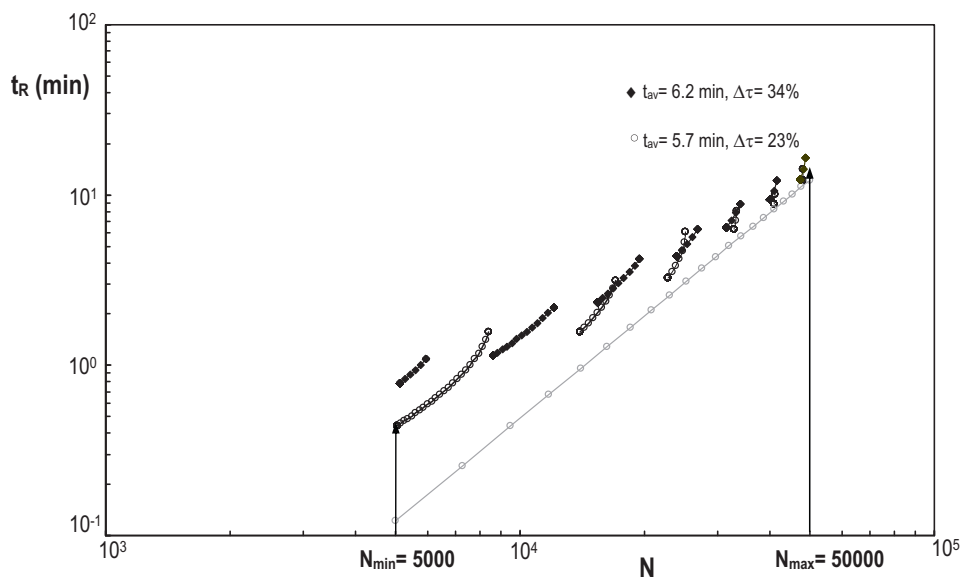
Fig. 5. Serially coupled multi-column systems to cover an efficiency range of 5000–50,000 plates: (♦) Optimal solution with a fixed particle size (d_p = 2.2 μm) and segment length (3 cm), (○) optimal solution with a variable particle size and segment length (segments 1–2: d_p, 1.8 μm, L = 3 cm, segment 3–5: d_p, 1.9 μm, L = 3 cm, segment 6: d_p, 2.2 μm, L = 3 cm). All other parameter values are given in the text (Section 3).

needs to be optimized for an N-value that is slightly larger than the average or the midway value is again a consequence of the fact that the highest efficiency points have the highest weight in the formation of the t_{av}-value.

3.1.3. Parallel triple column system

In a similar way, the analysis time for a specific efficiency range can be further reduced by introducing a third column to the system. In this case, the longest column should be designed based on N_{max} (again with α close to unity), whereas two intermediate values of N need to be selected (by minimizing t_{av}) for the design of the two shorter columns (which again should be used with an α-value close to unity). The best possible solution that can be obtained in this way for the efficiency range of N = 5000–50,000 is represented by the circles in Fig. 3. It can be noted that, while the triple-column solution coincides with the dual-column solution for the range N > 15,000, the addition of the third column segment allows to significantly reduce the analysis time for the low efficiency range.

As a general conclusion from Fig. 3, it can be said that, the more columns that are available in a parallel mode system, the closer the KS-limit can be approached. Eventually, if the number of columns would exceed 5–10, each optimized for a different efficiency range, the composite "stairway" curve would nearly perfectly coincide with the KS-limit. This is straightforward, since the KS-limit is precisely defined as the performance obtained if an individually optimized column length would be used for each different target efficiency.

3.2. Optimization of serially-connected column systems

When the different column segments are used in series, the optimization must take into account that the performance of the ith segment depends on the length and particle size of all previous segments. Since they are connected in series, the velocity that can be established in the ith segment not only depends on its own pressure-drop but also on that in the previous segments. To account for this, a serially-connected column optimizer was developed in MS Excel (see supporting material, SM, for full details). In this optimizer, handling up to ten segments, the performance of each column segment is calculated in separate worksheets, linked

together by adding the calculated variances and times of the previous segment to every new segment. This means that sheet 1 represents the performance that is obtained in a system consisting of one segment (segment 1), sheet 2 represents the performance of a system consisting of two segments (segment 1 + 2), and so on until ten segments are linked. In this calculation, the efficiency and pressure losses in the connection tubing used to couple the column segments are also taken into account (the length of the connection tubing is adapted to the length of each segment in such a way that the total length of each segment and its connection tubing is 20 cm).

Every sheet starts with a list of possible mobile phase velocities, for which first the total pressure drop is calculated by taking the pressure in the column (ΔP_{col}) and the connection tubing (ΔP_{tub}) into account:

$$\Delta P_{tot, n\,col} = \sum_{i=1}^{n} \Delta P_{col,i} + i \cdot \Delta P_{tub} + \Delta P_{\text{return capillary}} \tag{9}$$

Subsequently the peak variances caused by the individual column segments, calculated as:

$$\sigma_{t,col\,i}^2 = \frac{L_i \cdot H_i}{u_0^2}$$

wherein L_i and H_i are the length and plate height of the ith element (H_i is determined by the particle size used in this segment) are added to the peak variance contributions of the connection tubing (calculation: see SM), to yield:

$$\sigma_{t,n\,col}^2 = \sum_{i=1}^{n} \sigma_{t,col\,i}^2 + i \cdot \sigma_{t,tub}^2 + \sigma_{\text{return capillary}}^2 \tag{10}$$

Finally, the obtained variances and velocity data are used to determine the analysis time and the plate counts obtained for every mobile phase velocity on every segment:

$$t_{n\,col} = \sum_{i=1}^{n} \frac{L_i}{u_0} + 2 \cdot i \cdot \frac{L_{tub}}{u_{tub}} + \frac{L_{\text{return capillary}}}{u_{tub}} \tag{11}$$

