Transport Properties of Rolled, Continuous Stationary Phase Columns

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Continuous stationary phase columns consist of woven textile matrices of fibers rolled into a cylindrical configuration and inserted into a liquid chromatography column. This configuration allows separations to be carried out at interstitial mobile phase velocities in excess of 100 cm/min and pressures of up to 700 psig for stationary phases based on cellulose. Ordinarily, these conditions would cause compaction of a cellulose stationary phase to the point where flow is no longer possible. The packing of the column with cellulose as a continuous stationary phase enables these linear velocities to be achieved. Most importantly, this type of column allows the study of momentum transport and mass transfer in a media in which the mobile phase explores almost all of the void volumes in the column. The analysis of flow patterns in these columns has been modeled using elution patterns of both retained and unretained components, and plate height has been correlated as a function of velocities in the range of 1–100 cm/min. Engineering analysis of this type of chromatography column based on visual representation of the packed fibers by scanning electron microscopy, analysis of porosities using unretained (nonadsorbing) molecular probes, and application of momentum and mass transport equations is discussed.

Background

Rolled, continuous stationary phase columns consist of fibers which are aligned in the form of yarns, with the yarns woven into textiles. The textiles are rolled into a cylinder, and the cylinder is inserted into a liquid chromatography column (Figures 1 and 2). The column is capped using adjustable plungers to minimize dead volume at the column inlet and outlet (Figure 3). This column is connected to a liquid chromatography system in the same manner as a conventional column. The operational characteristics are also similar except that much higher flow rates are possible with the rolled stationary phase than with an equivalent column packed with loose fibers (3).

The concept of utilizing textile fabrics in the form of rolled stationary phases for liquid chromatography was first demonstrated for Nomex/Kevlar (22). The yarns had a characteristic diameter of 200–400 µm and were made from fibers consisting of 95% poly(m-phenyleneisophthalamide) and 5% poly(p-phenyleneeterephthalamide). The average fiber length was 45 mm, and the fiber width was 10–20 µm. There are approximately 90 000 fibers/g of the yarn. Nomex has good chemical resistance except for 50% NaOH at a temperature above 60 °C and hot concentrated acids. In addition to chemical resistance, Kevlar has an extremely high tenacity of approximately 23 g/denier, which is five times the strength of steel wire of the same weight and more than twice the strength of industrial nylon, polyester, or fiberglass. Consequently, the fibers were hypothesized to possess the mechanical strength to allow liquid to flow through a column packed with a textile made of Nomex/Kevlar when the fabric is inserted into the column in a rolled form (22).

Figure 1. Schematic diagram of a rolled stationary phase (RSP) consisting of a continuous stationary phase (textile).

A column packed with the Nomex/Kevlar was able to separate a four protein mixture of immunoglobulin G (IgG), bovine serum albumin (BSA), insulin, and β-galactosidase in 12 min when step gradients were used to elute the proteins (23). This first textile-based stationary

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particles (22):

$$H_{\text{total}} = A + B + \frac{DC_{\text{chem}}}{D} + C_{\text{chem}}$$

where $H_{\text{total}}$ is the total plate height, $D$ is the chromatographic velocity, and $A$, $B$, $C$, and $D$ are lumped parameters. At large velocities, the velocity terms in the denominators of eq 1 will dominate and the plate height expression becomes constant:

$$H_{\text{total}} \approx A + D$$

The coupling between flow and diffusion have been described by a number of investigators in a manner analogous to eq 2 (1, 7, 8, 19, 21, 22). van Krevelen and van den Hoed (19) suggested that eddy diffusion would extend from the interstitial space into intraparticulate pores, although not fully penetrating the pore. Their plate height expression had a similar form to eq 1 and reduces to a constant value at high velocity. Aleyan et al. (1) similarly described a velocity-independent plate height where flow occurs through the particle, while Giddings (7) had suggested a coupling theory where contributions from flow and diffusion act simultaneously. A summary of these results and their relation to a modified van Deemter equation is given by Yang et al. (23).

The packing of fibrous cellulose-based materials in columns had previously been reported in the literature for a variety of configurations as reviewed by Yang et al. (21). These included the following: yarn or fiber wound in a spiral manner around a rod (18); yarn or fiber in a parallel arrangement which is packed longitudinally (6, 13); randomly oriented fiber, yarn, fabric chunks, or fiber (17); and ordered diaz of batting and fabric (4, 16, 17).

