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Hydrodynamic flow in capillary-channel fiber columns for liquid chromatography

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Abstract

The flow characteristics of capillary-channel polymer (C-CP) fiber liquid chromatographic (LC) columns have been investigated. The C-CP fibers are manufactured with eight longitudinal grooves (capillary channels) extending the length of the fibers. Three C-CP fiber examples were studied, with fiber dimensions ranging from ≈35 μm to 65 μm, and capillary-channel dimensions ranging from ≈6 μm to 35 μm. The influence of fiber packing density and column inner diameter on peak asymmetry, peak width, and run-to-run reproducibility have been studied for stainless steel LC columns packed with polyester (PET) and polypropylene (PP) C-CP fibers. The van Deemter A-term was evaluated as a function of fiber packing density (≈0.3 g/cm^3–0.75 g/cm^3) for columns of 4.6 mm inner diameter (i.d.) and at constant packing densities for 1.5 mm, 3.2 mm, 4.6 mm, and 7.7 mm i.d. columns. Although column diameter had little influence on the eluting peak widths, peak asymmetry increased with increasing column diameter. The A-terms for the C-CP fiber packed columns are somewhat larger than current commercial, microparticulate-packed columns, and means for improvement are discussed. Applications in the area of protein (macromolecule) separations appear the most promising at this stage of the system development.

Keywords: Liquid chromatography; Stationary phase; Capillary-channel polymer fibers; A-term

1. Introduction

The most common chromatographic sorbents used in reversed-phase liquid chromatography (LC) separations are prepared with chemically modified microparticulate silica (e.g., C_{18} phases). Separation efficiency is increased as particle size is decreased due in part to enhanced diffusion of the solute within and between the particles [1]. The use of small diameter microparticulate silica results in increased column backpressure and the requirement for high-pressure pumps and compatible hardware [2]. These constraints place limits on the applicability of large-scale separations (e.g., preparative-scale chromatography) or other applications in which rapid flow separations are desired.

Attempts to utilize carbonaceous-fiber stationary phases to reduce backpressure and to create more cost effective columns have been reported. Jinno and co-workers filled microbore columns (1070 mm × 0.53 mm i.d.) with cellulose acetate fibers to separate a mixture of alcohols using an aqueous mobile phase at a flow rate of 1.0 μL/min [3]. The separation demonstrated the feasibility of using fibers as the chromatographic sorbent in LC separations. This approach was subsequently used to separate mixtures of akylbenzenes and polycyclic aromatic hydrocarbons (PAHs) [4]. PAH retention profiles were similar for fiber-based columns and particulate cellulose columns, suggesting that similar retention mechanisms exist for the two column types. Beyond their use in pressure-driven chromatographic separations, polymer-fiber stationary phases can also be employed in capillary electrochromatography (CEC) for the separation of neutral compounds [5].

Ladisch and co-workers prepared LC columns packed with rolled, textile fabric for use in size exclusion and ion exchange chromatography [6]. The authors separated an analyte mixture of immunoglobulin G, bovine serum albumin (BSA), insulin, and β-galactosidase in 12 min with a 95:5 Nomex:Kevlar fabric blend. The columns exhibited relatively low capacity and

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low efficiencies. In subsequent work, transport and mass transfer phenomena were studied in rolled fabric columns containing fiber blends of cotton, cellulose acetate, and polyester (PET, poly(ethylene terephthalate)) [7]. The individual fibers were relatively nonporous, which minimized pore diffusion effects. Peak efficiency was found to be independent of mobile phase velocity, suggesting an A-term dominant (eddy diffusion-limited) efficiency. The possibility of high mobile phase velocity (≈50 cm/min) separations without loss of peak efficiency was demonstrated.

Ladisch and co-workers continued the study of rolled-fabric columns for the rapid separation of proteins [8]. The authors demonstrated that such columns were mechanically stable at linear velocities of up to 100 cm/min. Furthermore, they found that the optimal plate height occurs at a relatively low packing density equivalent to approximately 0.55 g/cm^3, with the range investigated covering approximately 0.5 g/cm^3–0.74 g/cm^3. The authors attributed this decrease in peak efficiency with increased density to flow inhomogeneity resulting from inter-fiber channeling.

Marcus and co-workers recently described the use of capillary-channeled polymer (C-CP) fibers for use as stationary phases for reversed-phase liquid chromatographic (RPLC) separations [9–11]. The extruded C-CP fibers are manufactured with eight parallel channels on the periphery of each fiber [12]. These fibers possess 2.5–3.2 times the surface area of conventional fibers with a circular cross-section and having the same nominal diameter. The shape and size of the channels increase fiber wettability for hydrophobic polymers. This capillary structure is an efficient transport medium for pressurized flow, yielding relatively low backpressures (e.g., ≈3.5 MPa at 3 mL/min for a 306 mm × 4.6 mm column). Fibers are created by a spin-melt extrusion process which is applicable to a variety of polymers types, and analyte–polymer interactions can be selected for a given separation. Nelson and Marcus [10] separated proteins on a polypropylene C-CP column under conditions similar to those used with a packed C_4 column, and concluded that the C-CP columns can provide separations that are competitive with conventional particulate columns.

