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A column capacity study of single, serial, and parallel linked rod monolithic high performance liquid chromatography columns

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Abstract

The loading capacity of rod monolithic C18 columns was found to be sensitive to the injection volume, but essentially insensitive to the mass loading for a separation of oligostyrenes. When rod monoliths were coupled in series the injection volume loading increased, as too did the resolution of the oligomers, but at the expense of separation time. The volume load capacity of these serially connected monoliths was, however, not directly proportional to the number of columns connected. The volume load capacity was, however, directly proportional to the number of columns when the monoliths were connected in parallel and the flow stream split between each of the monolithic channels. When the number of monoliths in each channel equaled the number of monoliths that were connected in a single channel serial system the peak capacity and retention time was equivalent for both systems, but the volume load capacity of the parallel system was twice that of the serial connection each time the number of channels doubled. The results of this study indicate that parallel connection of rod monolithic columns may be useful for preparative scale and multidimensional separations where it is important that the volume load capacity is high.

Keywords: Column capacity; monolithic; HPLC; Multidimensional separational; Serial linked monolithic; Parallel linked monolithic; Preparative HPLC; Load capacity; Peak capacity; Volume load capacity; Monolithic channels rod monolithic HPLC columns

1. Introduction

With the ever-increasing demand placed upon the analyst to increase the speed of separation and analysis the chromatographer seeks higher flow rates. The chromatographer's bane, however, is the limitation associated with high back-pressures. Furthermore, we tackle more and more complicated samples and in doing so either longer columns are required, which is further detrimental to the speed, or we remain dissatisfied at our limitations in peak capacity. The same problems face preparative scale high performance liquid chromatography (HPLC), except here overload conditions, while increasing the sample capacity, lead to further reduction in peak capacity. In order to maintain high flow rates often the particle diameter is increased, but at the expense of resolution.

All separations in chromatography would benefit from high resolution, high column capacity, fast separations, and low back-pressures. These attributes would be great improvements in terms of isolation purity and increased sample throughput. One way of reducing back-pressure is to employ monoliths [1]. It is well known that the efficiency of separation on a rod monolithic column does not suffer the same detrimental separation performance that is observed in packed columns, and as the flow rate increases the comparative increase in back-pressure is only a fraction of that on a packed column of the same length. Therefore, the use of rod monoliths in HPLC seems to be an obvious advantage.

Multidimensional separation techniques at both the analytical [2–4] and preparative scale [5] are becoming increasingly popular. These methods of separation offer increased peak capacity by virtue of the expanded two-dimensional separation space. In some instances, a two-dimensional separation can be so designed such that it is tailored specifically to the isolation or analysis of a target compound [5]. Hence,

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the speed in separation can be gained without the subsequent requirement of high flow rates, and hence low back-pressures can be maintained. Having said that, however, not all two-dimensional separations are specifically orientated towards target compounds. Many two-dimensional separations involve the comprehensive analysis of a sample matrix [4]. In order to maintain an ordered two-dimensional separation the speed in the second dimension must be fast enough so that components heart cut from the first dimension do not undergo co-elution in the second dimension as a consequence of the wrap around effect. Hence, the problem is once again a limitation in back-pressure in the second dimension as flow rates are increased in order to maintain maximal resolution following a sufficient number of heart cutting sequences from the first dimension. The obvious answer is to incorporate monoliths into the second dimension [4].

It is well known that monoliths suffer from a two important limitations. The first being it is difficult to manufacture large volume solid rod monoliths [6]. Secondly, monoliths are subject to sample overload as a function of injection volume [7]. While this may not be of significance to analytical scale HPLC, it is very important to two-dimensional HPLC because it restricts the volume that can be heart cut from the first dimension to the second dimension that employs the monolith. Furthermore, if the rod monolith could be made in a sufficient size for preparative scale separation, the limited injection volume would reduce the productivity in separation for samples that were concentration limited.

An alternative approach that potentially overcomes the limited volume load capacity of a monolithic column is through the serial linkage of an array of monolithic columns. However, limitations still exist in meeting the high column capacity requirement [1,6,8]. Nevertheless, if this type of process is employed as the second dimension of a two-dimensional system, the limitation in peak capacity is almost unimportant, since each heart cut section to the second dimension contains only a fraction of the components of the entire sample that otherwise would have been analysed in a one-dimensional mode. Such an approach could feasibly be employed even with particle packed columns in situations were transfer volumes are limited due to immiscibility between solvents in each dimension, such as normal phase-reversed phase two-dimensional HPLC.

While studying channel capacity limitations with large signals and parallel channel applications in communication theory, also known as information theory (IT), it was realized that the IT concepts used in splitting or multiplexing large signals over multiple channels were applicable in chromatography separations [9,10].

Therefore, an HPLC experiment was designed using single, serially linked, and parallel linked monolithic columns to see if the initial contents of a temporarily overloaded column could be multiplexed into additional channels, separated, and then recombined without loss of analyte resolution. If successful, this would effectively simulate a single high capacity monolithic column of a selected phase. Although the approach is conceptually applicable to any phase, the phase must have the three properties of high resolution, low back-pressure, and fast separation. This approach has been used in the current study and we illustrate that the sample capacity and peak capacity can in fact be both increased through the application of serial and parallel linked monolithic columns.

