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Practical comparison of 2.7 μm fused-core silica particles and porous sub-2 μm particles for fast separations in pharmaceutical process development

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ABSTRACT

Fused-core silica stationary phases represent a key technological advancement in the arena of fast HPLC separations. These phases are made by fusing a 0.5 μm porous silica layer onto 1.7 μm nonporous silica cores. The reduced intra-particle flow path of the fused particles provides superior mass transfer kinetics and better performance at high mobile phase velocities, while the fused-core particles provide lower pressure than sub-2 μm particles. In this work, chromatographic performance of the fused-core particles (Ascentis Express) was investigated and compared to that of sub-2 μm porous particles (1.8 μm Zorbax Eclipse Plus C18 and 1.7 μm Acquity BEH C18). Specifically, retention, selectivity, and loading capacity were systematically compared for these two types of columns. Other chromatographic parameters such as efficiency and pressure drop were also studied. Although the fused-core column was found to provide better analyte shape selectivity, both columns had similar hydrophobic, hydrogen bonding, total ion-exchange, and acidic ion-exchange selectivities. As expected, the retention factors and sample loading capacity on the fused-core particle column were slightly lower than those for the sub-2 μm particle column. However, the most dramatic observation was that similar efficiency separations to the sub-2 μm particles could be achieved using the fused-core particles, without the expense of high column back pressure. The low pressure of the fused-core column allows fast separations to be performed routinely on a conventional LC system without significant loss in efficiency or resolution. Applications to the HPLC impurity profiling of drug substance candidates were performed using both types of columns to validate this last point.

1. Introduction

The ever-increasing demand for high sample throughput and fast analysis has driven HPLC users to search for breakthroughs in HPLC instrumentation and column technology. Ultra-high or very high pressure pump systems have been used to overcome the high pressure drop generated by small particles of packing materials [1,2]. The commercialization of higher pressure LC systems has accelerated applications of fast liquid chromatography in various fields, including drug discovery [3–6] and drug development [7,8], food industry [9] and environmental monitoring [10,11]. Recent advances in HPLC column technology have focused on various approaches to increase the speed of analyses [12–14]. Monolithic columns, for example, were introduced for their potential use at high mobile phase velocities due to decreased mass transfer effects over conventional fully porous particles [15,16]. The high porosity and small skeleton size of monolithic columns permit operation at high flow rates using conventional LC systems [17]. The disadvantages for the monolithic columns are their limited stationary phases commercially available and often low retention. Another approach of achieving fast separation is to use columns packed with sub-2 μm particles, which provide fast and efficient separations over conventional 3–5 μm particles [18,19]. Smaller particles result in flatter van Deemter curves, allowing for higher flow rates while still maintaining near maximum efficiencies. Unfortunately, the cost for the improved efficiencies is higher column back pressure. To realize the benefit of the sub-2 μm particles, instrumentation beyond conventional HPLC is usually required [12].

The development of fused-core or "superficially porous" silica particles was considered as a breakthrough in column technology aimed at reducing analysis times while maintaining column efficiencies and requiring relatively low back pressures [20,21]. The fused-core particles are made by fusing a 0.5 μm porous silica layer onto solid 1.7 μm core particles. A major benefit of the fused-core particles is the small diffusion path (0.5 μm) compared to fully porous particles (e.g., 1.8 μm). The reduced intra-particle flow path provides superior mass transfer kinetics and better performance at high mobile phase velocities [22,23]. Another feature

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that is unique to the fused-core particles is their narrow particle size distribution which facilitates packing of these columns with great ruggedness [24]. More importantly, 2.7 μm fused-core particles produce only approximately half the back pressure of the 1.8 μm particles, which makes it possible to use fused-core columns on conventional HPLC systems. In addition, columns packed with 2.7 μm fused-core particles can use 2 μm porosity inlet frits, the same frit typically used on columns with 3–5 μm particles. This makes these columns less susceptible to the plugging problems that are sometimes evident with most sub-2 μm columns with 0.5 μm inlet frits, especially for the pharmaceutical samples with complex matrices. As we hope to demonstrate in the present work, these characteristics of the fused-core particle columns can produce key advantages over the sub-2 μm particle columns, for many applications, with minimal impact on the separation speed, efficiency, and resolution.