The rolled stationary phase differs from these other approaches in that a single block of fabric constitutes the stationary phase. The yarns in the fabrics that are prepared and packed in this manner have orientations that are both parallel and perpendicular to the flow direction (21, 22). Most importantly, the experiments with cellulose-based fabrics showed that they also have sufficient mechanical stability to withstand mobile phase linear velocities of 100 cm/min or more when properly packed. The strength of the fibers and yarns in the cotton fabrics were sufficient to enable their use as chromatographic stationary phases. While the tenacity of Nomex/Kevlar is helpful, it is not necessary.

The demonstration of high linear velocities without destroying the stability of the stationary phases opened up the prospect of applying a wide variety of textile fabrics and fibers for use as chromatographic stationary phases. Both anion and cation exchange cellulose stationary phases were shown to be feasible. Different types of cellulose fabrics (100% cotton, ramie, cotton/polyester, and viscose rayon) as well as fabrics based on synthetic polymers (polyester, acrylic) were demonstrated to be capable of being packed and operated in chromatography columns (15, 20).

The different internal configuration of this type of stationary phase relative to columns packed with particulate materials led to investigations on transport properties of stationary phases consisting of 60% cotton/40% polyester as well as 100% cotton (cellulose). These textiles represent materials which are widely available in the industry. New methods for preparing and packing these materials made it possible to achieve the 1-10 mm plate heights that are often typical of process-scale
chromatography. Plate heights for analytical columns range from 0.1 to 0.01 mm or less. The difference in plate heights is a reflection of the difference in the goals of analytical chromatography compared to process chromatography.

The purpose of analytical chromatography is to completely resolve samples of unknown composition containing multiple components. Concentrations of some of the components are measured at levels of parts per billion or less (i.e., to nanomolar concentrations). Samples for analytical chromatography range from 1 to 10 µL in volume and are about 0.1% of the column volume. Peak dispersion broadens peaks and decreases their height so that they can become difficult to detect. Stationary phases that minimize dispersion and result in chromatograms with sharp, narrow peaks are needed to satisfy the combined requirements of resolution of a complex mixture and detection of trace components in a small sample volume. Columns with 10^4 to 10^6 plates per meter (i.e., plate heights of 0.1–0.01 mm) are therefore necessary for analytical chromatography. The small plate heights of analytical chromatography are achieved by packing columns with small particles. The combination of low throughput and high costs (on the order of $10,000/kg) of analytical stationary phases preclude their use for purifying larger volumes of most protein products in a cost-effective manner, although there are a few exceptions.

Process chromatography has the purpose of fractionating the product from the other impurities, where the chromatogram may consist of only two peaks. One peak is the product, while the other peak contains all of the other components. The compositions of the sample and amounts of the product and impurities change little from one batch to the next. The sample volumes are much larger than those for analytical chromatography and can be from 2 to 50% of the column volume. At these large volumes, the peak maximum is flat. Due to this plateau, dispersion at the leading and trailing edges has only a small effect on overall peak dilution. Column throughput and robustness are important because of the large volumes of product that must be processed in a predictable and reproducible manner from one batch to the next. Stationary phases with particle sizes of 50 to several hundred microns are used since the column pressure drop and stationary phase costs must be less than for analytical chromatography. Consequently, stationary phases for process chromatography that represent the optimum combination of resolution, cost, throughput, and operational characteristics will often consist of larger particle sizes but may also have 10× higher plate heights than materials used in analytical chromatography. Process chromatography has different objectives than analytical chromatography and, therefore, different criteria by which its performance is judged.

This analysis of the needs of process chromatography gives insights into motivations of engineering research on the scale-up and optimization of liquid chromatographic separation of biotechnology products. The ability to reproducibly pack textile-based continuous stationary phase columns enables further study of fiber-based chromatographic materials. It is now possible to systematically examine how orientation of the yarns relative to the flow of the mobile phase affect pressure drop and dispersion characteristics. Demonstration of rapid size exclusion chromatography illustrates the potential utility of rolled, continuous stationary phases based on textile fabrics.

Materials and Methods

Two types of textile materials were prepared for these studies and then packed in chromatography columns. One type was based on a fabric made of yarns which were a blend of 60% cotton and 40% polyester. The other type was based on 100% cotton.

Stationary Phase 1: The 60/40 fiber blend of cellulose (cotton) and polyester (poly(ethylene terephthalate) or PET) was obtained from Cotton Inc., P.O. Box 30087, Raleigh, NC 27612, under the name “60/40 cotton/polyester plain weave”. The average yarn and fiber diameters of this material were 200 and 13 µm, respectively, as measured by scanning electron microscopy. Specific surface areas were 1–2 m^2/g as measured by nitrogen permeation.