2. Experimental

2.1. Chemicals

HPLC grade methanol and dichloromethane was obtained from Labscan Scientific Australia. Polystyrene standards with molecular weights of 580 (*n*-butyl) Daltons were purchased from Polymer Laboratories. Chromolith^{TM} Performance RP-18e monolithic columns, 100 mm × 4.6 mm were purchased from Merck KgaA, 64271 Darmstadt, Germany.

2.2. Equipment

The HPLC system used for study was a Waters LC system incorporating a 717plus autosampler, a 600-pump and controller and two 2487 dual wavelength detectors. The system was controlled by Waters Millenium^{32} 4.00 software.

2.3. Chromatographic separations

Polystyrene standards were prepared in 100% dichloromethane at concentrations noted in the text. All separations were conducted using a 100% methanol mobile phase, which was sparged continuously with helium. As the solvent injection plug solvent was substantially stronger than that of the mobile phase, as is common in polymer chromatography, sample load is limited due to polymer solvation effects in the solvent plug, which increase as the injection volume increases. This solvation effect serves as a useful indicator as to the advantage of the parallel column design, highlighted later in the text. Flow rates varied in order to maintain a constant elution time and the exact flow rate of any system is noted in the text. Columns were connected in a manner that minimized dead volume. Column temperature was ambient ∼21 °C. Injection volumes varied and are also noted in the text. UV detection was set at 262 nm. Duplicate injections were performed for each experiment, except for the reproducibility data, which was repeat six times for each column.

3. Results

3.1. Single channel, single monolithic columns

A 5 μL injection of the polystyrene standard solution (2 mg/mL) on to a C18 monolithic column (10 cm in length) is illustrated in Fig. 1. In total, there are 10 oligomeric bands

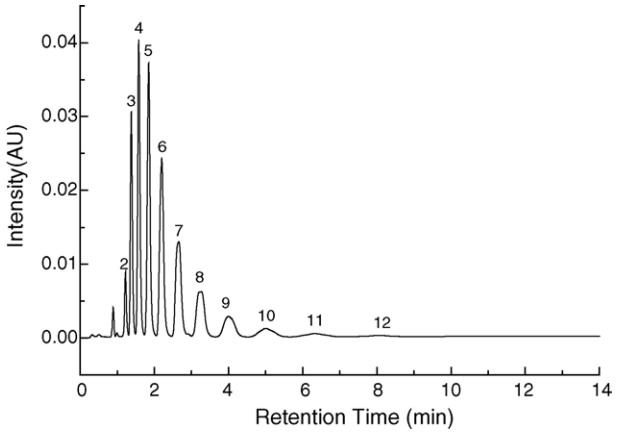

Fig. 1. Separation of *n*-butyl polystyrene oligomers on a C18 monolithic column (100 mm × 4.6 mm). Mobile phase 100% methanol, flow rate 2 mL/min, injection volume 5 μL, sample concentration 2 mg/mL.

observed. These are labeled 2–12 in accordance with the degree of polymerisation. The last band is, however, barely visible above the baseline, and as such the practical peak capacity of this isocratic separation is in the order of ten. Baseline resolution is observed between all oligomers and there is some peak distortion for the eighth oligomer, which is due to the partial separation of the diastereoisomers.

As the injection volume of the sample was increased from 5 to 20 μL the resolution between the oligomers decreased markedly, especially for the 20 μL injection. This is illustrated in Fig. 2. A slight degree of resolution is lost between the early eluting oligomers as the injection volume increased from 5 to 10 μL, but this was very minor. A more significant reduction was observed at 15 μL, while the resolution is clearly unsuitable at 20 μL.

Despite the monolithic column being sensitive towards injection volume, there was very little, if any, change in the resolution as the mass load was increased. Injections of 10 μL of the polystyrenes at concentrations from 1 to 40 mg/mL were tested, as illustrated in Fig. 3. The most significant difference across the five sample loads was an increase in retention as the mass load increased, even this was, however, slight.

These types of retention behaviour on monolithic columns, that is, the volume sensitivity and mass insensitivity, have been observed by other workers [7]. Such a problem is particularly important for the widespread application of monoliths as it limits their ability to be used in what we