Recently, Gritti et al. [22] demonstrated the efficiency advantages of fused-core columns over 3.0 porous packed columns. Cunliffe and Maloney [21,25] and Hsieh et al. [26] compared the column efficiency and pressure for fused-core columns and sub-2 μm porous particles. In the pharmaceutical industry, retention and selectivity behaviors and sample loading capacity of fused-core columns are of practical interest. In this study, these properties of the fused-core and the sub-2 μm totally porous particles are systematically evaluated. Column efficiency and pressure drop are also compared in the pharmaceutical background. Finally, applications to the HPLC impurity profiling of drug substance candidates and intermediates are demonstrated, directly comparing the performance of the fused-core column to that of the sub-2 μm column using a conventional HPLC system in pharmaceutical process development.

2. Experimental

2.1. Reagents and materials

HPLC grade acetonitrile (MeCN) and methanol (MeOH) were purchased from Fisher (Springfield, NJ, USA). Deionized water was purified using Hydro ultra pure water purification system (Garfield, NJ, USA). Benzophenone, benzylalcohol, benzylamine, butylbenzene, caffeine, pentylbenzene, phenol, phosphoric acid, o-terphenyl, toluene, triphenylene, and uracil were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Potassium phosphate (mono- and dibasic) was purchased from J.T. Baker (Phillipsburg, NJ, USA). Pharmaceutical related compounds, including cannabinoid receptor-1 antagonist (CB-1), a drug intermediate, and a drug substance candidate were synthesized by the Process Research Department, part of Merck Research Laboratories (Rahway, NJ, USA). The 10 mM phosphate buffer (pH ≈ 7) was prepared by dissolving 0.54 g of KH_2PO_4 and 1.04 g of K_2HPO_4 in 1 L of deionized water. The 0.1% phosphoric acid aqueous solution was prepared by dissolving 1 mL of 85% H_3PO_4 in 1 L of deionized water.

2.2. Instrumentation and chromatographic conditions

All the experiments were performed on either a Waters Acquity UPLC system (Milford, MA, USA) or an Agilent 1100 HPLC system (Palo Alto, CA, USA), equipped with a diode array detector.

The chromatographic performance of the fused-core particles (Ascentis Express C18, 2.1 (or 4.6) × 50 (or 100) mm, 2.7 μm column) and sub-2 particles (Zorbax Eclipse Plus C-18, 4.6 mm × 50 mm, 1.8 μm or Waters Acquity BEH C18, 2.1 mm × 50 mm, 1.7 μm column) were investigated in the experiments described below.

2.2.1. Retention/selectivity characteristics

The relationship between retention factor (k') and mobile phase composition was investigated for both polar (benzophenone) and non-polar (benzene) solutes using the fused-core and sub-2 μm particles and various (isocratic) mobile phase compositions containing acetonitrile levels ranging from 30 to 50% (v/v). The relationship between selectivity (α) and mobile phase composition was also investigated for polar (aniline and benzophenone) and non-polar (benzene and toluene) analytes using the fused-core and sub-2 μm particles and various mobile phase compositions with acetonitrile levels again ranging from 30 to 50% (v/v).

Selectivity behaviors of the two particles were further investigated using a modified column characterization protocol based on methodology developed by Tanaka's group [27] and later adopted for comparison of commercially available phases by Euerby and Petersson [28]. This protocol comprises several tests with the probe molecules designed to provide comparative information about hydrophobic and silanophilic properties of reversed-phase columns. The chromatographic parameters of interest measured in this study are briefly described below:

(a) Retention factor for pentylbenzene, k_{PB}: reflects the surface area and surface coverage. Chromatographic conditions: MeOH:H_2O (80:20, v/v), 1.0 mL min^{-1}, 40 °C, UV detection at 254 nm.
(b) Hydrophobic selectivity, α_{CH_2}: ratio of retention factors of pentylbenzene and butylbenzene, k_{PB}/k_{BB}; it measures selectivity between alkylbenzenes differentiated by one methylene group, which is dependent on the stationary phase surface coverage and the alkyl chain length of the ligand. The chromatographic conditions were the same as for the retention factor determination (*i*).
(c) Shape selectivity, $\alpha_{T/O}$: ratio of retention factors between planar triphenylene and non-planar o-terphenyl, k_T/k_O; it is influenced by the spacing of the ligand as well as functionality of the silylating agent used to make the stationary phase. The chromatographic conditions were as the same as for the retention factor determination (*i*).
(d) Hydrogen bonding capacity, $\alpha_{C/P}$: retention factor ratio between caffeine and phenol, k_C/k_P; this parameter is designed to trace hydrogen bonding interactions between surface silanols and caffeine. On phases with higher silanol activity, caffeine will observe stronger retention. The chromatographic conditions used were the same as for the retention factor determination (*i*), except the mobile phase consisted of MeOH:H_2O (30:70, v/v).
(e) Total ion-exchange capacity, $\alpha_{B/P}$, at pH 7.6: retention factor ratio between benzylamine and phenol, k_B/k_P; this parameter provides an estimate of total silanol activity. The chromatographic conditions used were the same as for the retention factor determination (*i*), except the mobile phase was methanol–water (20 mM KH_2PO_4, pH 7.6) (30:70, v/v).
(f) Acidic ion-exchange capacity, $\alpha_{B/P}$ at pH 2.7: retention factor ratio between benzylamine and phenol, k_B/k_P; this parameter is a measure of acidic activity of the surface silanol groups on the stationary phase. Chromatographic conditions were the same as for total ion-exchange capacity, except the mobile phase was methanol–water (20 mM KH_2PO_4, pH 2.7) (30:70, v/v).