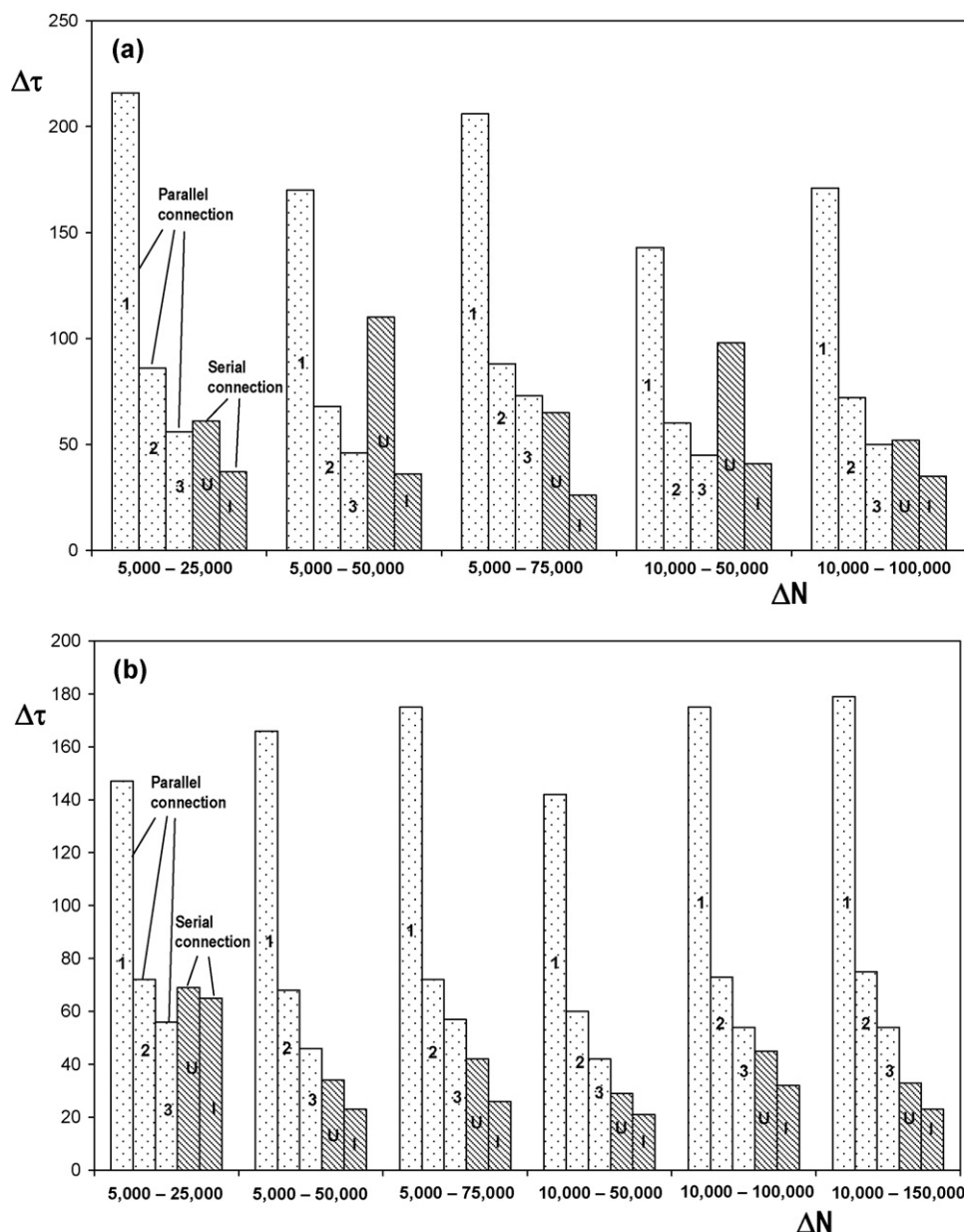$$N_{n\,col} = \frac{t_{n\,col}^2}{\sigma_{t,n\,col}^2} \tag{12}$$

Fig. 6. Relative deviation from the KS-limit ($\Delta\tau$) for the different optimized multi-column systems. Parallel connected columns: single column (1), dual column (2), triple column (3); serially connected columns: system with uniform particle size and segment length (U), system with individually optimized particle size and segment length (I), (a) solutions obtained at 400 bar and (b) 1200 bar.

Fig. 4a shows the different curves of analysis times versus efficiencies obtained in each of the ten sheets of the spreadsheet optimizer. The most leftward curve in Fig. 4a is for a system composed of one segment, the second curve corresponds to a system with two serially connected segments, and the most rightward one corresponds to a system composed of 10 serially connected segments.

To minimize the analysis time over a given range of efficiencies, the calculation presented in Fig. 4a is however not complete yet, as many of the solutions represented in Fig. 4a are useless or redundant and need to be removed by imposing certain constraints. Using a set of logical if-constructions in the same MS Excel file, these data points can be filtered out without needing any iterative solution. A first constraint is needed to set an upper limit on the maximum pressure that can be used ($\Delta P < \Delta P_{max}$). This maximum pressure is set equal to the maximum operating pressure of the experimental