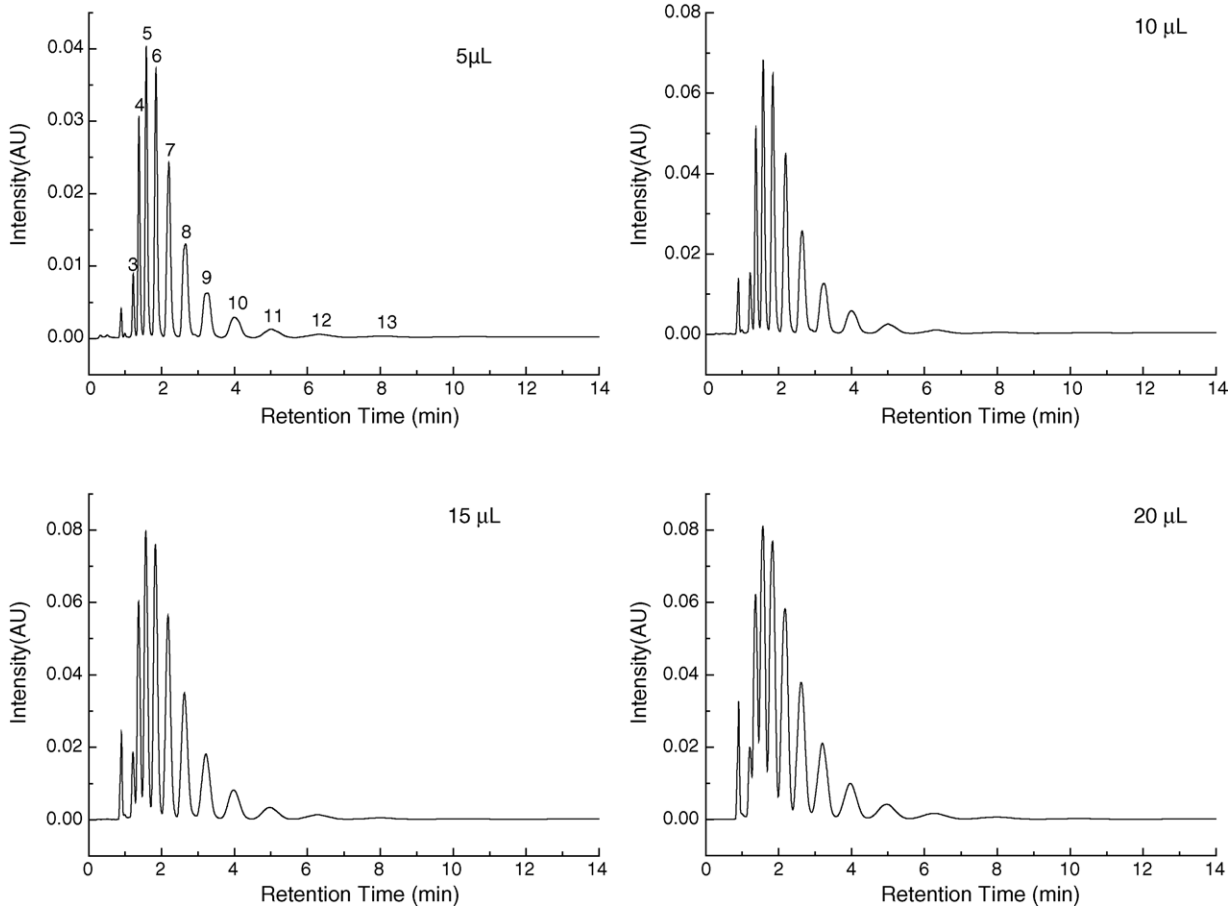

Fig. 2. Separation of *n*-butyl polystyrene oligomers on a C18 monolithic column (100 mm × 4.6 mm). Injection volumes: 5 μL (%fraction of void = 1.53), 10 μL (%fraction of void = 3.06), 15 μL (%fraction of void = 4.60) and 20 μL (%fraction of void = 6.12) as noted. Mobile phase 100% methanol, flow rate 2 mL/min, sample concentration 2 mg/mL.

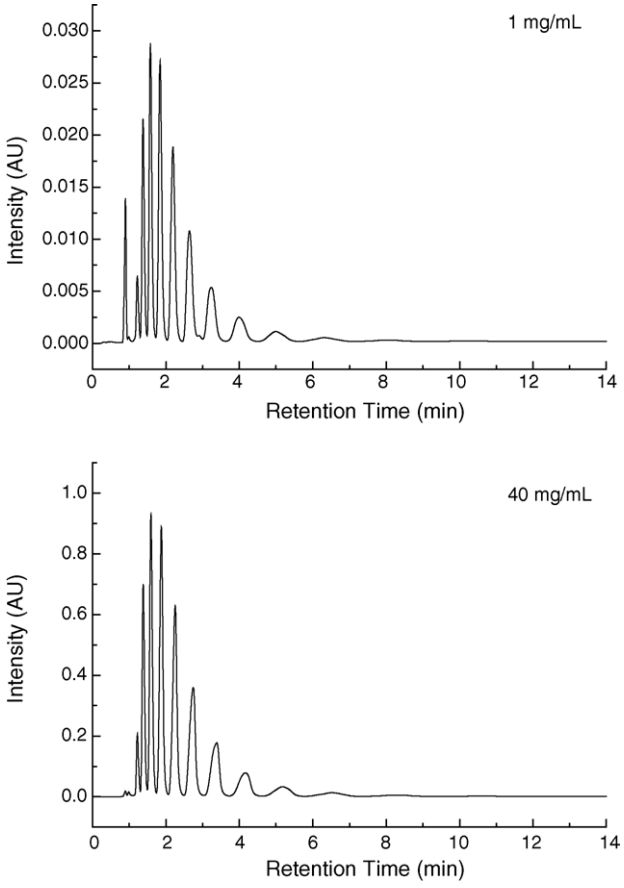

Fig. 3. Separation of *n*-butyl polystyrene oligomers on a C18 monolithic column (100 mm × 4.6 mm). Injection volumes 10 μL of a 1 and 40 mg/mL standard as noted. Mobile phase 100% methanol, flow rate 2 mL/min.