2.2.2. Sample loading capacity

For the determination of sample loading capacity of the two different particles, mobile phase compositions of 60:40 H_2O/MeCN or 65:35 H_2O/MeCN (v/v) were used for the 1.8 μm Eclipse Plus porous particles or for the 2.7 μm fused-core C18 particles, respectively, in order to obtain similar retention factors (flow rate = 0.5 mL min^{-1}, in both cases). A series of benzyl alcohol solutions ranging in concentration from 0.1 to 80 mg mL^{-1} were prepared in diluents having

the same composition as each of the two mobile phases. The compositions of the sample solutions were kept as similar as possible to the mobile phase to minimize any effects of solvent mismatch. In addition, a 2 μL injection volume was used for all injections of the benzyl alcohol standard solutions to minimize band broadening caused by higher injection volumes.

2.2.3. Column efficiency/van Deemter curve

A van Deemter curve was generated under isocratic conditions using water–acetonitrile (55/45, v/v) for 0.5 mg mL^{-1} benzophenone. The column temperature was set to 40 °C and the sample tray temperature was maintained at 25 °C in all cases. The flow rate on both columns was systematically varied from 0.1 to 1.5 mL min^{-1}, using 0.2 mL min^{-1} increments.

2.2.4. Column back pressure

An isocratic condition (50/50 H_2O/MeCN) was utilized to compare the back pressures generated by the fused-core and the sub-2 μm particles at different flow rates, ranging from 0.25 to 3.0 mL min^{-1}. The column temperature was set to 40 °C for this experiment. The linear velocity was calculated by dividing the column length (L, mm) by void time (t_0, s) at each flow rate. The void time was determined by using uracil as un-retained/void volume marker.

2.2.5. Applications

(a) A linear gradient mobile phase condition was employed to analyze a pharmaceutical intermediate and its impurities using the fused-core particle column (Ascentis Express C18, 4.6 mm × 50 mm, 2.7 μm) and sub-2 μm particles column (Zorbax Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 μm). The mobile phase of 0.1% H_3PO_4 (A) and acetonitrile (B) and a linear gradient from 65:35 A:B (v/v%) to 5:95 A:B (v/v%) over 6 min was employed. For a drug substance candidate, the mobile phase of 0.1% H_3PO_4 (A) and acetonitrile (B) and a linear gradient from 10:90 A:B (v/v%) to 5:95 A:B (v/v%) over 6 min was employed. For both compounds, sample concentration of 0.1 mg mL^{-1}, injection volume 3 μL, flow rate of 1.5 mL min^{-1} and column temperature of 40 °C were used for the method.

(b) Isocratic elution was utilized to compare the chromatographic performance of an Ascentis Express C18 column (4.6 mm × 50 mm, 2.7 μm) to that of a Zorbax Eclipse Plus C18 (4.6 mm × 50 mm, 1.8 μm) column, using a real-world developmental pharmaceutical compound (CB-1) and its related impurities (see Fig. 1). In order to achieve the same k' on each phase, the mobile phase, consisting of 0.1% H_3PO_4 (A) and MeCN (B), had a consistency of 40/60 A:B (v/v%) for the fused-core particle column and 36/64 A:B for the sub-2 μm porous particle column. In both cases, the detection wavelength was 220 nm, the flow rate was 2.5 mL min^{-1} and the column temperature was maintained at 40 °C. An injection volume of 5 μL was used for all injections, with samples prepared at a concentration of ~1 mg mL^{-1} in a diluent consisting of 30/70 H_2O/MeCN (v/v%).