equipment (column or system). A second restriction is needed to eliminate (N, t_R)-data points corresponding to the B-term region of the van Deemter curve. This restriction is implemented by putting an upper limit on the mobile phase velocity ($u_0 > u_{0,opt}$). In a next step, the obtained efficiencies are limited to a pre-set target range $N_{min} < N < N_{max}$ defined in the general sheet. Furthermore, some (N, t_R)-couples obtained on different column lengths can have the same value of time for one value of N or the same value of N for one value of time. In this case, the couples that provide the shortest analysis times and the highest efficiencies are retained whereas the rest is eliminated. The resulting set of curves is shown in Fig. 4b. As this filtering procedure eliminates the data series "sticking out" of the target range of efficiencies, it also automatically determines the number of segments that need to be coupled in series. In the example in Fig. 4, aiming at an efficiency range running between 5000 and 50,000, this number is equal to 7 (see Fig. 4b), whereas the
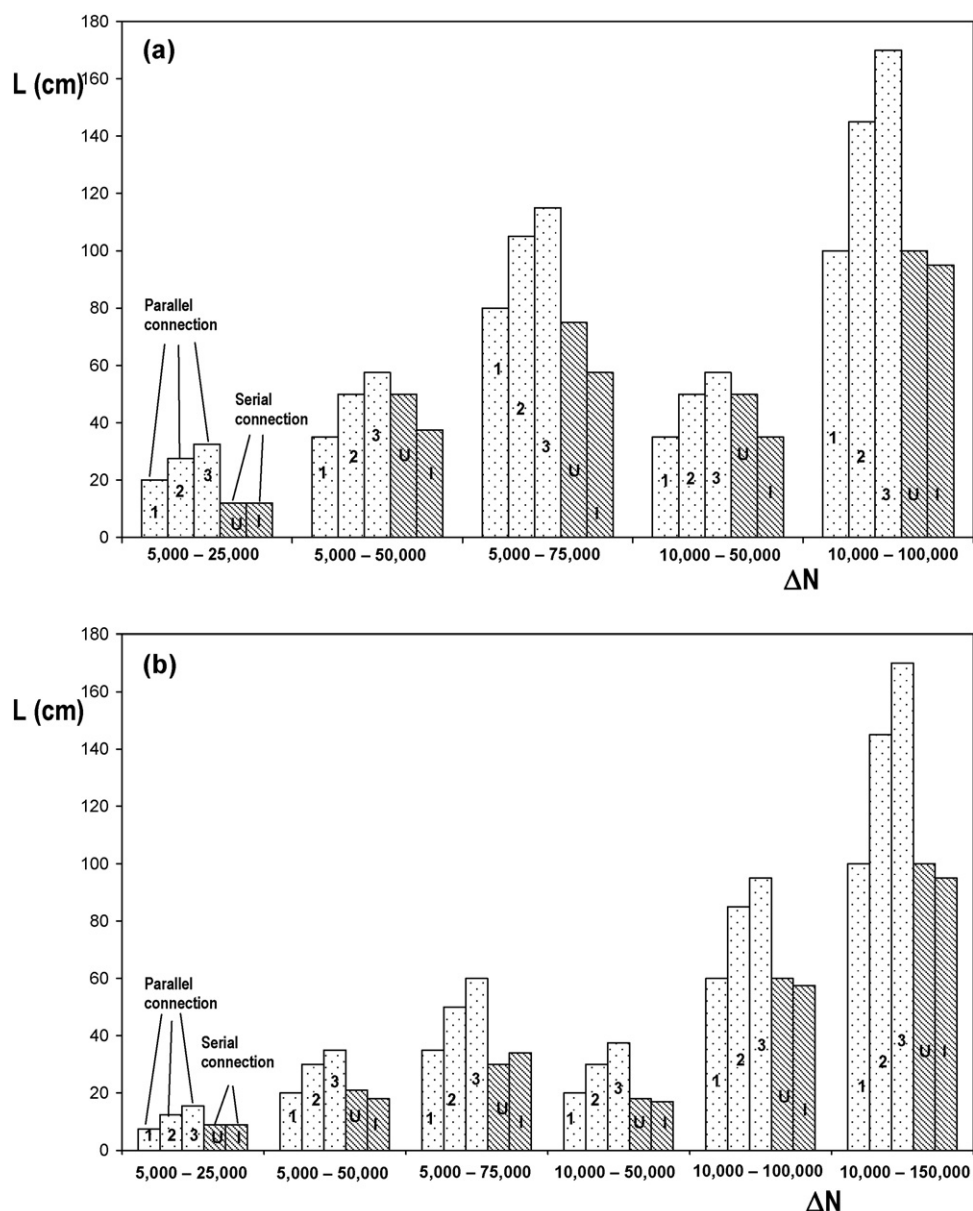
Fig. 7. Total column length for the different multi-column systems represented in Fig. 6. Parallel connected columns: single column (1), dual column (2), triple column (3); serially connected columns: system with uniform particle size and segment length (U), system with individually optimized particle size and segment length (I), (a) solutions obtained at 400 bar and (b) 1200 bar.

full set of segments is initially calculated for ten segments (corresponding to the number of sheets in the spreadsheet optimizer), as shown in Fig. 4a.

The spreadsheet optimization has been carried out for three different cases: (1) particle size and segment length are the same for every segment, (2) particle size is the same while the segment length is varied between different segments and (3) particle size and segment length are varied between different segments. These cases are discussed in below three sections. Again, only segment lengths and particle sizes that are commercially available were allowed for.

3.2.1. Fixed particle size and fixed segment length

It was our experience that the optimization of serially connected column systems is best started by taking the smallest available segment length for each of the segments. In this way, as many segments as possible can be used to cover a specific efficiency range,