Stationary Phase 2: The stationary phase consisting of 100% cotton fiber was bleached, mercerized cotton print cloth, style #400M. This was supplied by Testfabrics, Inc., 415 Delaware Ave., P.O. Box 26, W. Pittston, PA 18643. The average yarn and fiber diameters measured by scanning electron microscopy were 275 and 20 µm, respectively.

Stationary Phase Preparation: The textile fabric was scoured to remove antistatic coatings and any other substances which may have adsorbed to the fabric surface during its manufacture. Fabric was placed in a beaker containing a detergent solution of 2% AATCC (American Association of Textile Chemists and Colorists, Research Triangle Park, NC 27709) standard detergent #124, without optical brightener and 5% sodium bicarbonate (NaHCO₃). The liquid-to-fabric ratio was 50 mL of solution to 1 g of fabric. The solution was brought to a boil and held at a boil for 1 h. The fabric was then removed and thoroughly rinsed with deionized (DI) water.

Some of the fabrics were derivatized using a solution of 0.5 M 2,4-diethylaminoethyl chloride. The derivatization procedure for this textile gave a stationary phase
Figure 5. Column packing apparatus.

capable of attaining an equilibrium protein loading of 110 mg of bovine serum albumin/g dry weight of stationary phase, when measured using an initial bovine serum albumin (BSA) concentration of 2 mg/mL in 50 mM Tris buffer at pH 7.0 and ambient temperature. The BSA was from Sigma Chemical Co. (St. Louis, MO) and was a fraction V powder (98–99% purity) and was dissolved in 50 mM Tris buffer (pH 8) using Trizma Base (T-1500) from Sigma Chemical Co. (St. Louis, MO 63178).

Column Packing. The stationary phase was prepared in a cylindrical roll as illustrated in Figure 4 and then pulled through the column using the packing device of Figure 5. The fabric was inflated until it reached a temperature for 3 h or more before packing to ensure preshrinkage. The stationary phase was then trimmed to length after packing so that it was flush with the column end fittings.

The tubing used to make the HPLC columns packed with 100% cotton was 316 stainless steel having dimensions of 3/8 in. (9.525 mm) o.d., 0.306 in. (7.747 mm) i.d., and 0.035 in. (0.889 mm) walls. Columns were 3.94 in. (100 mm) in length and capped with Valco 3/8 to 1/16 in. end-fittings obtained from Altech Associates (Deerfield, IL 60015). The cotton/polyester (60:40) stationary phases were packed in 10 mm i.d. Superformance glass columns which were a gift from R & S Technology of Wakefield, RI 02880. These columns utilize plunger that can be adjusted to the bed length.

Three types of columns were packed to study the effect of weave orientation on flow properties and plate height. The fabrics were packed with the fabric oriented with warp parallel, fill parallel, or bias orientation with respect to the mobile phase flow (Figure 6). Warp yarns run lengthwise in a fabric, parallel to the selvage, and are typically thicker and stronger than fill yarns, since warp yarns must withstand the constant tension and abrasion from weft yarn insertion during the weaving process. Fill, known as weft or woof, are the yarns which run crosswise in a fabric, at right angles to the warp yarns.

Column Storage. Packed columns were flushed with at least 10 column volumes of 1 M NaCl and then capped and stored at room temperature until use. The purpose of storing the stationary phase in 1 M NaCl was to eliminate microbial contamination and preserve flow capacity of the rolled stationary phase columns when they were not in use. No appreciable change in flow characteristics were detected when columns were stored in this manner for up to 6 months.

Buffer and Eluents. Water: An in-house source for water was used. Tap water was deionized using commercial cationic and anionic exchangers, then either glass distilled using a Corning single effect still or passed through an ultrafiltration device. Resulting water was at room temperature and greater than 2 x 10^6 Ω cm. Water from this source was used in the HPLC system after being filtered through a 0.22 μm filter and then deaerated for 30–60 min using an aspirator-induced vacuum, until the formation of bubbles in the water stopped.

Phosphate Buffer. Phosphate buffer, 20 mM, pH 6.0, was prepared by following the procedure of Gomori (9). Sodium phosphate monobasic, monohydrate (NaH2PO4· H2O, product no. 7892) and sodium phosphate dibasic, heptahydrate (Na2HPO4·7H2O, product no. 7914) were from Baxter Scientific Products, 1210 Wauckegan Rd., McGraw Park, IL 60085, as supplied by Mallinckrodt, Inc. Sodium chloride was added to 20 mM phosphate buffer, pH 6.0, to varying concentrations to suppress binding of proteins to the anion exchanger, DEAE cellulose (ET) (60: 40). Sodium chloride (NaCl, product no. 7581-500) was also from Baxter Scientific.