The current study reports the flow properties of C-CP columns in terms of fiber packing density and column inner diameter, using uracil to probe A-term contributions to band broadening.

2. Experimental[1]

2.1. C-CP fiber characteristics

One polypropylene (PP) and two polyester (PET1 and PET2) C-CP fiber types were used in these studies (Fiber Innovation Technology (FIT), Johnson City, TN, USA). The fibers were produced under different extrusion conditions and although the overall geometries of the fibers are similar, the cross-sections differ in size. As illustrated by scanning electron micrograph (SEM) in Fig. 1, the fibers are manufactured with eight longitudinal open capillaries, with the overall shape being oval/rectangular rather than circular. The PET1 fibers were produced with a maximum cross sectional dimension of ≈35 μm, the PET2 fibers ≈65 μm, and the PP fibers ≈50 μm as determined by the SEM images. These different geometries result in different packing densities (i.e., number of fibers/column cross-section) as well as different column permeability and backpressure. For the purposes of this study, the influence of the surface chemistry of the two polymer types is neglected as the uracil probe compound is unretained in both cases.

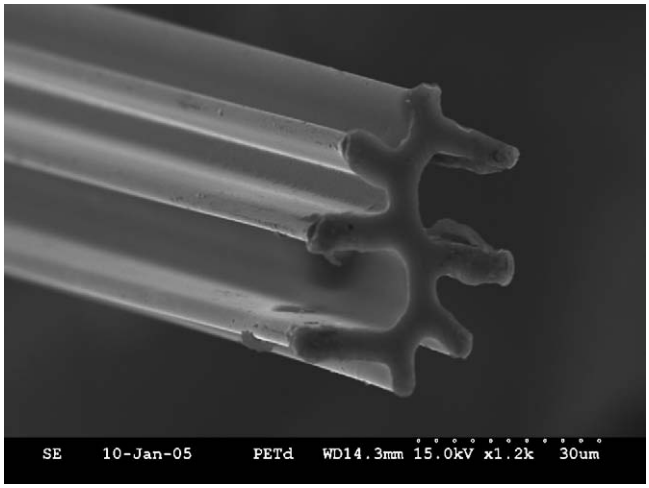

Fig. 1. Scanning electron microscope (SEM) image of a C-CP fiber depicting the eight capillary flow channels along the length of the fiber.

2.2. Column construction

The process of column packing is depicted in Fig. 2a. Stainless steel tubing, frits, and end-fittings were all purchased from Alltech (Deerfield, IL, USA). The tube diameters were 1.5 mm, 3.2 mm, 4.6 mm, and 7.7 mm, all with lengths of 250 mm. The C-CP fiber columns were prepared by winding the fibers onto a spool (≈71 cm circumference) that is mounted on an axle assembly and fit with a handle and a rotary counter. When the desired number of fibers (equal to twice the number of rotations) was obtained, the fibers were removed from the spool as a loop. A monofilament was looped around the fiber bundle and passed through the stainless steel column. The columns were positioned vertically on a manual cranking mechanism used to pull the attached fiber bundle through the column. The maximum packing density that can be achieved is limited by the strength of the monofilament and the C-CP fibers.

An SEM micrograph of the end of a C-CP fiber column is shown in Fig. 2b for polypropylene fibers packed in a 0.8 mm i.d. fluorinated ethylene propylene (FEP) tube. Contrary to earlier reports [9,10], the capillaries of each fiber do not exist as isolated tube-like entities within the column. Rather, orientation is such that the channel walls of adjacent fibers interdigitate (see