bringing the separation performance already as close as possible to the KS-limit from the start. The minimal segment length that was considered was 3 cm, as most column manufacturers nowadays offer such columns. When this segment length does not lead to a solution for the first segment, i.e., when the first depicted segment in the finally filtered solution is in fact segment number 2, the initial segment length needs to be increased. It was also found that the optimization procedure is best started using the particle size obtained from Eq. (8) for $\alpha = 1$ and $N = N_{max}$. If this particle size does not result in a satisfactory solution, i.e., one wherein the achievable minimal and maximal efficiencies are within 5% of the required efficiency range, the particle size needs to be increased stepwise until a satisfactory solution is obtained. Considering the same efficiency range as in Fig. 3 for the parallel system, the minimum particle size that is needed for this efficiency range is $d_p = 1.8$ μm. For a segment length of 3 cm, however, the maximum efficiency that can be obtained using this particle size is only $N = 42,500$. Increasing
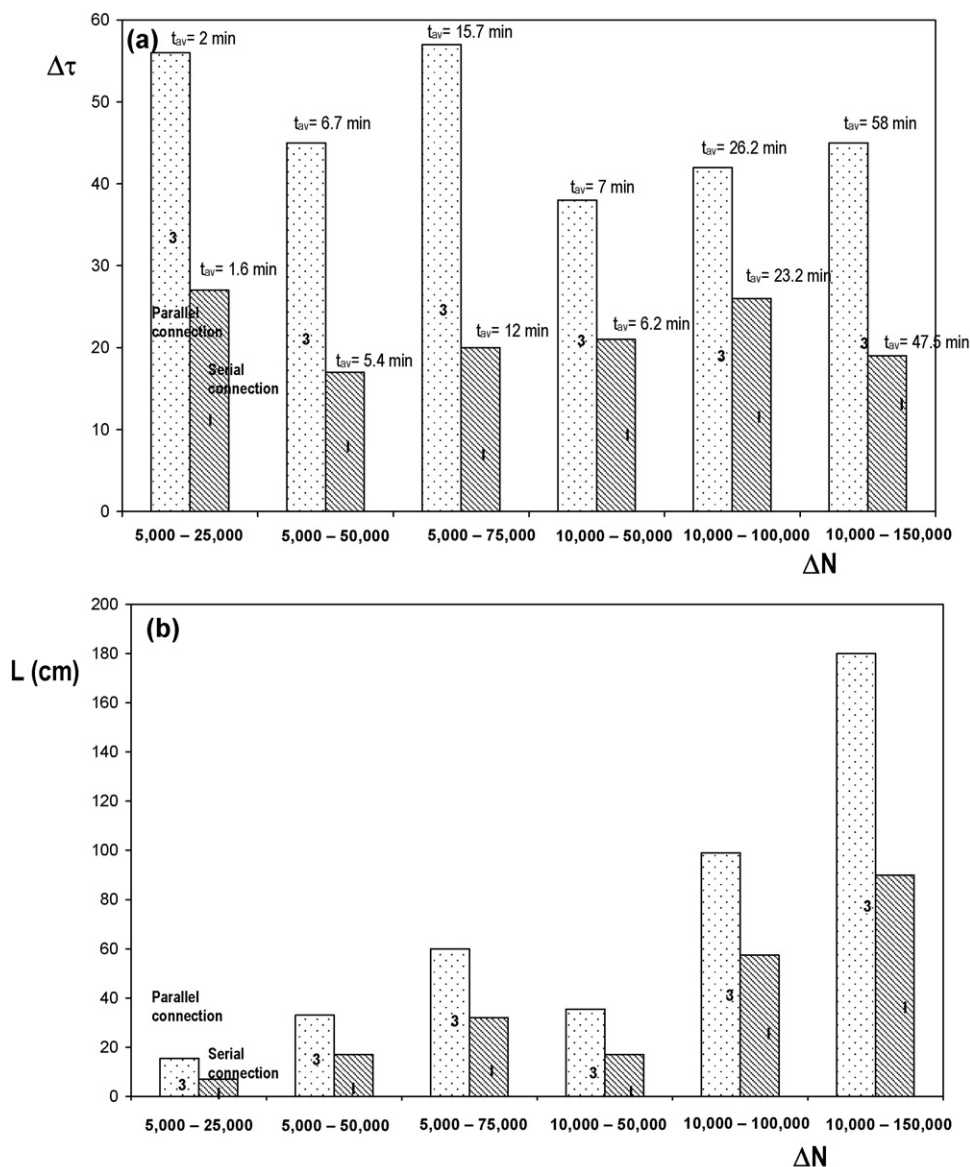
Fig. 8. (a) Relative deviation from the KS-limit ($\Delta\tau$) and (b) total column length for a parallel connected column system with three columns (3) and a serially connected column system with individually optimized particle size and segment length (I) considered at 1200 bar. In both cases, solutions yielding unconstrained segment lengths ($L \geq 1$) and particle sizes ($d_p \geq 1.5\,\mu m$) were allowed.

the particle size stepwise, a satisfactory solution is obtained for a particle size of 2.2 μm, allowing to cover the efficiency range in an average analysis time of 6.2 min. This solution is depicted in Fig. 5 (full symbols) and shows that 7 segments are needed to cover the entire range.

3.2.2. Fixed particle size and variable segment length

It was also observed that, for efficiency ranges wherein the final segment length is not the shortest length available, a further decrease in analysis time can be obtained by varying the length of the different segments. Again, the shortest possible analysis time will be obtained when the largest number of segments can be used. Practically, this means that the length of the first segment will be maintained, while the length of the subsequent segments is decreased. Obviously, a larger number of segments will require more connection tubing and hence lead to a higher system contribution. However, a careful selection of the dimensions of the connection tubing (as short as possible and with a small inner diameter (75–100 μm) allowed reducing this contribution to a minimum

(both the system pressure and the system band broadening never exceeded more than 5% of the total pressure or band broadening, even when using segments with a length of 3 cm, connected with 17 cm long connection tubing). Because the fixed particle size, fixed segment length-solution already made use of the shortest lengths available for the example shown in Fig. 5, no further decrease in analysis time could be obtained by altering the length of the individual segments. The fixed particle size, variable column length-case is therefore not represented in Fig. 5. An example where the use of variable lengths does improve the overall analysis time is given for a different target range ($N = 10,000$–$100,000$) in SM (Fig. S-7).

3.2.3. Variable particle size and variable segment length

A further decrease in analysis time can be obtained by optimizing the particle size as well as the segment length for every segment individually. Starting from the optimized fixed particle size, variable segment length solution, a new solution – wherein the total analysis time is reduced – can be found by decreasing the particle size of the first segment while simultaneously decreasing its

segment length (this follows again from the fact that smaller particles yield higher plate counts per meter column). Once a smaller particle size (and corresponding shorter segment length) leading to a satisfactory solution has been found, this new particle size (and segment length) is used in the next segment. If the solution is satisfactory (hence the efficiency range is still completely covered), the same (d_p, L)-combination is used in the subsequent segment. If the solution is not satisfactory, the particle size is increased stepwise until the efficiency range is completely covered again. The same process is then repeated (hence the new particle size is used in the subsequent segments and increased if necessary), until all segments have been re-evaluated.

The open symbols in Fig. 5 show the best serially-connected system to cover the efficiency range $5000 < N < 50,000$ when simultaneously optimizing both particle size and segment length. The average analysis time needed to cover this range is 5.7 min. Compared to the fixed particle size, fixed segment length-solution (closed symbols) a clear decrease in analysis time is observed, especially in the large efficiency part of the range. The achievable average time is only 23% larger ($\Delta \tau = 23\%$) than would be achievable with a hypothetical column operating systematically on the KS-limit line.