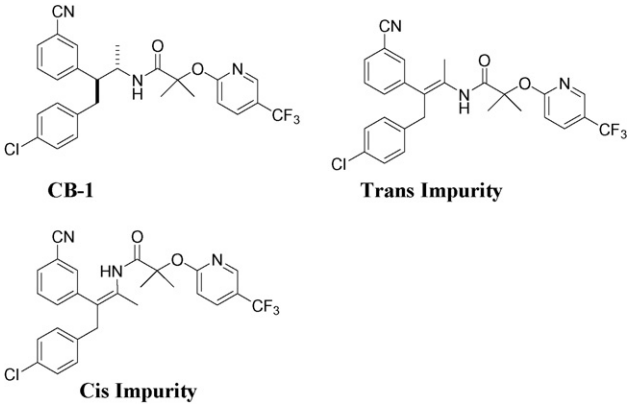

Fig. 1. Structures of CB-1 and its impurities.

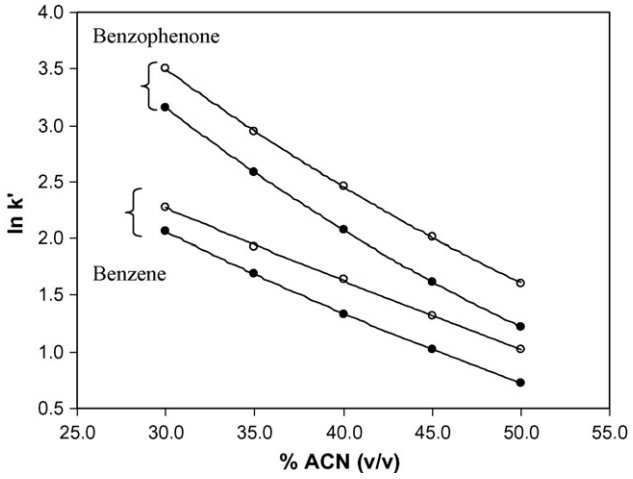

Fig. 2. Relationship of the retention factor k' and the MeCN concentration in the mobile phase. Benzene and benzophenone are the test solutes, respectively. Key: (●) Ascentis Express C-18, 4.6 mm × 50 mm, 2.7 μm column; (○) Zorbax Eclipse Plus C-18, 4.6 mm × 50 mm, 1.8 μm column.

2.3. Data acquisition and analysis

For all the experiments on HP 1100, Atlas Chromatographic System was used for instrument control, data collection, and data analysis. For UPLC, Atlas was only used to collect and process the chromatograms.

3. Results and discussion

3.1. Retention

The retention characteristics of the fused-core particles were compared to those of the sub-2 μm particles. Fig. 2 shows the relationship between retention factor and mobile phase composition for benzene and benzophenone, respectively, on these two stationary phases. The data indicate that the retention factor on the fused-core particle column (Ascentis Express) is lower than that of sub-2 μm particle column (Zorbax Eclipse Plus). The lower retention factors on the fused-core particle could be due to lower accessible surface area (lower carbon loading) compared to Zorbax sub-2 μm column. However, similar retention has previously been reported for the fused-core and Acquity 1.7 μm column [21], which suggests that there might be a difference in retention characteristics between Zorbax C18 phase and the Acquity phase. In any event, the difference in the retention factor for the fused-core and sub-2 μm particle Zorbax columns could be compensated simply by adjusting the composition of the mobile phase. For example, similar retention factors can be achieved using either the fused-core or sub-2 μm column and mobile phase conditions of 60/40 and 56/44 (water to acetonitrile, % v/v, isocratic elution), respectively.

3.2. Selectivity

Fig. 3 shows the relationship between the stationary phase selectivity (α) and the mobile phase composition for non-polar compounds (benzene and toluene) and polar compounds (aniline

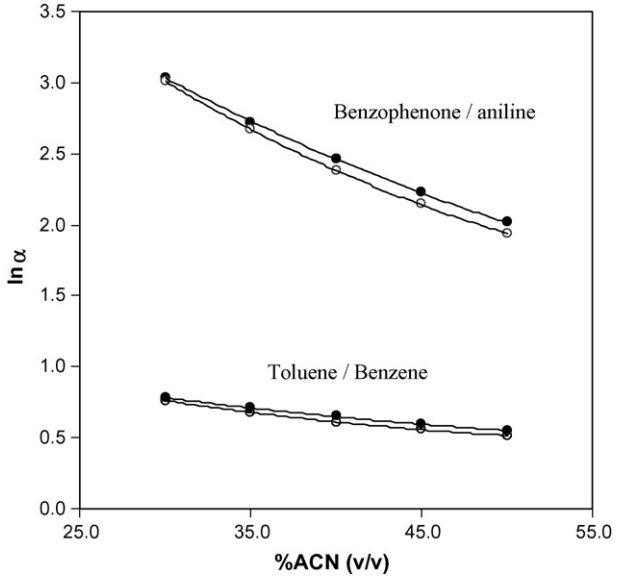

Fig. 3. Relationship of the selectivity α and the MeCN concentration in the mobile phase for toluene/benzene and benzophenone/aniline solutes, respectively. Key: (●) Ascentis Express C-18, 4.6 mm × 50 mm, 2.7 μm column; (○) Zorbax Eclipse Plus C-18, 4.6 mm × 50 mm, 1.8 μm column.