[1] Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

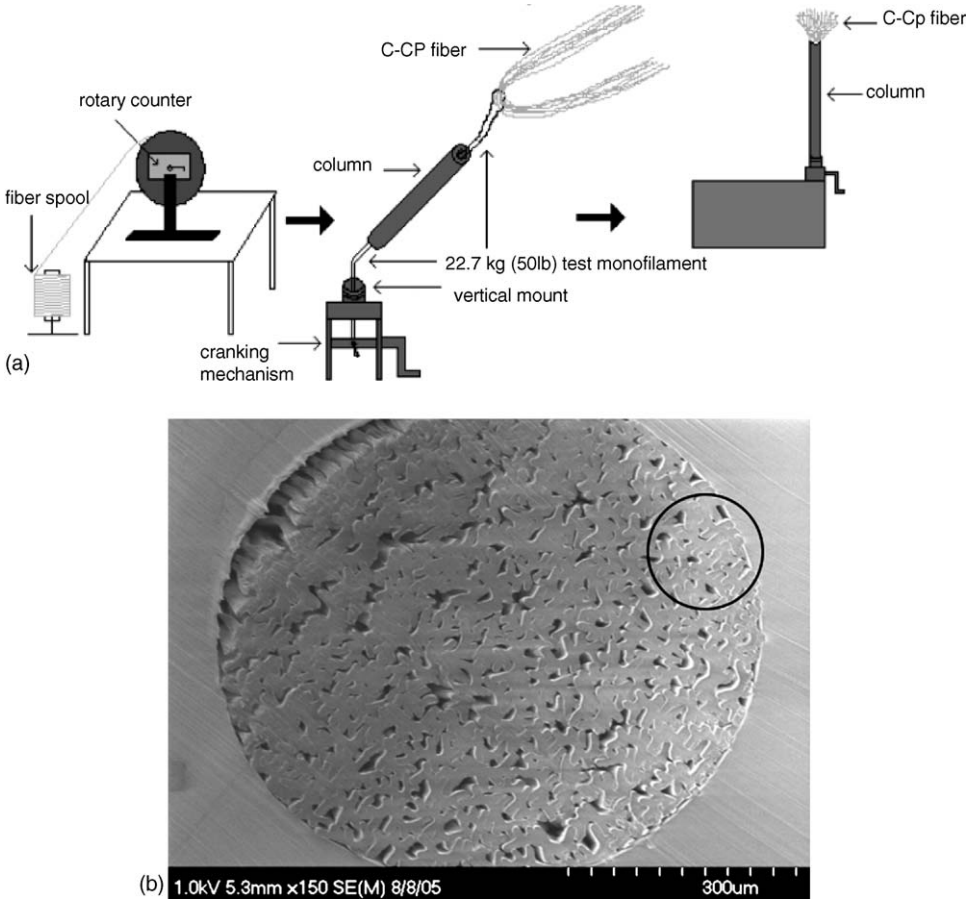

Fig. 2. (a) Depiction of the column packing procedure. The C-CP fibers are connected to a spool that is attached to a rotary counter. A nylon monofilament is looped around the C-CP bundle, and is guided through the tubing. The column is packed by pulling the bundle through the stainless steel tubing with a crank/pulley apparatus. (b) SEM micrograph of an end of a C-CP fiber column.

the circled region in Fig. 2b). Regions of low packing density are clearly evident that would serve as channels with relatively low flow impedance. Obviously, situations of inhomogenous packing density will lead to greater variance in the mobile phase velocity profile, packing within polymer tubing and use of radial compression greatly remedies this situation [13]. It should be noted that the sample shown in Fig. 2b was prepared for fibers trimmed using surgical steel scalpels. Micrographs of fibers cut at room temperature using simple razor blades (as is the case for the columns described here) show appreciation deformation of the fiber and channel structure. The effect of this process is likely added turbulence at the column inlet and will be reflected in the hydrodynamic (A-term) characteristics.

As a result of the packing procedure, the C-CP fibers experience longitudinal stretching when pulled through the stainless-steel tubing but relax to the original length when exposed to organic solvents such as acetonitrile (ACN) and methanol (MeOH) and subsequently allowed to dry. The stretching of the PET1 and PET2 fibers is much more pronounced (0.8 cm–1 cm) than for the polypropylene fiber (0.02 cm). The rate of fiber relaxation was determined by soaking the C-CP fiber packed columns in an organic solvent. The fiber columns were submerged in either ACN or MeOH solvent for 10-min intervals, removed, and allowed to dry overnight and the overall length measured. In practice, fiber relaxation to the original lengths was observed after the 30-min exposure, with longer exposures not affecting net shrinkage. Each of the fiber columns employed in the studies presented here was subjected to a 30-min relaxation cycle in ACN, allowed to dry, the fibers trimmed flush with the tubing ends with a razor blade, and the frits and end-fittings attached. For some measurements, an asterisk-style flow distributor and 2-μm stainless-steel frit was utilized at the top of the column to distribute the flow.