Tris Buffer. Tris buffers, 50 and 100 mM, both pH 8.0, were prepared using Trizma Base (T-1503) obtained from Sigma Chemical Co., P.O. Box 14028, St. Louis, MO 63178. The pH was adjusted using hydrochloric acid (HCl, 9535-33) obtained from J. T. Baker Inc., 222 Red School Ln., Phillipsburg, NJ 08865. Sodium chloride was added to 50 and 100 mM Tris buffer, pH 8.0, to a concentration of 1 M to suppress binding of proteins to the anion exchanger, DEAE cellulose (ET) (60:40).

Molecular Probes. Molecular probes were used for investigating and analyzing relative polarities and dispersion characteristics of the packed columns. Deute-
Table 1. Poly(ethylene glycol) of Various Molecular Weights Available from J. T. Baker

<table>
<thead>
<tr>
<th>MW of PEG</th>
<th>average MW</th>
<th>product no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>180-210</td>
<td>U214</td>
</tr>
<tr>
<td>300</td>
<td>265-315</td>
<td>U215</td>
</tr>
<tr>
<td>400</td>
<td>380-420</td>
<td>U216</td>
</tr>
<tr>
<td>600</td>
<td>570-630</td>
<td>U217</td>
</tr>
<tr>
<td>1000</td>
<td>960-1050</td>
<td>U218</td>
</tr>
<tr>
<td>1450</td>
<td>1300-1600</td>
<td>U220</td>
</tr>
<tr>
<td>3350</td>
<td>3000-3700</td>
<td>U221</td>
</tr>
</tbody>
</table>

* The term, average MW, is representative of the range in which the nominal molecular weights for these products can occur and does not reflect the distribution of molecular weights for these probes.

rium oxide (D2O, 99.8 atom % D, catalog no. 15,185-2) and PEG 14,000 (catalog no. 20,246-0) were obtained from Aldrich Chemical Co., Inc., 1001 West Saint Paul Avenue, Milwaukee, WI 53233. Specific molecular weights of poly(ethylene glycol) (PEG) polymer [CAS 25322-68-3] were obtained from J. T. Baker Inc., 222 Red School Lane, Phillipsburg, NJ 08865 (See Table 1).

Polyethylene glycol 10,000 (P-8667), polyethylene glycol 20,000 (P-8673), and polyethylene glycol 40,000 (P-8670) were obtained from Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178. Dextran [CAS 9004-54-0] of various molecular weights, 9000 through 2 x 10^6, were also obtained from Sigma (see Table 2).

Polyethylene oxide 4,000,000, product no. 4039, was obtained from Polysciences, Inc., Washington, PA 18976-2590. Poly(ethylene oxide) (CAS 25322-68-3) is a synonym for poly(ethylene glycol). Poly(ethylene glycol) manufactured by these three vendors was supplied through Union Carbide.

D2O was injected in an undiluted form. NaCl was prepared in concentrations of 5 mg/mL. Fresh solutions were prepared every 2-4 weeks to avoid microbial contamination. Solutions [0.1% (w/v), 10 mg/mL] of each polyethylene glycol, glucose, and dextran probe were prepared. All probe solutions and D2O were stored at 4 °C. The BSA used in this work was fraction V powder 88-99% (product number A-6793, from Sigma Chemical Co., St. Louis, MO).

Liquid Chromatography System. Characterization of Nomura P. A HPLC system was used to characterize the performance of the columns. For flow rates of 10 mL/min or less, a programmable single-head pump by ISC0 (model no. 2350) was used. The pump delivered 0.05 mL/min from a 1-L reservoir and directed flow to a pressure release check valve, a 7125 Rheodyne injector, through the column and finally to a detector. Pulse dampening and pressure measurements were performed by internal components of the ISC0 pump. For flow rates from 10 to 240 mL/min, a Novaprep 9000 programmable two-head pump system (gift of R&S Technologie, Wagelsfeld, RE) was used in place of the ISC0 pump.

Molecular probes were detected using either a 401 or 403 Waters Differential Refractometer. Protein samples were measured at a wavelength of 280 nm using either an ISC0 V-UV–vis detector or a Hitachi L-4000 UV detector. The analog signal from the detectors was either recorded on a Linear 1200 strip-chart recorder or digitally collected on a data acquisition device from Computer-Boards, Inc.