consider could be the two most important avenues of chromatographic separation where their speed of separation could be most significant. That is: (1) preparative scale separations, where the speed of separation on monoliths would lead to increases in production rate and (2) multidimensional separations, where high speed of separation in the second dimension could quite literally be the difference between being able to undertake a comprehensive analysis or not. In both these separation modes the injection volume is an important aspect of the method of analysis. In preparative chromatography large injection volumes are required to maximise sample load and overcome solubility limitations of the sample. While in multidimensional separations, heart-cut volumes could vary from 50 μL to more than 1 mL, depending on the separation problem. Quite obviously these types of sample load regimes would negate the use of the monolith.

This brings us to the concept of the present study. Here, we propose that in order to increase the capacity, both in regards to the peak capacity and the sample load capacity, that monoliths could be coupled serially and/or in parallel. A serial couple increases the number of theoretical plates, which is directly related to the resolution, while a parallel couple effectively allows splitting of the flow stream and hence a decrease in the apparent sample load in accordance with the number of parallel flow channels. The ability to couple monoliths rather than conventional packed particle columns stems from the vastly increased permeability of the monolith. Hence, columns can be stacked in series with substantially less back-pressure. Splitting the flow stream results in an overall reduction in flow through each of the parallel streams, but the flow rate can be increased in order to maintain constant elution time. These column formats are illustrated in Fig. 4, which illustrates the case of a single monolithic column, two serially coupled monolithic columns, four serially coupled monolithic columns, two parallel flow streams each of which contain two serially coupled monolithic columns, and four parallel flow streams each containing a single monolithic column. Each of these five flow regimes were tested in the course of this study and the results are presented following. In the case of the parallel flow streams, we have maintained systems that contain four monolithic columns in all cases.

Using this approach to increase peak capacity and sample load capacity does, however, rely significantly on the reproducibility of the manufacture of the individual monolithic columns. Following the splitting of the sample through parallel columns the flow may be required to re-converge through a single outlet. Band broadening as a result of column-to-column variation may be significant and as a result decrease the effectiveness of this separation approach. With this in mind we undertook a very basic test of column-to-column reproducibility. This test was in no way as intensive as those undertaken by Kele and Guiochon [11], but nevertheless served to illustrate the degree of retention reproducibility that is attained in our system. In total four monolithic columns were employed. The reproducibility of the oligomeric separation was tested using a 5 μL injection of a 40 mg/mL sample. The results in Table 1 show the injection-to-injection reproducibility on each of the monolithic columns and the column-to-column reproducibility. The results depicted in Table 1 only include the oligomers $n = 2$–7 because the peak splitting that occurs as a result of the partial isomer separation for oligomer eight and above biases the result. In general, the column-to-column reproducibility resulted in a relative standard deviation that was up to twice that of the RSD observed for most injection-to-injection reproducibility tests. Hence, band broadening is expected as a result of the flow stream splitting, but its significance will be shown to be largely less important than would have been expected.

3.2. Single channel, multiple monolithic columns

As the number of monolithic columns that were serially coupled increased, the resolution, as expected increased. This can be easily observed by evaluating the baseline separation between oligomers, and also by observing the increase in the partial separation of the isomers for the oligomer 8 as labeled in Fig. 5. Hence, the peak capacity of the system has increased as a result of coupling columns. Likewise, at constant flow rate, the separation time increased in accordance with the number of serially coupled columns. That is the retention