4. Results and discussion

To quantify how much gain in analysis time can be obtained by switching from a single column to a multi-segmented column system, the above optimization procedures have been applied to a number of different cases, considering different available pressures (400 bar and 1200 bar) and considering different efficiency ranges. In a first series of comparisons (Section 4.1), only column lengths and particle sizes that are commercially available are allowed for (see first paragraph of Section 3.1 for considered length and diameter values), and only fully porous particles are considered. In a second series of comparisons (Section 4.2), all particle sizes and column lengths (rounded of to 0.1 μm and 1 cm, respectively) are allowed for, as long as $L \geq 1$ cm and $d_p \geq 1.5$ μm. Finally, also the difference in dynamic efficiency range between a given type of fully porous and superficially porous particles is considered (Section 4.3).

4.1. Serial versus parallel connection (commercially available L and d_p only)

For each efficiency range, the best possible single column, and parallel-connected dual and triple column solutions are compared to the serially-connected column solution with uniform particle size and fixed segment length (index U in Fig. 6) and the serially-connected column solution wherein particle size and the segment length have been individually optimized for each segment (index I in Fig. 6). For each of the solutions, the total column length, the average analysis time needed to cover the efficiency range and the relative % deviation from the KS-limit have been determined. As a summary of the results, the relative % deviation from the KS-limit for the different column systems is graphically represented in Fig. 6. The full detail of the solutions (including the optimal values for d_p and L of each segment) is presented in Table 1 ($P = 400$ bar) and Table 2 ($P = 1200$ bar). Only realistic solutions (hence particle sizes and column lengths that are commercially available) were considered. Note that the number of segments for the serially-connected columns is variable for each solution, whereas this is not the case for the parallel-connected systems.

A general observation from the optimized particle sizes in Tables 1 and 2 is that, for any of the considered multi-column systems, the size of the particle diameter tends to increase as the required range in efficiencies increases. This is because long columns are needed to cover large efficiency ranges. Because the small particles have low permeabilities, they reach the maximum backpressure in relatively short column lengths and can therefore not be operated in columns that are long enough to cover the large efficiency ranges. Compared to the 400 bar solutions, the particle sizes that can be used at 1200 bar are much smaller (due to the larger available pressure) and the segment lengths correspondingly shorter. This consequently leads to much shorter analysis times compared to the 400 bar solutions.

Considering the different parallel systems, Fig. 6 (light bars) shows that, the larger the number of columns that can be used in parallel, the closer one can get to the KS-limit, in agreement with the findings in Fig. 3. Comparing the segmented column systems in Fig. 6 (dark bars), the KS-limit is always better approached by switching from a serially-connected system with uniform d_p, L (bars with index U) to the system with individually optimized d_p, L (bars with index I). The most important conclusion from Fig. 6 is that the serially-connected system with individually optimized segments in general approaches the KS-limit the best, with an average deviation of some 25–30%. This holds for both the 400 and the 1200 bar case (note that both cases have a different KS-limit, since this limit is proportional to ΔP_{max}, see Eq. (2)). There is one exception, where the parallel system with 3 columns is slightly better (1200 bar, range 5000–25,000). This is however only due to the considered lower limit on the applicable column length, as can be concluded from the following section, where this limitation is lifted and where the serially-connected system is best for all considered efficiency ranges.

Translating the $\Delta \tau$-values into absolute average analysis times (which is done in Tables 1 and 2 for all the different considered ranges), the serially-connected system with individually optimized segment lengths can for example cover the range of 5000–50,000 (dynamic range of 10) in an average time of 5.7 min (when the last analyte elutes with a retention factor $k = 10$), and the range of 5000–75,000 (dynamic range of 25) with an average time of 12.6 min when operating the system at 1200 bar. At 400 bar, these values are 18.8 and 37.9 min, respectively. The strong reduction of the average analysis time that can be realized by going from 400 to 1200 bar emphasizes the large advantage of UHPLC- over HPLC-conditions when a large range of efficiencies needs to be covered (average analysis time drops from 37.9 to 12.6 min when a range of 5000–75,000 needs to be covered).

Fig. 7 shows the corresponding total column length for each column solution, as a measure for the total amount of silica that is required for each case. From Fig. 7, it is evident that the serially-connected system is also more economic in terms of total amount of silica needed. The total column lengths needed to cover the different efficiency ranges with the different column solutions is consistently larger for the parallel connection-case with $n = 3$ (light bar with index "3") than for the two serially connected cases (dark bars). Obviously, parallel systems with $n = 4, 5, 6,\dots$ would allow to get closer to the KS-limit solution (and would finally outperform the serially-connected system), but this would occur at the expense of even a much larger required total column length. This will inevitably increase the price of the total column system and will also lead to set-ups that are much less practical to use.

4.2. Serial versus parallel connection (L and d_p available in increments of 1 cm and 0.1 μm, respectively)

The solutions considered so far were obtained assuming that the length and particle size of every column segment are restricted to dimensions that are commercially available. Assuming that column segments would be available with any length between 1 and 10 cm (in increments of 1 cm) and with particle sizes between 1.5 μm and

Table 1
Full detail of the column solutions for different efficiency ranges at 400 bar.