Analysis of columns began with recording elution profiles for various probes at various flow rates. PEG 200, PEG 3350, PEG 20,000, and dextran 506,000 were selected as exploratory probes because of their different sizes. Each probe was injected three times into the column at the prescribed flow rates. Typically, a single-column analysis required 120 injections (=10 flow rates x 4 probes x 3 replicates).

The peak recorded by the strip-chart recorder was digitized on a Calcomp 9500 digitization board. Digital information was analyzed using Fortran programs which determined solute velocities and plate heights as described by the following equation:

The Scanning Electron Microscopy. Microphotographs of the stationary phase were taken using a scanning electron microscope. The samples were 1 cm² and were sputter-coated with 8-10 nm of gold/palladium with a Hummer 6.2 sputtering system. The scanning electron microscope was a JEOL JSM-T300 scanning electron microscope. Instrument voltage was either 10 or 15 kV.

Measuring Packing Density. The measurement of packing density in an operating packed stationary phase column required that the column be sacrificed. Exact measurements were obtained by removing the packed stationary phase from the column and measuring the stationary phase’s dry weight and the column volume which it occupied. Alternatively, the packing and column is oven-dried and the weight of the packing is determined from the difference of the combined weight of the column and packing and the tare weight of the empty column weighed prior to packing. In either case, the column is no longer suitable for use. An approximate measurement is obtained from the area of fabric packed and from measuring the height of area of unpacked fabric.

Chromatographic Parameters. Dimensionless, or reduced, plate heights and velocities have been calculated using the definition of Honwath and Lin (12). The equation for the Archimedes' spiral:

\[ r = d_0 \cdot \theta^{2/\pi} \]  
(3)

where \( r \) is the internal radius of the column, \( d_0 \) is the thickness of the fabric in its packed state, and \( \theta \) is the angular measure of revolutions required to roll the fabric (radians).

If the Archimedes' spiral is represented as the graph of a polar equation where \( r = f(\theta) \) and \( f \) is smooth over the interval \([16, 32] \), then the length, \( L \), of the curve from
\[ \theta = \Theta_1 \text{ to } \theta = \Theta_2 \text{ is given by} \]

\[ L = \frac{2}{\alpha} \left[ \frac{\left[ f(\theta) \right]^2 + \left[ f'(\theta) \right]^2}{\pi} \right] d\theta \tag{4} \]

Therefore, if eq 3 is assumed to provide a suitable model for fabric length within the column, then substituting a form of eq 3 into eq 4, where \( \alpha \) is the thickness factor, \( \alpha = d_{ab}/2\pi \), gives

\[ L = \frac{\pi}{\alpha^2} \left( \frac{R_1^2}{\alpha^2} + 1 \right) \]

\[ + \frac{\alpha}{2} \left[ \frac{R_2^2}{\alpha^2} + \frac{R_1^2}{\alpha^2} + 1 \right] \tag{5} \]

The thickness of the packed fabric, \( d_{ab} \), is difficult to measure directly and requires that the thickness of unpacked fabric be measured with calipers and be related to the packed thickness based on packing a test column with known lengths of fabric. Equation 5 can then be used for estimating the length of fabric for subsequent columns or columns of different diameters.

A 5 mm i.d. column was packed with 11.5 cm of 60:40 cellulose/polyester fabric, and eq 5 was used to determine the correlation for the thickness of the packed fabric. The thickness of the packed fabric, \( d_{ab} \), was found to be 0.170 72 mm and was less than the 0.2 mm measured for unpacked fabric using calipers. Table 3 summarizes the lengths of fabric necessary to pack columns of larger diameters based on this equation. These values were calculated from eq 5 and found to be good estimates for the fabric lengths utilized in packing 10- and 26-mm columns.

A shell model can also be used to estimate the required length. This results in a less complex form of an equation for estimating fabric length required for a given column diameter. The shell model represents the rolled fabric as concentric circles or shells around a center point with each circle having a radius of one fabric thickness greater than the circle immediately to its interior, rather than as a continuous segment of fabric. The radius of the rolled cylinder, \( r \), would be given by

\[ r = n_i d_{ab} \tag{6} \]

where \( r \) is the internal radius of the packed column, \( n_i \) is the total number of theoretical shells, and \( d_{ab} \) is the thickness of the packed fabric. The length of fabric required for each shell, \( n_i \), is

\[ L = 2\pi n_i \frac{1}{2} d_{ab} \tag{7} \]

The total length of required fabric is obtained by summing the lengths for each shell:

\[ L = n_i^2 \pi d_{ab} \tag{8} \]

Substituting eq 7 into eq 8 gives the solution

\[ L = \frac{\pi}{\alpha^2} \frac{R_1^2}{\alpha^2} + \frac{R_2^2}{\alpha^2} + 1 \tag{9} \]

Lengths of fabric calculated from eq 9 are summarized in Table 3 and are similar to those obtained using the Archimedes’ spiral, differing by only 0.003% of the total length. Table 3 compares estimates of lengths of fabrics calculated from eq 5 and 9.