An issue in the use of polymer substrate stationary phases is the potential for swelling upon exposure to different mobile phase environments. This possibility was studied by determining the column void volume and specific permeability of each type of fiber (PET and PP). Columns of each fiber type were soaked individually overnight (∼12 h) in H_2O, MeOH and 20 mM Tris buffer media and the results presented in Table 1. In no case did the void volumes change by more than 2%; this of course bodes well for chromatographic consistency. Only in the case of PET fiber exposure to MeOH did the specific permeability value change by as much as 10%. Based on these results, little or no fiber swelling has occurred and excellent overall stability has been demonstrated. Certainly, these sorts of studies will be relevant in each instance of introducing a different base polymer/solvent combination. Experience to this point suggests that

Table 1
Assessment of C-CP fiber column swelling before and after ~12-h exposure to typical RP-HPLC solvent media

Fiber type	H_2O		MeOH		20 mM Tris buffer	
	Before	After	Before	After	Before	After
PET						
V_o	2.52 mL	2.56	2.56 mL	2.70	2.69 mL	2.62
B_o	1.96×10^{-8} cm^2	1.85	8.18×10^{-9} cm^2	9.22	2.63×10^{-9} cm^2	2.56
PP						
V_o	2.11 mL	2.09	2.27 mL	2.34	2.35 mL	2.31
B_o	5.39×10^{-9} cm^2	4.75	3.81×10^{-9} cm^2	4.02	6.69×10^{-9} cm^2	6.47

the physical stability to reversed-phase solvents appears quite good.

2.3. Sample preparation and solution delivery

Stock solutions of reagent-grade uracil and copper(II)chloride (Aldrich, Milwaukee, WI, USA) were prepared in 18.2 MΩ/cm water at a concentration of 10 μg/mL. Methanol was used as the mobile phase for the elution of the solute in each of the chromatographic experiments and water was used in the determination of the specific permeability. The chromatographic system consisted of a high-performance liquid chromatography pump with a six-port injector valve and a dual wavelength absorbance detector. 1 μL, 10 μL, 20 μL, and 50 μL injection loops were used for sample introduction. The chromatograms were recorded using a data acquisition system and processed using a worksheet. The data presented is the mean of triplicate injections, with the error bars in each plot representing one standard deviation.

3. Results and discussion

3.1. Physical characteristics of C-CP fiber columns

Two column properties related to fluid flow were initially studied: the interstitial fraction and specific permeability. Table 2 lists the physical characteristics of the various densities of the PET1, PET2, and PP C-CP fiber-packed columns of 25 cm length and 4.6 mm i.d. All measurements were determined under conditions of a constant linear velocity (68 cm/min).

Fiber mass within the column was determined by subtracting the mass of the empty column from the mass of the packed column. The range of packing densities that was prepared was limited by the tensile strength of the fibers and the nylon monofilament used to pull the bundle through the tubing. Up to ≈3 g of C-CP fiber could be packed in the column in each case. The fiber packing density (ρ), in g/cm^3, was determined by dividing the mass of fiber (m) in the column by the total volume (V) of the column shown in Eq. (1):

$$\rho = \frac{m}{V} \tag{1}$$

As indicated in Table 2, the much smaller diameter of the PET1 fibers results in a much greater number of fibers packed within the column for a given stationary phase mass. The mass of fibers per column is also a function of the polymer density for the respective fibers.

The interstitial fraction (ε_i) of each column was determined using Eq. (2), where V_o is the void volume for each column, r the inner diameter of the tubing (0.46 cm), and L the column length (25 cm):

$$\varepsilon_i = \frac{V_o}{\pi r^2 L} \tag{2}$$

V_o was determined by filling the pre-weighed columns with methanol. The mass of MeOH inside the column was converted to an equivalent volume (cm^3). An inverse relationship is observed between the density and the interstitial fraction. The packing densities for the fibers are comparable to conventional packed-bed columns; however, the interstitial fraction values are <0.3 for the most densely-packed fiber columns. Surprisingly, given the relatively low ε_i values, low column backpressures (<5 MPa) at flow rates of approximately 1 mL/min are still realized.

Specific permeability (B_o) relates resistance of a packed column (or empty tube) to fluid flow. The specific permeability (cm^2) is defined by Eq. (3) where F is the mobile phase flow rate, η the viscosity of the mobile phase, L the length of the column, r the radius of the column and ΔP the pressure drop across the column [2]:

$$B_o = \frac{F\eta L}{\pi r^2 \Delta P} \tag{3}$$

Pressure-drop values for C-CP columns operating at a constant linear velocity of 68 cm/min are listed in Table 2. In general, column backpressure increases with increased packing density. This increase in backpressure may result from restricted inter-fiber flow due to the close proximity of the fibers. This is exacerbated at the highest packing densities where dramatic increases are seen for PET2 and PP. Here, twisting, kinking and physical deformation of the fibers may shut off flow paths. Further study including detailed microscopy and analysis of flow rate versus pressure characteristics is certainly in order. In comparison to conventional LC columns, the permeability of C-CP fiber columns is large even for the most densely packed C-CP fiber columns. Column backpressures do not exceed 13 MPa (at 68 cm/min) for the polypropylene column with an interstitial fraction of 0.13, and backpressure values are typically less than 4 MPa for columns with interstitial fractions >0.3. High column permeability and low interstitial fractions for the C-