N-range	$d_{p,opt}$ (μm)	L (cm)	L_{total} (cm)	t_{av} (min)	$\Delta\tau$
(1) One segment (N_{max})					
5000–25,000	3.5	20.0	20.0	12.1	216
5000–50,000	3.5	35.0	35.0	37.3	170
5000–75,000	5	80.0	80.0	92.0	206
10,000–50,000	3.5	35.0	35.0	37.3	143
10,000–100,000	5	100	100	150	171
(2) Two segments ($N_{max,1}$, $N_{max,2}$)					
5000–25,000	3.5 + 1.9	20.0 + 7.50	27.5	7.20	86
5000–50,000	3.5 + 2.5	35.0 + 15.0	50.0	23.2	68
5000–75,000	5.0 + 3.0	80.0 + 25.0	105	56.5	88
10,000–50,000	3.5 + 2.5	35.0 + 15.0	50.0	24.5	60
10,000–100,000	5.0 + 3.5	100 + 45.0	145	94.9	72
(3) Three segments ($N_{max,1}$, $N_{max,2}$, $N_{max,3}$)					
5000–25,000	3.5 + 1.9 + 2.0	20.0 + 7.50 + 5.00	32.5	6.00	56
5000–50,000	3.5 + 2.5 + 2.0	35.0 + 15.0 + 7.50	57.5	20.1	46
5000–75,000	5.0 + 3.0 + 2.2	80.0 + 25.0 + 10.0	115	52.1	73
10,000–50,000	3.5 + 2.5 + 2.0	35.0 + 15.0 + 7.50	57.5	22.3	45
10,000–100,000	5.0 + 3.5 + 3.0	100 + 45.0 + 25.0	170	83.0	50
(4) Serially-connected system: fixed segment length, fixed particle size					
5000–25,000	2.5	3.00	12.0	6.20	61
5000–50,000	5.0	10.0	50.0	29.0	110
5000–75,000	5.0	15.0	75.0	49.6	65
10,000–50,000	5.0	5.00	50.0	30.4	98
10,000–100,000	5.0	20.0	100	83.8	52
(5) Serially-connected system: variable segment length, variable particle size					
5000–25,000	1.7 + 1.9 + 2.5	3.0	9.00	5.30	37
5000–50,000	3.5 + 3.5 + 3.5 + 3.5 + 3.5 + 3.5 + 5.0	5.0 + 5.0 + 5.0 + 5.0 + 5.0 + 5.0 + 7.5	37.5	18.8	36
5000–75,000	3.5 + 3.5 + 3.5 + 3.5 + 3.5 + 5.0 + 5.0	7.5 + 7.5 + 7.5 + 7.5 + 7.5 + 10 + 10	57.5	37.9	26
10,000–50,000	3 + 3 + 3 + 3 + 3 + 3 + 5 + 5	10 + 3 + 3 + 3 + 3 + 3 + 3 + 3	35.0	21.7	41
10,000–100,000	5 + 5 + 5 + 5 + 5 + 5 + 5 + 5 + 5 + 5	20 + 20 + 20 + 5 + 5 + 5 + 5 + 5 + 5 + 5	95.0	74.6	35

Table 2
Full detail of the column solutions for different efficiency ranges at 1200 bar.

N-range	$d_{p,opt}$ (μm)	L (cm)	L_{total} (cm)	t_{av} (min)	$\Delta\tau$
(1) One segment (N_{max})					
5000–25,000	1.5	7.50	7.50	3.20	147
5000–50,000	1.9	20.0	20.0	12.3	166
5000–75,000	2.2	35.0	35.0	27.5	175
10,000–50,000	2.0	20.0	20.0	12.4	142
10,000–100,000	3.0	60.0	60.0	50.7	175
10,000–150,000	3.5	100	100	112	179
(2) Two segments ($N_{max,1}$, $N_{max,2}$)					
5000–25,000	1.5 + 1.5	7.50 + 5.00	12.5	2.20	72
5000–50,000	1.9 + 1.5	20.0 + 10.0	30.0	7.70	68
5000–75,000	2.2 + 1.7	35.0 + 15.0	50.0	17.2	72
10,000–50,000	2.0 + 1.5	20.0 + 10.0	30.0	8.20	60
10,000–100,000	3.0 + 2.0	60.0 + 25.0	85.0	31.9	73
10,000–150,000	3.5 + 2.5	100 + 45.0	145	70.1	75
(3) Three segments ($N_{max,1}$, $N_{max,2}$, $N_{max,3}$)					
5000–25,000	1.5 + 1.5 + 1.5	7.50 + 5.00 + 3.00	15.5	2.00	56
5000–50,000	1.9 + 1.5 + 1.5	20.0 + 10.0 + 5.00	35.0	6.70	46
5000–75,000	2.2 + 1.7 + 1.5	35.0 + 15.0 + 10.0	60.0	15.7	57
10,000–50,000	2.0 + 1.5 + 1.7	20.0 + 10.0 + 7.50	37.5	7.30	42
10,000–100,000	3.0 + 2.0 + 1.5	60.0 + 25.0 + 10.0	95.0	28.3	54
10,000–150,000	3.5 + 2.5 + 3.0	100 + 45.0 + 25.0	170	61.8	54
(4) Serially-connected system: fixed segment length, fixed particle size					
5000–25,000	1.8	3.0	9.00	2.20	69
5000–50,000	2.2	3.0	21.0	6.20	34
5000–75,000	2.5	7.5	30.0	14.3	42
10,000–50,000	1.9	3.0	18.0	6.60	29
10,000–100,000	3.0	10	60.0	26.7	45
10,000–150,000	3.5	10	100	53.4	33
(5) Serially-connected system: variable segment length, variable particle size					
5000–25,000	1.7 + 1.8 + 1.8	3.0 + 3.0 + 3.0	9.00	2.10	65
5000–50,000	1.8 + 1.8 + 1.9 + 1.9 + 1.9 + 2.2	3.0 + 3.0 + 3.0 + 3.0 + 3.0 + 3.0	18.0	5.70	23
5000–75,000	2.2 + 2.2 + 2.3 + 2.5 + 2.5 + 2.5 + 2.5 + 2.5 + 2.5 + 2.5	5.0 + 5.0 + 3.0 + 3.0 + 3.0 + 3.0 + 3.0 + 3.0 + 3.0 + 3.0	34.0	12.6	26
10,000–50,000	1.7 + 1.8 + 1.9 + 1.9 + 1.9	5.0 + 3.0 + 3.0 + 3.0 + 3.0	17.0	6.20	21
10,000–100,000	2.5 + 2.5 + 2.5 + 2.5 + 2.5 + 2.5 + 2.5 + 3.0 + 3.0 + 3.0	7.5 + 5.0 + 5.0 + 5.0 + 5.0 + 5.0 + 5.0 + 5.0 + 7.5 + 7.5	57.5	24.2	32
10,000–150,000	3.0 + 3.0 + 3.0 + 3.0 + 3.5 + 3.5 + 3.5 + 3.5 + 3.5 + 3.5	7.5 + 7.5 + 10 + 10 + 10 + 10 + 10 + 10 + 10 + 10	95.0	49.5	23