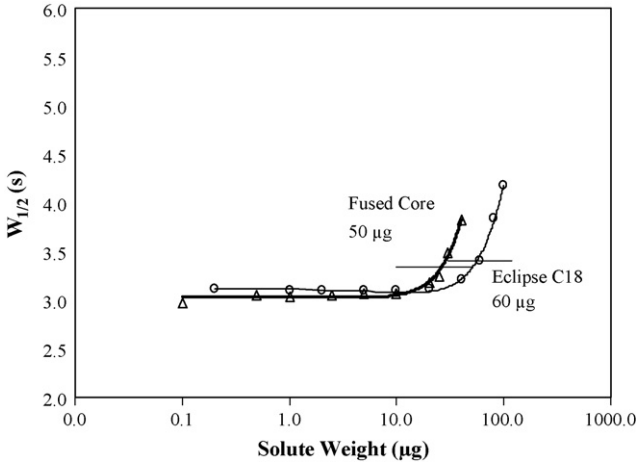

Fig. 4. Comparison of sample loading capacity for porous sub-2 and semi-porous fused-core particles using the peak width at half-maximum measured for various solute amounts. See text for details. Key: (△) Ascentis Express C-18, 4.6 mm × 50 mm, 2.7 μm column; (○) Zorbax Eclipse Plus C-18, 4.6 mm × 50 mm, 1.8 μm column. The retention factors were 0.62 and 0.61 for the Eclipse Plus C18 (porous particles) and Acsentis Express C18 (fused-core particles) column, respectively.

and benzophenone), respectively, as it pertains to the fused-core and sub-2 μm particle columns. The plots indicate that both the semi-porous fused-core and porous sub-2 μm particles have slightly higher selectivity for both the polar and non-polar compounds that were tested and the difference is more significant at high organic concentrations.

The selectivity properties of the fused-core particle and sub-2 μm porous particle phases were further evaluated using modified column characterization protocol based on methodology developed by Tanaka and coworkers [27] and later adopted for comparison of commercially available phases by Euerby and Petersson [28]. The summary of characterization data obtained using this protocol is presented in Table 1. From this investigation it was found that the two columns of interest have a comparable surface coverage and hydrophobic selectivity, as judged by comparable values of respective pentyl benzene retention factors and methylene selectivity values. Other selectivity tests, specifically those probing hydrogen bonding, residual silanols and ion-exchange capacity, also showed no major differences between these columns. On the other hand, a remarkable selectivity difference was observed in the triphenylene/o-terphenyl test, which serves as an indicator of the shape selectivity. The α (T/O) value for the fused-core C18 particles is ~30% higher than that for the 1.8 μm, C18 porous particles. The difference observed in shape selectivity between the two stationary phases might be attributable to differences in the ligand spacing caused by variations in surface silanization chemistry and/or the silica particle morphology [29].

3.3. Sample capacity

The sample capacity of a column is one of the most important criteria that needs to be investigated during the evaluation of any new stationary phase for use in pharmaceutical process development. That is because the sample capacity of a column can significantly affect the linear dynamic range of a given analyte. Columns with higher sample capacity usually provide a greater linear dynamic range. It is well known that the acceptable loading capacity of a packed analytical column is related to the carbon loading percentage [30]. However, the total volume of the fused-core in the superficially porous particles (1.8 μm) that does not provide any retention can be estimated to be ~25% of the 2.7 μm porous particle. This suggests that the sample loading capacity (all other factors being considered equal) for the fused-core column should be in the realm of approximately 25% lower than that of the sub-2 μm particle column. Fig. 4 shows the relationship of width at half peak height ($W_{1/2}$) with approximate sample amount injected, for both the porous and semi-porous particles. Little increase (~10%) in $W_{1/2}$ was observed up to 60 μg for the sub-2 porous particle column and up to 50 μg for fused-core particles, using benzyl alcohol as the solute. The result suggests that a column packed with fully porous particles can provide a slightly higher linear dynamic range than that packed with fused-core particles. The difference, however, is not deemed to be significant for most small-scale/analytical applications.