Table 2
General properties of capillary-channeled (C-CP) fiber packed 4.6 mm × 250 mm columns

	Fiber mass (g)	Fiber packing density (g/cm^3)	Interstitial fraction (ε_i)	Flow rate (mL/min)	Pressure drop (MPa)	Specific permeability (cm^2)
PET1 (# of fibers)						
10,500	1.80	0.433	0.71	1.99	1.37	3.64×10^{-9}
13,500	2.09	0.503	0.65	1.80	0.81	5.57×10^{-9}
18,000	2.57	0.619	0.55	1.54	0.81	4.77×10^{-9}
22,500	2.89	0.696	0.39	1.07	1.01	2.65×10^{-9}
24,000	2.99	0.719	0.29	0.80	0.91	2.21×10^{-9}
PET2 (# of fibers)						
5,000	1.97	0.434	0.66	1.83	0.71	6.46×10^{-9}
5,900	2.19	0.482	0.59	1.65	1.12	3.69×10^{-9}
6,800	2.50	0.551	0.55	1.54	1.01	3.82×10^{-9}
7,700	2.86	0.630	0.49	1.36	4.66	7.32×10^{-10}
8,600	3.15	0.694	0.29	0.83	4.05	5.14×10^{-10}
Polypropylene (# of fibers)						
4, 500	1.22	0.294	0.69	1.94	0.97	5.02×10^{-9}
6, 300	1.50	0.361	0.60	1.68	0.76	5.54×10^{-9}
8, 100	1.75	0.421	0.50	1.41	1.12	3.16×10^{-9}
13, 500	2.80	0.674	0.29	0.83	1.42	1.46×10^{-9}
15, 300	3.06	0.737	0.13	0.35	12.36	7.10×10^{-11}

A constant linear velocity (U_o) of 68 cm/min was maintained throughout the study.

CP fiber columns is a consequence of the fluid flow paths set up between the adjacent fibers and their interdigitated capillary walls. Using an average permeability value of 5×10^{-9} cm^2 and an interstitial fraction of 0.5, the Kozeny–Carman equation equates these columns to packed-bed columns having nominal particle diameters of ≈14 μm [2]. The validity of comparisons with packed-bed columns is questionable, but it does provide an interesting point of reference. Alternatively, this combination of flow rates and backpressure is typical of an open tube of ∼5 μm inner diameter. Operation under conditions that yield such low backpressures suggest the future use of "capillary" column structures of narrow bore. Preliminary studies of such columns having lengths of 1 m and inner diameters of ≈1 mm show promise for applications [13]. The use of flexible polymer tubing in those columns allows the possibility for radial compression to improve the packing quality (i.e., elimination of channels of the sort seen in Fig. 2b). In this case, plate heights and resolution are substantially improved by virtue of improved hydrodynamics and increased phase ratios [13].

3.2. Role of linear velocity on band broadening

Using the van Deemter equation for the height equivalent to a theoretical plate (H) (Eq. (4)), one can extract the major contributing factors to band broadening [2,14]:

$$H = A + \frac{B}{U} + CU \tag{4}$$

where A represents the so-called "eddy diffusion" term, B represents molecular diffusion in the longitudinal direction, U the mobile phase linear velocity, and C represents the mass transfer between the mobile and stationary phases. A plot of H versus U facilitates evaluation of the limiting broadening mechanisms. An unretained solute (uracil) was used to assess the contributions from the A-term in Eq. (4). To confirm that uracil is not retained on the C-CP fibers, its elution characteristics were compared to aqueous cupric chloride ($CuCl_2$), which exists as a hydrated cation in solution and is not retained by the hydrophobic fibers. Uracil is indeed a viable test compound to study column hydrodynamics as its elution time (t_o) and peak width ($w_{1/2}$) are virtually identical to the Cu-salt. Being unretained, issues related to mass transfer and its potential contributions to band broadening can be ignored, and so only A- and B-term components are of relevance. Based on an estimated diffusion coefficient for uracil (in water at 20 °C) of 1×10^{-5} cm^2/s, B-term broadening is ≈10^{-4} mm and can also be considered insignificant [2].