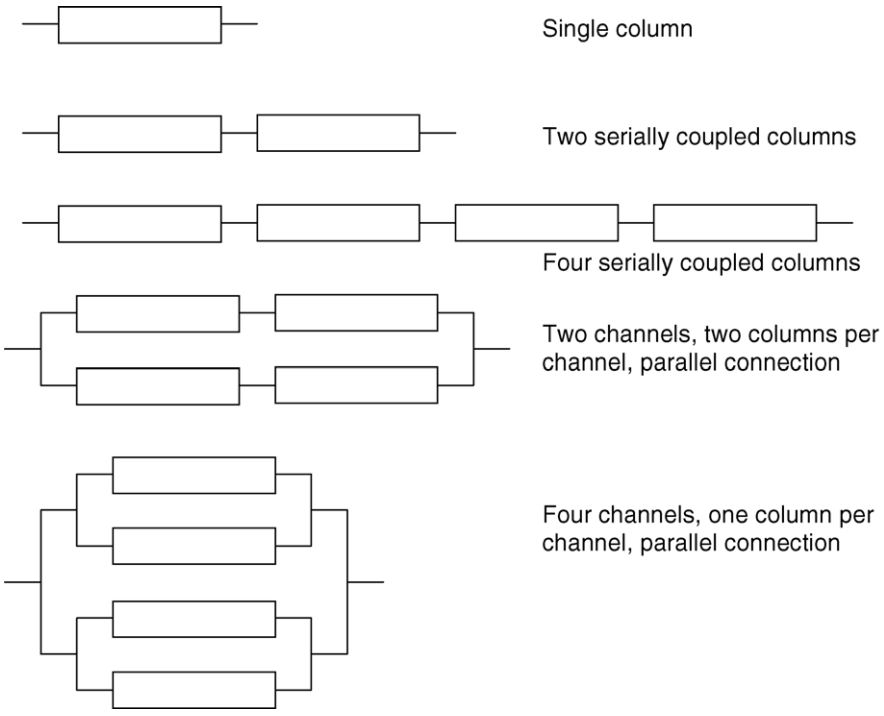

Fig. 4. Illustration of the coupling of columns in series and in parallel.

time for a two-column system was twice that of a single column system etc, although flow rates of 8 mL/min through a four-column section (40 cm bed length) were not possible due to high pressure shut down in the system. Fig. 5 illustrates the separation of the oligomers on a single monolithic column (Fig. 5a) in comparison to the four serially coupled monoliths (Fig. 5b). In each case, the separation remained insensitive to the mass load, but was sensitive to the volume load as shown by the separations illustrated in Fig. 6 where the injection volume is increased from 15 to 80 μL on the four serial column system. The injection volume that could be applied to the column at which point overload (as defined in Fig. 2) became significant increased as the number of 10-cm sections increased. However, this increase was not uniform as the peak broadening was observed for the 40 μL injection volume (Fig. 6b), which resulted in peak splitting for the 60 and 80 μL injection volumes (Fig. 6c and d, respectively). This is most easily observed for the lower order oligomers for which isomer resolution has not been apparent. We believe this peak splitting was a result of sample break through caused by the sample being dissolved in a stronger solvent (dichloromethane) than the mobile phase

Table 1
Mean retention times, and relative standard deviations and resolution values (Rs) of the oligostyrenes (n = 2–7) eluting from the four monolithic columns

Oligomer	Column 1 mean R_t (min) (Rs) (six repetitions)	Column 2 mean R_t (min) (Rs) (six repetitions)	Column 3 mean R_t (min) (Rs) (six repetitions)	Column 4 mean R_t (min) (Rs) (six repetitions)	Average overall mean R_t (min) (Rs) (four columns)	RSD (%)
Void	0.33	0.33	0.32	0.32	0.33	1.41
2	1.15 (1.92)	1.15 (1.91)	1.14 (1.83)	1.15 (1.69)	1.15	0.45
3	1.31 (2.01)	1.32 (1.90)	1.30 (1.95)	1.31 (1.77)	1.31	0.49
4	1.52 (2.11)	1.53 (2.07)	1.51 (2.04)	1.52 (1.87)	1.52	0.51
5	1.79 (2.12)	1.80 (2.06)	1.78 (2.05)	1.80 (1.95)	1.79	0.55
6	2.16 [a]	2.18 [a]	2.13 [a]	2.16 [a]	2.16	0.85
7	2.64 [a]	2.66 [a]	2.61 [a]	2.63 [a]	2.64	0.85

[a] Data not included because diastereoisomer separation skews oligomer peak to peak resolution.

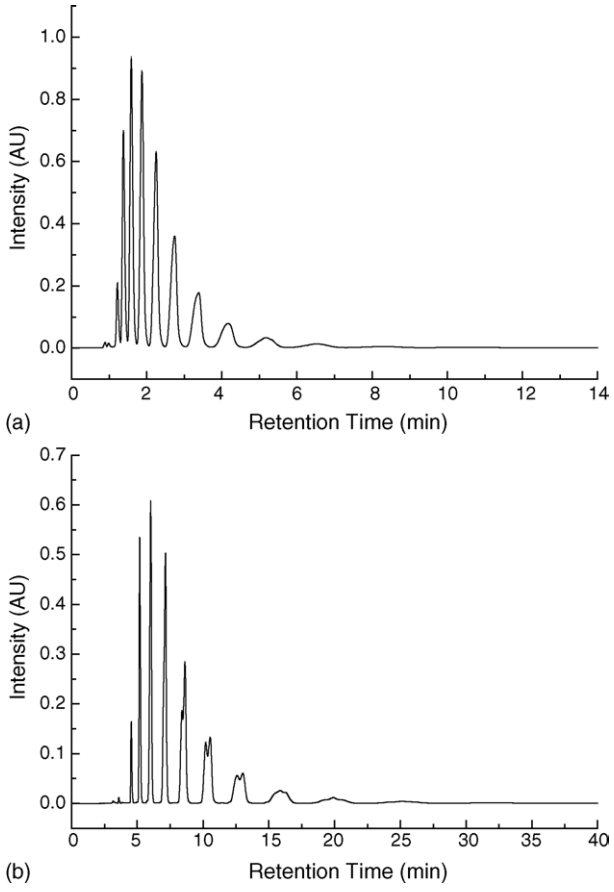

Fig. 5. Separation of *n*-butyl polystyrene oligomers on (a) a single C18 monolithic column (100 mm × 4.6 mm) and (b) four serially coupled C18 monolithic columns (total bed length 400 mm). Mobile phase 100% methanol, flow rate 2 mL/min, injection volume 10 μL, sample concentration 40 mg/mL.