3.4. Column efficiency

Reducing the particle size of the packing in HPLC columns has been one of the most important strategies for many column manufacturers to increase speed of analysis. A smaller particle size (d_p) results in higher efficiency (N) per unit length and therefore can provide higher resolution ($N \propto 1/d_p$). To investigate the chromatographic performance of the two different stationary phases (semi-porous fused-core and porous sub-2 μm particles), van Deemter curves were plotted using column efficiencies calculated at linear velocities raging from 0.6 to 10 mm s^{-1} with benzophenone used as a test analyte. The mobile phase composition was slightly adjusted so that the two columns would

Table 1
Selectivities of the fused-core particle and sub-2 μm porous particle phases.

Column/selectivity	α CH_2	α T/O	α C/P	α B/P, pH 2.7	α B/P, pH 7.6
Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 μm	1.47	1.16	0.45	0.08	0.29
Acsentis Express C18, 4.6 mm × 50 mm, 2.7 μm	1.49	1.41	0.42	0.09	0.33

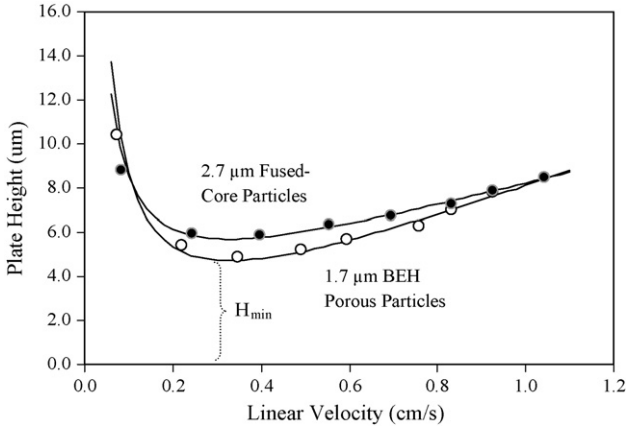

Fig. 5. van Deemter curves for the 2.1 mm × 50 mm columns packed with the 2.7 μm semi-porous/fused-core particles and the 1.7 μm BEH porous particles, respectively. Benzophenone was used for the study; see text for details.

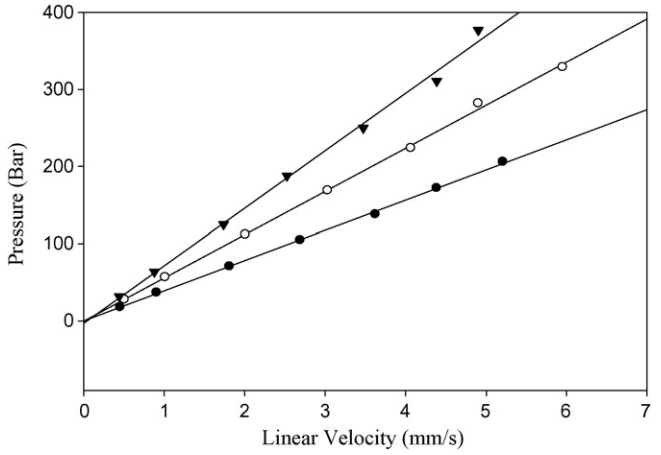

Fig. 6. Variation in pressure drop observed for the fused-core and sub-2 μm particles, with varying linear velocity. Key: (●) Ascentis Express C-18, 4.6 mm × 50 mm, 2.7 μm column; (○) Ascentis Express C-18, 4.6 mm × 100 mm, 2.7 μm column; (►) Zorbax Eclipse Plus C-18, 4.6 mm × 50 mm, 1.8 μm column.

have similar retention factor values ($k' \sim 5$). As shown in Fig. 5, the 1.7 μm particles are more efficient (lower theoretical plate heights) than the 2.7 μm fused-core particles at the optimal linear velocity and above (0.3–1.0 cm s^{-1}). The efficiency obtained with the fused-core particles is on average ~85% of the 1.7 μm porous particles at this range of linear velocities. Additionally, it is estimated that the slope or the C-term of the curve for the fused-core particles is ~25% lower than that for the 1.7 μm articles due to the lower mass transfer resistance for the fused-core particles [22]. This leads to an intersect between the two curves at 1 cm s^{-1}. The result suggests that the fused-core particles should be more favorable for fast separation at high flow rates. However, the minimum plate height (μm) for the fused-core particles is ~18% greater than that for the 1.7 μm BEH porous particles probably due to the greater particle size of the fused-core packings [12].