Fig. 3 shows the van Deemter plots for the three C-CP fiber columns of lowest packing density. The term "apparent plate height" is used here to emphasize the fact that the test compound is not retained on the fibers. The fiber packing densities for the PET1, PET2, and PP, columns were 0.284 g/cm^3, 0.434 g/cm^3, and 0.294 g/cm^3, respectively. The linear velocities here represent volume flow rates from 1 mL/min to 10 mL/min. The A-term is the dominant band broadening process, as indicated by the relative flatness of the plots. In the case of packed-bed columns,

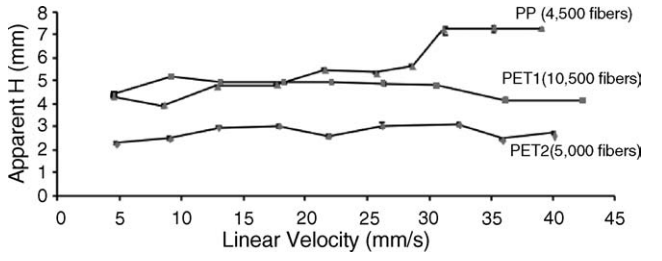

Fig. 3. van Deemter plot of uracil for PP, PET1, and PET2 C-CP fiber columns. Fiber number densities: PP = 4500 fibers, PET1 = 10,500 fibers, and PET2 = 5000 fibers. The mobile phase was methanol and the injection volume was 20 μL.

A-term band broadening is attributed to multiple (tortuous) paths traversed by solute molecules during elution, and is related to packing quality [2,14,15].

Unlike particulate-based columns, fluid flow in C-CP columns has no appreciable radial component to minimize flow nonuniformities. Variations in flow velocity can be attributed to differences within the inter-fiber spaces (ranging from single-micrometers for the iterdigitated channel walls to gross channels as seen in Fig. 2b) and fiber/wall voids. This is a significant issue associated with the relatively collinear flow channels. Hartwick and co-workers found in their study of multicapillary columns [15], the variance in channel diameter must be <0.5% to achieve column efficiencies similar to that of particle packed columns.

3.3. Role of packing density on band profiles

The influence of packing density on band profiles was also studied for the C-CP columns. The packing density, and the associated inter-fiber channels and/or restrictions, are expected to have a strong effect on band profiles. Peak widths and asymmetry values were evaluated as a function of fiber packing density for 20 μL uracil injections at a constant linear velocity of 68 cm/min and column diameter of 4.6 mm i.d. As expected, a gradual increase in peak width ($W_{0.10}$) is observed for increased packing density at a constant linear velocity, while the elution time (t_R) for the uracil is constant. The broadening is assumed to be due to increased restrictions in the interfiber regions in the column as the fiber packing density increases. The product of the flow rates and corresponding peak widths ($F^*W_{0.10}$) as a function of packing density is relatively constant for different packing densities (<25% variation), suggesting no other major forms of flow perturbation. Any increase in flow restrictions, crimping, or stangnant zones will naturally lead to a decrease in uniformity of the flow profile and an increase in peak asymmetry (Eq. (5)). Ladisch and co-workers [8] found similar results while studying the optimum packing characteristics of rolled, continuous stationary phases.

Peak asymmetry was further studied as a function of packing density. Peak asymmetry (A_s) was determined by the ratio of the width of the tail of the peak to the width of the front of the peak as shown in Eq. (5) [2]:

$$A_s = \frac{t_p}{f_p} \quad (5)$$

where t_p represents the width of the peak tail and f_p the width of the peak front at 10% peak height. Peak asymmetry values are plotted as a function of C-CP fiber packing density in Fig. 4. The most symmetric peak shapes were observed at lower stationary phase densities. Peak tailing increased with increased packing density, which is consistent with the idea that flow restrictions become more problematic as packing density increases. Relatively small increases in peak asymmetry were observed for PET1 and PET2 phases, whereas more appreciable changes were found for the PP C-CP phase, possibly related to its much lower melting point than PET (160 versus 280 °C). At the highest packing density, the PP C-CP column exhibited appreciable fronting, and A_s is reduced as a consequence. It might be imagined that the friction and heating at the walls during the fiber pulling process could melt the periphery fibers and eliminate flow near the column wall. Increased peak asymmetry was observed in multiple preparations of high-density PP C-CP columns and it seems likely that the mechanical properties of the polypropylene fibers are the source of the difference. Tailing at higher packing densities for PET1 and PET2 phases are likely due to stagnant flow paths created as the interfiber distances are squeezed. We are hopeful that further microscopic investigations of the fiber surfaces will lend insights to the changes in flow characteristics.

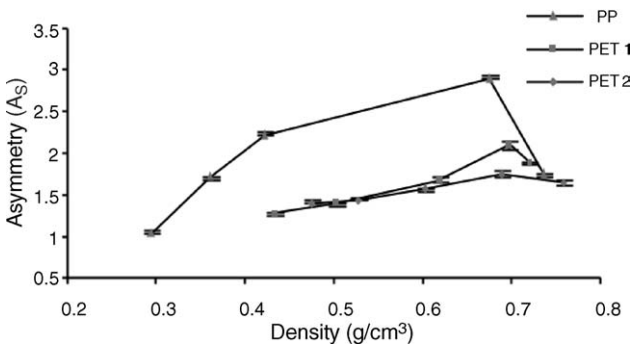

Fig. 4. Effects of fiber packing density on eluting uracil peak asymmetry at a fixed linear velocity of 68 cm/min. The mobile phase was methanol and the injection volume was 20 μL.