5 μm (in increments of 0.1 μm), a further decrease in analysis time is possible. Fig. 8 shows how close the KS-limit can be approached for the best parallel connection case (i.e., for $n = 3$) and for the best serial connection case (i.e., for individually optimized segments). Table S-1 in the SM provides the corresponding construction and performance details. By also providing the t_{av}-times, an indication of the absolute times is given. Again, the serial connection case outperforms the parallel-connection case in terms of speed (Fig. 8a), and needs less silica material (cf. Fig. 8b). For example the serial connection system covering the 10,000–100,000 plate range, with segment lengths of 7.5 cm (segment 1, 9–10) and 5 cm (segments 2–8), and respectively filled with particle sizes of 2.2 μm (segment 1), 2.4 μm (segment 2), 2.5 μm (segments 3–7) and 3 μm (segments 8–10) seems a very attractive system, allowing to finish a set of randomly selected samples requiring a separation efficiency between 10,000 and 100,000 plates in an average time of 23 min per analysis (for a retained component with $k = 10$). Also the system where the segment length and the particle sizes are constrained to the commercially available values (see Section 4.1) is already

Table 3
Values for a, b and c, obtained by fitting the experimental plate height data to a reduced van Deemter model. The confidence intervals and quality of the fit (R^2) are given. Experimentally determined ϕ-values for each of the considered supports are also shown.

	Fully porous ($d_p = 1.8$ μm)	Superficially porous ($d_p = 2.7$ μm)
a	1.063 ± 0.165	0.745 ± 0.069
b	6.603 ± 0.155	5.615 ± 0.218
c	0.053 ± 0.015	0.036 ± 0.003
R^2	0.999	0.997
ϕ	518	586

very attractive. Having segment lengths of 7.5 cm (segments 1, 9–10) and 5 cm (segments 2–8), filled with particle sizes of 2.5 μm (segments 1–7) and 3 μm (segments 8–10), this system needs an average analysis time of 24 min (see Table 2), which is only slightly larger than that of the unconstrained solution. The deviation from the KS-limit is, however, more pronounced ($\Delta \tau = 32\%$ versus 26%).
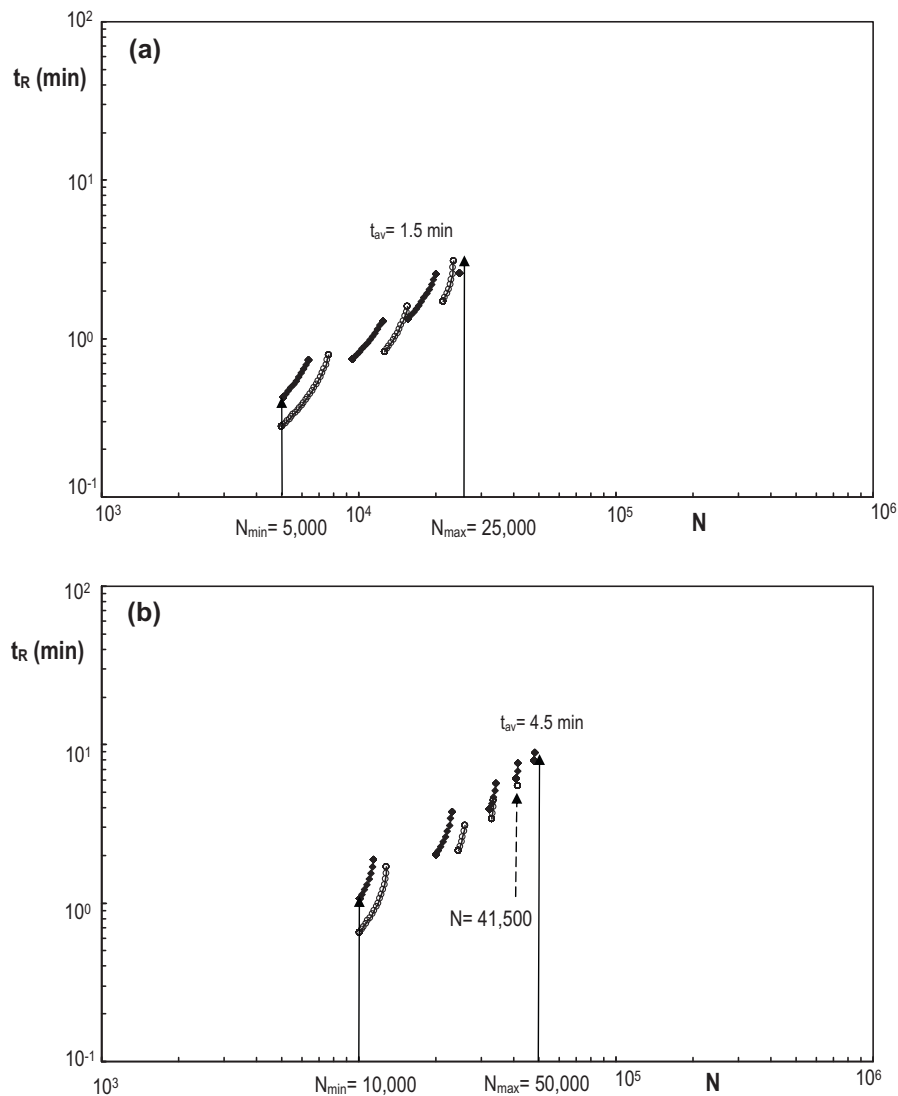

Fig. 9. Performance limits of commercially available column supports: (○) fully porous particles with a particle size of 1.8 μm and (♦) superficially porous particles with a particle size of 2.7 μm for an efficiency range of (a) $N = 5000$–25,000 and (b) $N = 10,000$–50,000 ($P_{max} = 600$ bar for the superficially porous particles and $P_{max} = 1000$ bar for the fully porous particles). Both particle types were available in lengths of 3 and 5 cm. For the solution in (a), both the fully porous particles and the superficially porous particles were used in lengths of 3 cm. For the solution in (b), the fully porous particles were used in lengths of 5 cm (segments 1–2) and 3 cm (segments 3–4), the superficially porous particles in lengths of 5 cm (segments 1–3) and 3 cm (segments 4–5). The a, b, c and ϕ-values of each support are shown in Table 1, $k = 7$, $\eta = 0.87\ 10^{-3}$ Pa.s, $D_{mol} = 6.9 \times 10^{-10}$ m^2/s.