3.5. Back pressure

Although column efficiency increases as the particle size decreases ($N \propto 1/d_p$), column back pressure (ΔP) increases at a greater rate than efficiency as particle size decreases ($\Delta P \propto 1/d_p^2$). Therefore, for most applications, high resolution LC with small particles (sub-2 μm) often requires high pressure systems [1]. For this reason, a particle with high efficiency that generates low backpressure would be a more suitable candidate for conventional LC systems. The 2.7 μm fused-core particles which are made with a porous layer surrounding a solid core potentially offer a lower-pressure alternative with only slight sacrifice in column performance compared to 1.8 μm particles [25,26].

Fig. 6 shows a comparison of back pressure for the fused-core and sub-2 μm particle columns. The slopes of the plots in this figure represent column pressure drops at increasing linear velocities, using 50/50 (v/v) MeCN/H_2O as the mobile phase. The fused-core particle provided, on average, ~55% of pressure drop compared to a column of the same length packed with 1.8 μm porous silica particles. Interestingly, the decrease in pressure drop for the fused-core column is less than theoretically predicted $(1.8/2.7)^2 \times 100\% = 44\%$. This could be ascribed to the rough surface of the fused-core particles, which provides greater flow resistance and thus higher pressure than the smooth surface of particles with the same diameter [29]. In other words, the decrease in pressure due to the larger particle size of the fused-core particles (2.7 μm vs. 1.8 μm) was partially counteracted by the greater roughness of the particle surface. Additionally, even doubling the column length of the fused-core particle column (to 100 mm) still produced 12% less back pressure as compared to the 1.8 μm totally porous particle column (50 mm) under the same conditions. This finding indicates that fused-core particle columns deliver a much better efficiency per unit pressure relative to sub-2 μm particle columns.

3.6. Applications

The applicability of the fused-core particles as an alternative to the porous sub-2 μm particles was demonstrated by comparison of impurity profiles of selected pharmaceutical compounds and intermediates. Fig. 7 shows two chromatograms of the intermediate obtained on both the fused-core (Ascentis Express 4.6 mm × 50 mm, 2.7 μm) and sub-2 μm (Zorbax Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 μm) particle packed column. Note that the chromatographic conditions were identical for the two chromatograms. Although there was slightly less retention on the fused-core column, the average resolution for the three critical impurities and the main peak (compounds **1**–**4**) was 2.9 for both columns. Additionally, the selectivity for the four com-

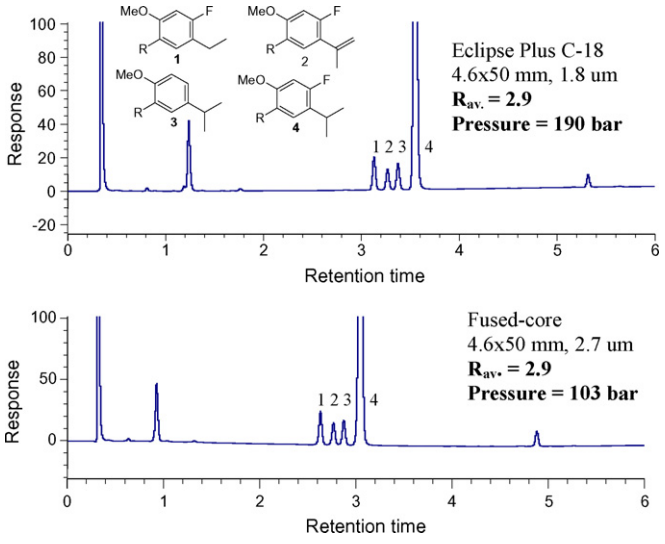

Fig. 7. Chromatograms of a pharmaceutical intermediate and its impurities using a 50 mm, 1.8 μm packed column and a 50 mm fused-core column, respectively. 1.5 mL/min flow rate.

Table 2
Comparison of the retention and selectivity factors of the Ascentis Express C-18, 4.6 mm × 50 mm, 2.7 μm (fused-core particle) column and the Zorbax Eclipse Plus C-18, 4.6 mm × 50 mm, 1.8 μm (porous particle) column in separating CB-1 and its related impurities; see text for details.

	t_R (min)		k'		α (the neighboring peaks)		R (the neighboring peaks)	
	Fused-core	1.8 μm porous	Fused-core	1.8 μm porous	Fused-core	1.8 μm porous	Fused-core	1.8 μm porous
Void	0.19	0.19	n/a	n/a	n/a	n/a	n/a	n/a
CB-1	1.40	1.40	6.37	6.37	n/a	n/a	n/a	n/a
Trans	1.81	1.76	8.53	8.26	1.34	1.30	1.91	1.98
Cis	1.53	1.52	7.05	7.00	1.11	1.10	3.75	3.62

n/a: not applicable.