3.4. Role of column diameter

Column diameter influences solvent consumption and detection limits in LC. For constant linear velocity flow conditions, little chromatographic difference is observed in the efficiency of packed-bed columns ranging in size from 1 mm to 7 mm i.d., except in instances of mass or volume overload [2]. The influence of column diameter on band profile has been studied for each type of C-CP fiber at constant linear velocity flow conditions. Columns were prepared at the lowest packing densities listed in Table 2 for each fiber type as they provided the peak symmetry closest to unity. The peak profile of uracil was evaluated for 1.5 mm, 3.2 mm, 4.6 mm, and 7.7 mm inner diameter columns. The sample injection volumes were varied in proportion to the column volume.

As expected, the elution times for the uracil marker were unchanged at constant linear flow velocity. In general, peak widths were observed to increase with increasing column diameter as seen in Fig. 5a. Larger peak width changes occurred for columns prepared with the two smaller-diameter C-CP fibers (PP and PET1) than with columns prepared with the largest diameter (PET2) fibers. Greater variability in peak width was also observed for the PET2 columns.

Unlike particulate-based substrates, flow paths in the C-CP fiber columns are oriented longitudinally, and radial transport is achieved primarily though diffusion. Solute bandwidth should not necessarily increase as a consequence of radial diffusion if the longitudinal flow is uniform across the column cross-section. However, if longitudinal flow varies across the face of the column (e.g., flow is greater at the center of the column than at the

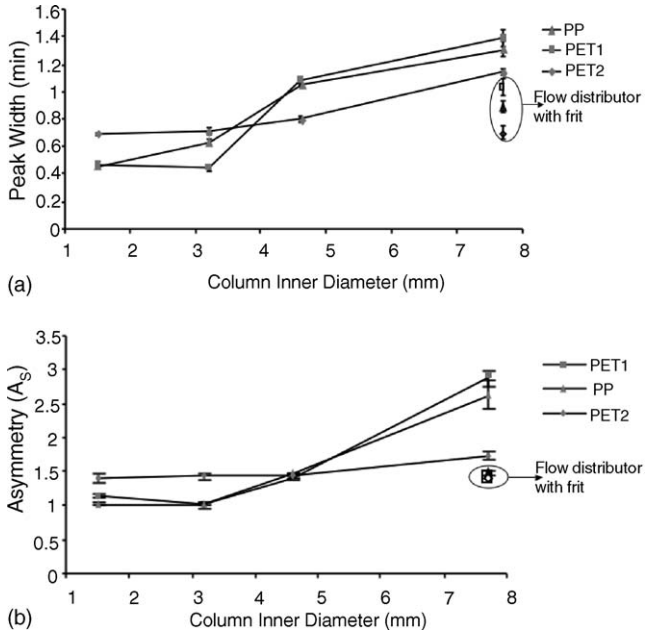

Fig. 5. Effects of column inner diameter on eluting uracil peak profiles at a fixed linear velocity of 68 cm/min. (a) Peak width (10% definition) and (b) peak asymmetry. The mobile phase was methanol and the injection volumes were varied in proportion to column volumes.

column walls) then band broadening will result. This model is consistent with band broadening even in the absence of radial diffusion. For microparticulate substrates, flow would be directed through a frit to be distributed across the face of the column, and band broadening due to flow inhomogeneity would be minimized. For C-CP fiber columns with longitudinally oriented flow paths, flow inhomogeneity may be more problematic as wetting across the face of the fiber bundle at the inlet may be restricted. To test this hypothesis, measurements of band broadening were made with and without the use of a flow distributor in combination with a frit. Peak widths were significantly reduced with the use of the flow distributor/frit combination (see Fig. 5a). It can be noted that the use of flow distributors is common for large-diameter, packed-bed columns used in preparatory scale separations.

Similar trends in peak asymmetry were observed as a function of column diameter (see Fig. 5b). Without the use of a flow distributor, more symmetric peak shapes resulted for small diameter columns than for large diameter columns. Inclusion of the flow distributor improved peak symmetry for large diameter columns, and peak profiles were comparable to those obtained with smaller diameter columns. It is interesting to note that a more symmetric peak shape was observed for the 7.7 mm i.d. PET2 column than for comparable columns with PET1 or PP fibers. The fibers of PET2 are larger than PET1 or PP and result in higher specific permeability (Table 2). Radial fluid transport is less restricted, and flow inhomogeneity is reduced. As such, only a modest (<15%) increase in peak asymmetry occurs as the column diameter is increased. This observation is consistent with trends in Fig. 4, where increased packing density is correlated with increases in both peak widths and asymmetry values.