(methanol) (this peak splitting is not to be confused with the diastereoisomer resolution apparent for oligomer 6 and above in Figs. 5b and 6a). As a consequence serial connection of monolithic columns did not allow for an increase in sample load as a simple function associated with the sum of each individual section.

3.3. Dual channel, multiple monolithic columns

The dual channel system contained two columns in each channel, a total of four columns. Flow stream from the injector was diverted via a Tee piece to each channel using identical pieces of pre column tubing. At the column outlet the flow streams were reunited in the reverse manner. The total flow rate was doubled to correspond to a 50% splitting in the flow rate through each channel, thereby maintaining a constant elution time. The results of the column reproducibility depicted in Table 1 illustrate the degree of column-to-column reproducibility. In addition they show that in this group of four columns there were essentially two pairs of columns that had very similar retention behaviour. That is the retention of oligomer 2, for example, on columns 1 and 2 eluted at 1.15 (value rounded up) and 1.14 (value rounded down) min, while for columns 3 and 4 elution occurred at 1.15 (value rounded down) and 1.15 (value rounded down) min, respectively. Consequently when the system was separated into dual channels columns from each group were used in each channel. That is columns 1 and 3 formed channel 1 and columns 2 and 4 formed channel 2. In this manner, the ability to reunite the flow streams with minimal band broadening was improved. Obviously as the number of channels increases and the number of columns in each channel increases such fortuitous column assignment may be less likely and band broadening may become an issue. At any rate, the separation of the oligostyrenes on this dual channel system is shown in Fig. 7. The two chromatograms depicted in Fig. 7 are collected from detectors located in each respective channel. The overlaid chromatograms illustrate that there is virtually coincident elution of each component from both channels and hence virtually no band broadening would result from the splitting of the flow stream through the two respective channels. The retention time and resolution of the oligomers is exactly equivalent to the single channel two-column system, however, the volume sample load is twice that of the single channel two-column system. Furthermore, the sample load (at the volume where overload is beginning to be observed as defined in Fig. 2) is greater than the single channel, four-column system (60 μL), as no peak splitting was observed because effectively 30 μL was applied to each channel. An additional advantage was that the separation was achieved in half the period of time. Some very slight band distortion is observed for the higher order oligomers at this sample load, but this has not appeared to affect the separation. Of course, if separation time is considered an unimportant factor, the peak capacity of the single channel, four-column system exceeds that of the dual channel, two columns per channel system as long as sample load is not important and small injection volumes can be employed. The difference between these systems becomes more important as the sample volume load increases.

3.4. Quad channel, single monolithic columns per channel

As we only had access to four monolithic columns, the Quad channel system contained only a single monolith in each channel. Nevertheless, this is sufficient to illustrate the application of flow splitting and the subsequent effect on peak capacity and volume load capacity. In this system, flow was split through three Tee pieces located between the injector and each channel. Identical pieces of pre-column tubing were used to minimise variation. The total flow rate was increased four fold over that for a single column system in order to maintain constant elution time. As we were unable to reunite the flow due to a lack of a sufficient number of Tee pieces we instead monitored the detection at each channel. Fig. 8 illustrates an overlay of the detection responses recorded for the oligostyrenes eluting in each of the four respective chan-