4.3. Case study: superficially porous versus fully porous particles

Finally, also the performance limits of fully porous and superficially porous supports were compared. In this case, superficially porous particles, with a particle size of 2.7 μm were compared with fully porous particles with a particle size of 1.8 μm. The maximum pressure was set to 600 bar for the superficially porous particles and to 1000 bar for the fully porous particles. For each of the supports, the a, b, c and ϕ-values were determined experimentally as explained in the experimental section (see Table 3) and entered in the spreadsheet optimizer. Two efficiency ranges were considered for each of the particle types: $N = 5000$–25,000 (Fig. 9a) and $N = 10,000$–50,000 (Fig. 9b). In both cases it was assumed that both supports were available in segment lengths of 3 and 5 cm. Each segment length was subsequently individually optimized until the best possible solution (yielding the lowest deviation from the KS-limit) was obtained for each particle type. It must be remarked that both support types have different KS-limits, as can be deduced from the fact that they have different values of a, b, c and ϕ.

In the case where an efficiency range of $N = 5000$–25,000 needs to be covered (Fig. 9a), both the optimized systems of fully porous particles and superficially porous particles allow doing this in an average analysis time of 1.5 min, despite the fact that the superficially porous particles are operated at a much lower pressure (600 bar versus 1000 bar for the fully porous particles). These findings agree well with results recently presented in literature [14]. The deviation from their proper KS-limit, however, is slightly higher for the fully porous particles ($\Delta\tau = 100\%$) than for the superficially porous particles ($\Delta\tau = 90\%$). The superficially porous particles also need to be operated in a larger number of segments (3 segments versus 2 segments for the fully porous particles) for this particular range, making their total column length, however, only slightly longer ($L = 11$ cm) compared to that of the fully porous particles ($L = 10$ cm).

In the case where an efficiency range of $N = 10,000$–50,000 needs to be covered, Fig. 9b shows that the superficially porous particles allow covering a larger efficiency range than the fully porous particles. This is because the superficially porous particles have a higher permeability compared to the fully porous particles ($K_{v0} = 1.2 \times 10^{-14}$ m^2 versus 6.3×10^{-15} m^2 for the fully porous particles), which (over)compensates the fact that the superficially porous particles are used at a lower operating pressure. Due to the pressure drop limitation the maximum plate count that can be obtained with the fully porous particles is only $N = 41,500$, therefore these particles do not even allow to cover the pre-set efficiency range entirely. The superficially porous particles on the other hand allow covering the efficiency range in an analysis time of 4.5 min. Under the current pressure constraints, the superficially porous particles are therefore the better choice when samples with a larger degree of complexity need to be analyzed.

5. Conclusions

Whereas a single column can only cover a range of efficiencies of a factor of 3–4, multi-column systems (either operated in a parallel or a serially connected way) can cover a range running up to a factor of 10–25.

The serial connection mode offers a better compromise between average speed and amount of required silica (total required column length) than the parallel connection mode for all efficiency ranges running from 5000–10,000 plates up to 75,000–150,000 plates. Even though assuming an additional connection tubing length of up to 17 cm between each segment, the contribution of the extra connection tubing never exceeded more than 5% of the total pressure or band broadening. Furthermore, the serial connection mode is also more economical in terms of the required total column length, i.e., volume of silica particles.

The parallel connection mode could in principle be made as performant as the serial connection mode, but this would require the use of a larger set of columns ($n = 5$–10). Because each column segment is used individually in the parallel connection mode, this would require a large set of relatively long columns, making the total length of the system impractically large. In the serial connection mode, the longest column lengths are made by coupling the shorter ones together, which is of course much more economic in terms of total required column length.

Considering an UHPLC operation at 1200 bar, the best possible serial connection system can get within about 15–25% of the absolute speed limit (i.e., the Knox & Saleem-limit) of the employed particles, whereas a three-column parallel system can only get to within 40–50% of the speed limit, while needing 50–100% more total column length. The performance of the serial connection systems is quite sensitive to the number and the lengths of the individual segments making up the system. These should optimally be numerous (and thus relatively short), indicating the need for the development of compact designs for multi-column systems.

Serially-connected systems with individually optimized segment lengths can cover the considered wide efficiency ranges with a high average speed, especially under UHPLC conditions. For example, it should be possible to cover a range of 5000–75,000 theoretical plates (dynamic range of 25) with an average time of 12.6 min when using a 1200 bar instrument. At 400 bar, this would still require 37.9 min, pointing at the fact that the construction of wide-efficiency range systems would be one of the application areas where the advantages of UHPLC-conditions would be most fully realized.

Considering an efficiency range of 10,000–100,000 at 1200 bar with segments that are restricted to dimensions that are commercially available, an average time of 24 min would be necessary per analysis. This is only slightly more than the average required analysis time if segments with unconstrained dimensions would be available ($t_{av} = 23$ min).

For separations requiring relatively low efficiencies ($N = 5000$–25,000), it has been demonstrated that superficially porous 2.7 μm and fully porous sub-2 μm particles allow to cover this range in the same average analysis time, despite the fact that the superficially porous particles are operated at a much lower maximum pressure ($P_{max} = 600$ bar versus $P_{max} = 1000$ bar). Due to their higher intrinsic permeability, the superficially porous particles moreover allow to cover wider efficiency ranges than fully porous 1.8 μm particles. These findings suggest that it would be worthwhile to expand the allowable operating pressure of superficially porous particles to that of sub-2 μm particles (i.e., 1000 bar and above).

Acknowledgement

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.11.010.

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