**Pressure Drop.** Pressure drops were found to be the lowest when the stationary phases were packed in a bias configuration (Figure 6). The pressure drop for the warp parallel was significantly higher than either the fill parallel or bias configurations, while the bias was still lower than the fill parallel configuration (Figure 7). A similar trend was observed for a 172-mm column of cotton/polyester 60:40 stationary phase (Figure 8). The pressure drops were linear at interstitial velocities ranging from about 10 up to 300 cm/min for these columns. On this basis the bias orientation was used for packing columns with the DEAE cellulose/polyester (60:40) columns.

Darcy’s Law was found to describe the pressure drop vs flow characteristic of the columns, as well as to provide a quantitative measure of permeability which can be used to compare different porous supports regardless of bed
Table 4. Comparison of Permeabilities

<table>
<thead>
<tr>
<th>stationary phase</th>
<th>characteristic dimension (d₀)</th>
<th>permeability, $K \times 100$ cm/min</th>
<th>intrinsic permeability, $\kappa \times 100$ darcys</th>
</tr>
</thead>
<tbody>
<tr>
<td>cellulose (cotton)</td>
<td>yarn, $\mu$m</td>
<td>fiber, $\mu$m</td>
<td></td>
</tr>
<tr>
<td></td>
<td>275</td>
<td>20</td>
<td>0.67</td>
</tr>
<tr>
<td>cellulose/polyester (60:40)</td>
<td>200</td>
<td>13</td>
<td>2.5</td>
</tr>
<tr>
<td>DEAE cellulose/polyester (60:40)</td>
<td>200</td>
<td>13</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Dimensions or volumetric flow rates. Darcy’s Law is

$$Q = \frac{KA(h_2 - h_1)}{\Delta \rho g}$$

(10)

where $Q$ is the flow rate, $K$ is the hydraulic conductivity, $A$ is the cross-sectional area, $\Delta \rho$ is the density difference, $g$ is the acceleration due to gravity, and $\Delta \rho g$ is the pressure drop. A graph of Darcy’s equation for data obtained with DEAE cellulose/polyester (60:40) shows the expected linear relationship with the slope of the line giving the values of $K$ for Tris-HCl and DI water as the mobile phases (Figure 9). The permeabilities for the DEAE cellulose/polyester, cellulose/polyester (both 60:40), and cotton (80% cotton) are summarized in Table 4. The results show that the derivatized form gives a lower permeability, although we do not know the cause.

**Comparison of Height Equivalents of a Theoretical Plate (HETP).** The HETPs for all three stationary phases were evaluated by injecting 100-μL samples of PEG 20,000 at 10 mg/mL in aqueous buffer. The HETP for the cotton print cloth (100% cotton) stationary phase gave nearly constant plate heights when measured using PEG 20,000 over a range of interstitial velocities (Figure 10) as did the cotton/polyester (60:40) stationary phase for dextran (MW = 506 000) and PEG 200 (Figure 11). The DEAE derivatized cotton/polyester (60:40) gave the lowest plate height (of about 2 mm), with PEG 200 showing an increasing plate height with respect to increasing flow rate (Figure 12a). The difference between the two probes is more obvious (Figure 12b) when the data are replotted as reduced plate height as a function of reduced velocity. Comparison of these data of underderivatized cellulose/polyester (Figure 13a,b) shows that the plate height decreases at interstitial velocities below about 5 cm/min (Figure 13a), with the plate height for the smaller probe (PEG 200) having a larger slope than PEG 20,000 (Figure 13a,b). The column, which gives the data of Figure 13, was packed with bias oriented stationary phase and reproducibly indicates configuration rela-
tive to warp or fill parallel stationary phases (compare Figures 11 and 13). The increase in plate height of the low molecular weight probes in both materials shows that the smaller probe probably exposes more of the intrafiber porosity than does the higher molecular weight probe which is excluded from the stationary phase. This is consistent with the pore size distribution curve which was measured for DEAE/cotton/polyester 60:40 using D₂O, glucose, PEG, and dextran probes dissolved in deionized water at 2 mg/mL (Figure 14). Column efficiency is a function of the dispersion which occurs for a solute moving through the packed bed. Plate counts were measured for increasing flow rates to observe the ef-
ciency of the systems being investigated. Probes of differing molecular weights, and hence hydrodynamic radii, were utilized to determine plate heights corre-
sponding to chromatographic velocities of up to 50 cm/ min. Explanations of flow rate independent plate heights are given by Hamaker and Ladosch (27).