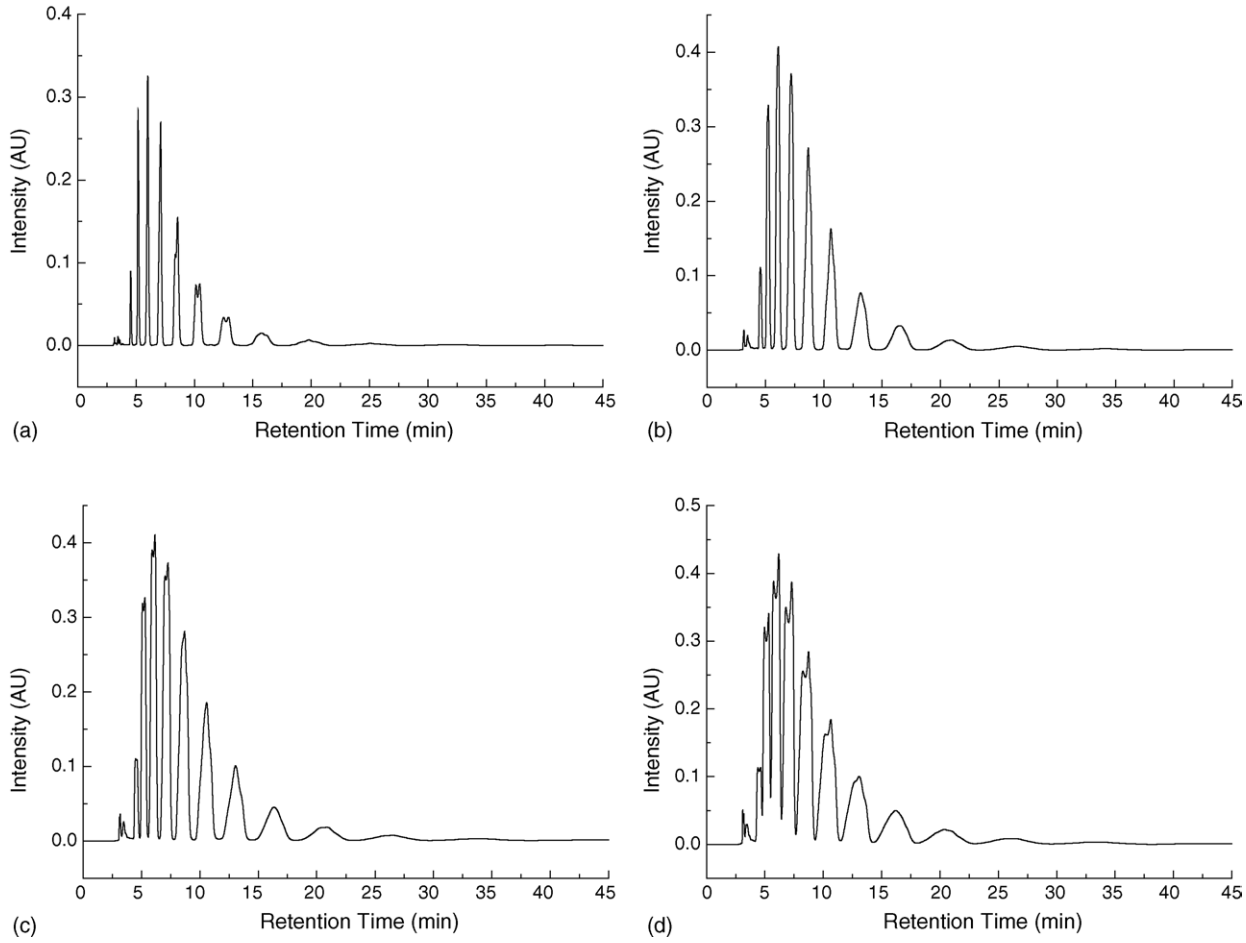

Fig. 6. Separation of *n*-butyl polystyrene oligomers on four serially coupled C18 monolithic columns (total bed length 400 mm). Mobile phase 100% methanol, flow rate 2 mL/min, sample concentration 10 mg/mL: (a) injection volume 15 μL (%fraction of void = 1.15); (b) injection volume 40 μL (%fraction of void = 3.06); (c) injection volume 60 μL (%fraction of void = 4.60); (d) injection volume 80 μL (%fraction of void = 6.12).

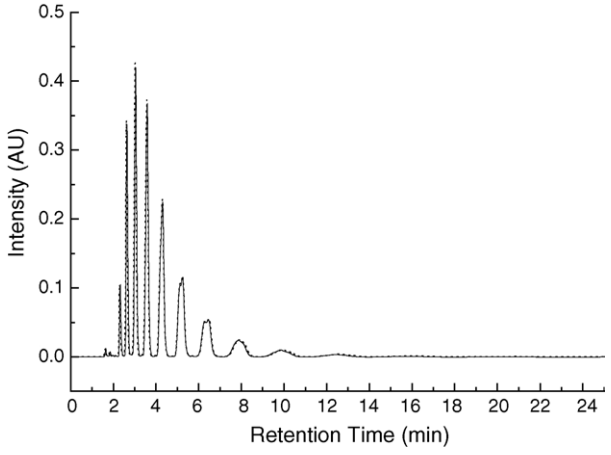

Fig. 7. Separation of *n*-butyl polystyrene oligomers on a dual channel, 2 columns per channel coupled C18 monolithic system. Mobile phase 100% methanol, flow rate 4 mL/min, injection volume 20 μL, sample concentration 10 mg/mL.

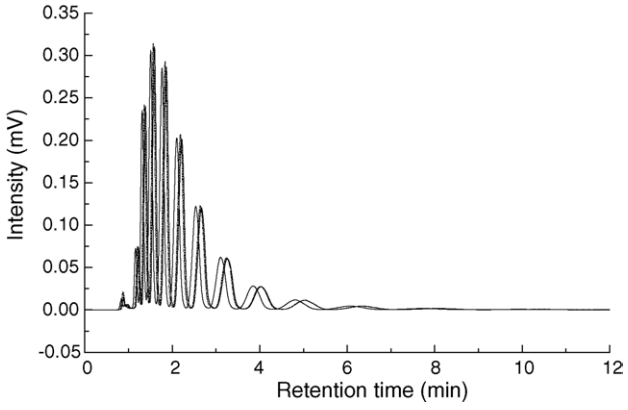

Fig. 8. Separation of *n*-butyl polystyrene oligomers on a quad channel, 1 column per channel coupled C18 monolithic system. Chromatograms illustrate the elution profiles observed from the four separate channels. Mobile phase 100% methanol, flow rate 8 mL/min, injection volume 40 μL (%fraction of void = 3.06), sample concentration 10 mg/mL.