It is clear that there will be trade-offs between the kinetic effects of radial diffusion across the column profile, the length of the column, and the mobile-phase velocity. These effects are fairly well described for packed-bed columns [16], but will likely be more important for the C-CP fiber columns. The use of on-column visualization studies as described by Guiochon and coworkers [17,18] should provide a better understanding of column hydrodynamics.

4. Conclusions

The influence of packing density and column diameter of capillary-channeled polymer (C-CP) fiber LC columns has been evaluated from the elution characteristics of an unretained test compound (uracil). C-CP fibers of PET1, PET2, and polypropylene having different diameters and channel sizes were investigated. The unique geometry of the C-CP fibers facilitates efficient fluid transport with high specific permeability. At constant linear velocity, peak widths increase as a function of packing density, while distortions of peak shape reflect the likely occurrence of restrictions and stagnant zones. Changes in peak profiles as a function of column diameter are attributed to flow inhomogeneity effects within the column. Use of a distributor-frit combination for the largest diameter columns greatly reduces these effects.

Based on the data presented here, it is clear that much improvement is required to approach the column efficiencies (at least in terms of A-term broadening) of commercial packed-bed columns. On the other hand, previous chromatographic studies have shown that the separation quality of the C-CP fiber columns is comparable to commercial packed-bed columns in reversed-phase, gradient protein separations [10,19]. In these sorts of separations, aspects of phase ratio and mass transfer (which lead to high efficiencies in small molecule separations) are minimized in lieu of the selectivity of the stationary phase. As such, these applications are likely the best fit for the qualities of the C-CP fiber columns (at least as demonstrated to date). Follow-up studies to determine the ultimate roles of packing density and column diameter on chromatographic quality for retained species are currently underway. While these parameters do not appreciably affect the hydrodynamics, differences in the elution characteristics of retained species are expected as the phase ratios and stationary phase surface area are changed. Other areas of investigation include studies of the loading capacity of the C-CP fiber columns and use of microbore columns with use of radial compression at lengths on the meter scale [13]. In addition, C-CP fibers of different geometry and higher surface porosity are also likely paths of development.

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References

[1] R.P.W. Scott, Small Bore Liquid Chromatography Columns: Their Properties and Uses, John Wiley & Sons Inc., New York, 1984.
[2] U.D. Neue, HPLC Columns: Theory, Technology, and Practice, Wiley-VCH, New York, 1997.
[3] Y. Kiso, K. Jinno, T. Nagoshi, J. High Resolut. Chromatogr. 9 (1986) 763.
[4] Y. Kiso, K. Takayama, K. Jinno, J. High Resolut. Chromatogr. 12 (1989) 169.
[5] K. Jinno, H. Watanbe, Y. Saito, T. Takeichi, Electrophoresis 22 (2001) 3371.
[6] Y. Yang, A. Velayudhan, C.M. Ladisch, M.R. Ladisch, J. Chromatogr. 598 (1992) 169.
[7] K. Hamaker, S.L. Rau, R. Hendrickson, J. Liu, C.M. Ladisch, M.R. Ladisch, Ind. Eng. Chem. Res. 38 (1999) 865.
[8] C. Li, C.M. Ladisch, Y. Yang, R. Hendrikson, C. Keim, N. Mosier, M.R. Ladisch, Biotechnol. Prog. 18 (2002) 309.
[9] R.K. Marcus, W.C. Davis, B.C. Knippel, L. LaMotte, T.A. Hill, D. Perahia, J.D. Jenkins, J. Chromatogr. A 986 (2003) 17.
[10] D.M. Nelson, R.K. Marcus, J. Chromatogr. Sci. 41 (2003) 475.
[11] R.K. Marcus, D.M. Nelson, R.D. Stanelle, P. Brown, Am. Lab. 37 (13) (2005) 28.
[12] W.A. Haile, B.M. Phillips, Tappi J. 78 (1995) 139.
[13] R.D. Stanelle, R.K. Marcus, Anal. Bioanal. Chem., in press.
[14] J.C. Giddings, Unified Separation Science, John Wiley & Sons Inc., New York, 1991.
[15] R.F. Meyer, P.B. Champlin, R.A. Hartwick, J. Chromatogr. Sci. 21 (1983) 433.
[16] J.H. Knox, G.R. Laird, P.A. Raven, J. Chromatogr. 122 (1976) 129.
[17] R.A. Shalliker, B.S. Broyles, G. Guiochon, J. Chromatogr. A 994 (2003) 1.
[18] R.A. Shalliker, B.S. Broyles, G. Guiochon, J. Chromatogr. A 826 (1998) 1.
[19] D.M. Nelson, R.K. Marcus, Protein Pept. Lett., in press.