The sharp cutoff in pore size distribution combined with the stable flow properties made it possible to achieve a size exclusion separation of BSA from NaCl at mobile phase flow rates of 0.5 and 10 mL/min. The separations

![Figure 9. Graphical representation of permeability, K, DEAE cellulose/PET (60:40).](image)

![Figure 10. Comparison of HETP values for different packing orientations for columns of the same dimension: 7.76 x 100 mm; cotton print cloth; probe, PEG 20,000.](image)

at the two flow rates were essentially equivalent (com-
pare parts a and b of Figure 15). These separations were
carried out using a mobile phase of 50 mM Tris at pH 8, with a salt concentration of 500 mM NaCl to suppress adhesion of protein on the stationary phase. This separation capability led to development of conditions to rapidly separate an unfolded protein from its denaturants in a manner which promotes efficient refolding (10).

**Discussion of Transport Properties. Particle Reynolds Number, $Re_p$.** The transition from laminar flow to turbulent flow within a packed bed is not as well defined as for an open tube. It is assumed that turbulence in packed beds develops gradually as $Re_p$ increases from 1 to 100 (13). Even at low Reynolds numbers, there is a lateral movement of the fluid elements between the stagnant and mobile liquid phase. This results in a substantial convective diffusivity at high velocities which is analogous to eddy diffusivity in turbulent flow. Consequently, the flow profile can be approximated as plug flow.

The Reynolds number for chromatographic media, $Re_p$, is usually assumed to be on the order of 1 or less, corresponding to a "creeping flow" regime. The Reynolds
number for porous media is defined as (11)

\[ R_e = d_p \rho \nu (1 - \varepsilon_v) \]  

(11)

where \( d_p \) is the particle diameter (m), \( \nu \) is the superficial velocity (m/s), \( \rho \) is the density of mobile phase (e.g., 1008 kg/m³ for water), \( \mu \) is the dynamic viscosity (e.g., 1.05 × 10⁻⁶ Pa·s for water), and \( \varepsilon_v \) is the interstitial void fraction. \( R_e \) is approximately 2 for a flow rate of 120 mL/min of H₂O. The void fraction was based on the dextran.

Figure 11. Comparison of HETP values for different packing orientations for columns of the same dimension: 10 × 172 mm, cellulose/PET (60:40).

Figure 12. Plate heights. Column: EM15, 10 × 105 mm; DEAE cellulose/PET (60:40). Eluent: DI H₂O. Sample volume: 500 µL. Detection: R.I. Panel a is a comparison of H and \( \nu \) and panel b reduced plate height (h) vs reduced velocity. (Note: the symbol Ω denotes inside diameter.)

Figure 13. Plate heights. Column: EM14, 10 × 161 mm, scoured cellulose/PET (60:40). Eluent: DI H₂O. Sample volume: 500 µL. Detection: R.I. Panel a is a comparison of H and \( \nu \) and panel b reduced plate height (h) vs reduced velocity. (Note: the symbol Ω denotes inside diameter.)

Figure 14. Porosity distribution curve. The eluent was DI water, with 100-µL sample injection of 10 mg/mL PEG, dextrans, and other molecular weight probes dissolved in water. The flow rate was 10 mL/min. The column bed volume is 8.25 mL (10 mm i.d. × 105 mm long). 66,300 probe and was assumed to be excluded from intraparticle pores.

Kozeny–Carman Equation. The Kozeny–Carman equation relates the specific permeability, or intrinsic permeability, \( \kappa \), to the particle size and void fraction of a porous medium. For spherical supports, the Kozeny–Carman expression is given:

\[ \kappa = \frac{d_p^4}{180(1 - \varepsilon_v)^2} \]  

(12)

where \( \varepsilon_v \) is the interstitial void fraction = 0. The intrinsic
A working description of pressure drop caused by fluid flux in a rolled stationary phase system.