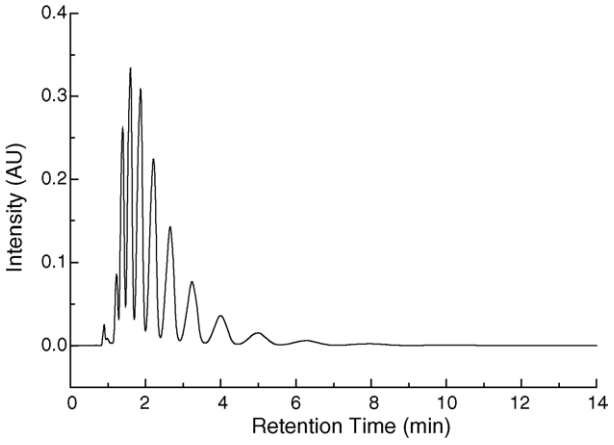

Fig. 9. Separation of *n*-butyl polystyrene oligomers on a quad channel, 1 column per channel coupled C18 monolithic system. Chromatogram illustrates the elution profiles from a single channel only. Mobile phase 100% methanol, flow rate 8 mL/min, injection volume 60 μL, sample concentration 40 mg/mL (%fraction of void = 4.60).

nels. The injection volume was 40 μL, which was below that set for the onset of the sample volume overload conditions for a four-column system in accordance with the results in Fig. 2. Three of the channels overlaid almost perfectly, while the forth was quite distinctly different and surprisingly not in agreement with the anticipated results from the reproducibility data given in Table 1. This probably indicates that the flow streams are not ideally split such that equal volume flow rate is established along each channel and is undoubtedly related to the permeability of each monolith. In accordance with the Dual channel, two columns per channel system, volume overload was obtained at 60 μL. The advantage of the four channel system was, however, that the elution time when volume overload is initially observed (i.e. peak capacity equal between each of the three systems) is half that of the dual channel system. The corresponding chromatograph at volume overload (60 μL) (illustration from one of the four channels only) is illustrated in Fig. 9. In comparison to Fig. 7, we clearly see the reduction in retention time.

4. Discussion

Increasing the peak capacity (hence resolution) in a system can be achieved by increasing the column length. In the case of monolithic columns, which are sensitive to the volume load, the increase in column length also increases the volume load that can be achieved, but this is not simply a summation function as factors such as sample break through from the injection plug ultimately limit the maximum injection. Hence, sample load is not directly related to the column length. Furthermore, increasing the column length in order to increase sample load has an adverse affect on the retention time and such a system, if it were to be applied in preparative chromatography, would prove to be detrimental to the production rate. Likewise both the limited load volume and the increase in retention associated with the increase in column length would adversely affect the ability of the monolith to be employed in the second dimension of a multidimensional system.

Another way to increase the sample load capacity is to divert flow through multiple monolithic channels. The load capacity effectively doubles as the number of channels doubles. Hence, for two systems that contain an identical bed length, but one contains four channels, the resolution (peak capacity) and separation time will be exactly equivalent (provided the flow rate through each section is held constant). However, when these systems are operated at maximum volume sample load the four-channel system will allow for an increase in sample load by a factor of four times that of the single channel.

The scope for maximising sample load can be further increased through the multi-channel system by simply increasing the number of columns (or column length) in each channel. At that point in time, the multi-channel system vastly outperforms the single channel system.

A disadvantage of the multi-channel system may be seen in the band broadening associated with the re-converging of the flow streams. However, in preparative chromatography the aim is rapid isolation. Detection across all channels is not essential, rather one channel could be monitored and at the appropriate time each flow stream through the respective channels could be diverted to a collection vessel. In the case of multidimensional analysis, it is not essential also that the flow streams reconverge as an analysis could be performed on a single stream, that is unless the dilution of the flow as a result of stream splitting introduces a detection sensitivity issue.

5. Conclusion

Serially linked, parallel linked, and parallel serially linked combinations of rod monolithic C18 HPLC columns were studied as a function of injection volume and mass loading to explore the concept of a multi-channel serially linked rod monolithic C18 HPLC column as a single preparative rod monolithic C18 HPLC column. The chromatographic behaviour of these linked monolithic columns was examined by analysing oligostyrene standards with four combinations of linked Chromolith^{TM} RP-18e columns: single channel, single monolithic columns; single channel, multiple monolithic columns, dual channel, multiple monolithic columns; and quad channel, single monolithic columns.

Higher peak capacities and higher sample load capacities were observed when the initial contents of a single temporarily overloaded rod monolithic HPLC column were diverted into additional parallel serially linked channels. In addition, the high speed of separation and low back-pressures typical of rod monolithic HPLC columns were preserved. Some band broadening was seen when reconverging flow streams of multi-channels depending on the column configurations and

the number of channels. However, this experiment has shown that parallel connection of serially linked rod monolithic columns may be useful in preparative and multidimensional separations where high volume load capacities are needed.

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