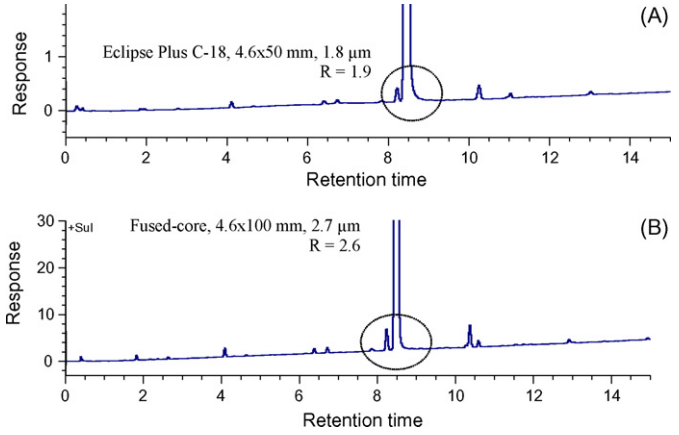

Fig. 8. Chromatograms of a drug substance candidate using a 50 mm, 1.8 μm packed column and a 100 mm fused-core column, respectively. 1.5 mL/min flow rate.

pounds was very similar. This is in agreement with the fact discussed in Section 3.2 that hydrophobic/polarity selectivity is similar for both columns, since the major difference in structure of the four compounds is their alkyl chain lengths or molecular weights.

Since the fused-core particles generate about 45% less back pressure as compared to the sub-2 μm particle column, it was possible to double the length of the fused-core column in order to achieve a better separation on Agilent 1100 HPLC system. Fig. 8 shows two chromatograms of a drug candidate using the fused-core (Ascentis Express 4.6 mm × 100 mm, 2.7 μm) and sub-2 μm (Zorbax Eclipse Plus C18, 4.6 mm × 50 mm, 1.8 μm) particle packed column. The resolution of the critical pair of peaks is 1.9 from a 50 mm, 1.8 μm porous packed column, compared to 2.6 from a 100 mm, 2.7 μm fused-core column. The latter would not be possible without UHPLC capability using a100 mm column packed with porous sub-2 μm particles.

A second application dealing with the analysis of a different pharmaceutical compounds, in this case CB-1 and its structurally closely related process impurities ("*Cis*" and "*Trans*", see Fig. 1), demonstrated the potential usefulness of fast LC methods developed using fused-core technology. As described in Section 2, the mobile phase conditions were adjusted on both the fused-core and sub-2 μm particle columns in order to achieve the same retention factor (6.37; see Table 2) for CB-1 on each phase. As expected, that required ~4% (v/v) more MeCN in the mobile phase for the porous particle packed column than the fused-core one. In addition, the selectivity of *trans* to CB-1 and *cis* to *trans* for the fused-core particles is slightly higher. This is in agreement with greater "shape" selectivity of the fused-core particles discussed in Section 3.2. Regardless, Table 2 clearly demonstrates a case where the fused-core column achieves an equivalent-to-slightly better separation of the three components than the porous sub-2 μm particle column. Finally, the slightly better separation for the fused-core packed column was achieved at a much lower column back pressure (166 bar vs. 298 bar). In both cases, the (isocratic) analytical separation of the test mixture was performed in less than 2 min.

4. Conclusions

The chromatographic performance of a fused-core particle (2.7 μm) Ascentis Express C18 column was compared to porous sub-2 μm particle columns (Zorbax Eclipse Plus C-18, 4.6 mm × 50 mm, 1.8 μm or Waters Acquity BEH C18, 2.1 mm × 50 mm, 1.7 μm). It was found that the fused-core particles, bonded with C18 alkyl chains, had a very similar selectivity to the sub-2 μm Zorbax C18 phase, but provided a better shape selectivity, based on the results using the Tanaka test protocol. Solute capacity and overall retention were slightly compromised relative to the porous sub-2 μm particles. The key advantages of the fused-core particle columns for pharmaceutically relevant analyses is their substantially lower back pressures which allows them to be used at much higher flow rates than porous sub-2 μm particle phases for fast LC applications, or the column length to be increased to improve separation efficiency without exceeding the capabilities of conventional HPLC equipment.

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