The first term of the right-hand side of eq 16 is a function of structural parameters (23):

$$\frac{d^2}{\mu} = \frac{c_s \gamma}{M_s^2}$$

where $c_s$ is Kozeny’s constant and varies according to the geometrical shape of the individual channels in the model and $\gamma$ is the tortuosity of those channels and is defined as

$$\gamma = \left(\frac{L}{L_d}\right)^2$$

where $L$ is the length of the straight line connecting two ends of a tortuous tube and $L_d$ is the true length of the tortuous tube. $M_s$ is the specific area of the particulate represented as spheres of uniform radius:

$$M_s = \frac{\text{particle surface area}}{\text{volume}} = \frac{4\pi r^2}{\frac{4}{3} \pi r^3} = \frac{3}{r}$$

where $r$ is the radius of the sphere. However, for a rolled stationary phase column, the individual fibers and yarns can be represented as infinitely long cylinders:

$$M_s = \frac{2\pi r^2}{\pi r^2} = \frac{2}{r_c}$$

where $r_c$ is the radius of the cylinder and $l$ is the length of the cylinder. If the structural constant, $c_p$, is assumed to be $\sim l$, which is a valid assumption given the near circular cross section of the fibers and yarns, the Kozeny–Carman equation becomes

$$\kappa = \frac{d_p^2}{8 \left(1 - \epsilon^2\right)}$$

For a void fraction of $\epsilon = 0.60$ and a yarn diameter of $d_p = 0.02 \text{ cm} (200 \mu\text{m})$, the predicted permeability of 684 darcys is much greater than the experimentally determined values for $\kappa$ of 0.1–0.4. If the characteristic particle diameter is represented as the fiber diameter, $d_p = 0.0013 \text{ cm}$, the predicted value for intrinsic permeability, 2.89 darcys, is still greater than experimental values; however, it is within 1 order of magnitude for cellulose/PET (60:40). This is significant because eq 19 is very sensitive to $d_p$ and $\kappa$ values ($d_p = 0.0013 \text{ cm}$ and $\epsilon = 0.46$ will give a $\kappa$ value of 0.74). This result is consistent with the hypothesis that flow occurs between the fibers, although a pressure drop measurement, itself, provides only partial evidence. A higher than expected pressure drop can also be due to obstructions.

**Blake–Kozeny Equation and the Ergun Equation.**

The Blake–Kozeny equation is obtained by substituting the Kozeny–Carman expression and the relation for intrinsic permeability into eq 15:

$$\frac{\Delta P}{\Delta l} = \frac{\epsilon^3}{80 \left(1 - \epsilon^2\right)}$$

The Blake–Kozeny is generally valid for void fractions, $\epsilon$, less than 0.5 and for laminar flow defined by the regime, $Re_\infty < 10$, where $v_c$ is the superficial velocity or the approach velocity.
For flows in the turbulent regime (Reₚ > 1000) the Burke–Plummer equation defines flow:

\[ \frac{\Delta p}{\Delta l} = \frac{1.75 \nu_d^2 e}{d_p e^2} (1 - \varepsilon) \]  

(21)

While the results discussed in this paper did not reach the flow velocities described by the Burke–Plummer equation, eq 21 is useful. When combined with the Blake–Roxeny equation a suitable description for flow occurring for all Reₚ values is obtained. This expression is called the Exrg equation and gives pressure drop for flows corresponding to Reynolds numbers between 10 and 1000:

\[ \frac{\Delta p}{\Delta l} = \frac{180 \nu_d^2 e}{d_p^3 e^3} (1 - \varepsilon)^2 + \frac{1.75 \nu_d^2 e}{d_p e^2} (1 - \varepsilon) \]  

(22)

Equation 22 is needed for combinations of particle dimensions and flow rates which give a flow characteristic in this range of Reynolds numbers.

Conclusions

The transport properties of rolled stationary phases packed in liquid chromatography columns enable operation of fibrous beds of cotton cellulose and cotton/PET blends at fluid velocities approaching 300 cm/min to be achieved. Analysis of the flow regimes show that the pressure drops follow Darcy's equation and that the particle Reynolds number is in the laminar region even at fluid velocities considered to be extraordinarily high in the context of liquid chromatography. The construction of the textile stationary phase imparts hydraulic stability, as well as velocity independent column efficiency. The fibers that are integrated into yarns, which in turn are woven to form the textile, enable a rolled stationary phase to be formed which is pull-extruded into chromatography columns compatible with existing bench- and preparative-scale chromatography systems. Experimental measurement and theoretical analysis of transport properties of rolled stationary phase columns show that fiber orientation affects both pressure drop and plate height. A bias configuration, where yarns are oriented at an angle of 45° relative to the flow direction, gives the best results.

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References and Notes


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