Chapter 11

Biotextiles — Monoliths with Rolled Geometrics

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11.1 INTRODUCTION

Stationary phases that are formed from textiles are a continuous, interconnected fibrous matrix in the form of yarns and fabric. The fibers are assembled into yarns and the yarns are woven into fabric. Since individual fibers have exhibited poor flow properties when used as stationary phases, rolled fabric stationary phases have been developed. Rolled stationary phases enable a long bed length to be attained while retaining good flow properties [1,2]. This kind of stationary phase orients the fabric into a three-dimensional structure through contact between adjacent layers of fabric where the fabric [1,3,4] supports the fibers (Fig. 11.1(a)) [5] assembled into the yarns (Fig. 11.1(b)) [6], and the woven fabric (Fig. 11.1(c)) [6]. This is a type of monolithic material since there are no distinct or individual particles packed into the column. Further, since the material is a textile, and it is used to fractionate biomolecules, we have called this material a biotextile.

11.2 WOVEN MATRICES (TEXTILES) AS STATIONARY PHASES

11.2.1 Chemistry

11.2.1.1 Potential for derivatization

The use of fibers as chromatographic stationary phases provides a range of chemistries available for packed columns for industrial bioseparations. Cellulosic, polyphenylene, and poly(ethylene terephthalate) fibers separate proteins. Acrylic fibers exhibit hydrophobic and ionic interactions with some types of dye molecules [1,3,4]. Silica, polypropylene, polyamide, carbonaceous, and other textile fibers represent additional scaffolds upon which biospecific ligands could be grafted or ion-exchange capacity derivatized. The surface chemistries of fibers used in the textile industry can be both hydrophilic (cellulose) and hydrophobic (polyester, aramid, and acrylic). Fig. 11.2 shows some of the structures.
Hydrophilic fibers with the appropriate porosities could prove useful as stationary phases for size exclusion chromatography. Derivatized forms of cellulose are viable for ion-exchange chromatography of proteins. Hydrophobic or reversed-phase interactions are possible with aromatic, vinyl copolymer, and carbonaceous fibers. These enable chromatographic separations to take place on the basis of interactions of the biomolecules with hydrophobic or ionic groups on the stationary phase when elution is carried out with an appropriate mobile phase.

This method of construction contrasts with another type of monolithic column developed by Svec et al. [7] and Hjertén et al. [8]. In their case, the column is prepared by filling it with a polymerization mixture containing porogens. Polymerization entraps the porogens that are subsequently removed by a solvent that is pumped into the column. Dissolution of the porogens leaves a porosity through which eluent flow occurs. Sharp peak and rapid separations are achieved since convective transport dominates diffusive factors throughout the column [9].

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Fig. 11.2. Chemical structures of (a) cellulose (cotton), (b) poly(ethylene terephthalate) (polyester), and (c) DEAE cellulose.
11.2.2 Forming cylindrical columns

11.2.2.1 Fabric preparation

All fabric is cut in a 45° diagonal (bias) direction [10] into swatches 20 cm wide and 60–100 cm long. Previous work on rolled stationary phases showed that columns rolled in the bias direction had a lower pressure drop and a smaller height equivalent of a theoretical plate (HETP) than did columns prepared from fabric rolled in the warp or filling direction [2]. The column is more stable than particulate packings such as Sephadex at higher flow rates (Fig. 11.3). Prior to rolling, the fabrics are scoured to remove antistatic coatings and any other substances that may have adsorbed to the

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fabric surface during its manufacture. This is accomplished by boiling the fabrics in a dilute detergent solution with sodium bicarbonate for one hour. The fabric is then removed and thoroughly rinsed with deionized (DI) water. All prepared fabrics are stored in DI water at 0–4°C until rolled and packed into a column.

11.2.2.2 Column rolling and packing

The fabric is immersed in DI water at room temperature for 3 h or more before packing in order to ensure pre-shrinkage. The wet fabric is then rolled into a cylinder and packed into a 10 mm (internal diameter) × 200 mm (length) glass tube as indicated in Fig. 11.4 in order to form a liquid chromatography column. The stationary phase is trimmed to length after packing so that one end of the rolled phase is flush with the column end fittings. Plungers are added on each end of the column in order to avoid dead volume [11, 12] (Fig. 11.4).

11.3 CHARACTERIZATION

11.3.1 Pressure drop

The structural rigidity of an underivatized rolled stationary phase is demonstrated by the pressure drop curve in Fig. 11.3. Stable operating pressures were achieved at flow rates up to 100 mL/min (18,000 cm/h) of DI water through a column with an inside diameter of 10 mm. At 25 mL/min, pressure drop of the Sephadex column [13] was 0.2 MPa/cm or about 3.7 MPa over the length of the bed. The particle Reynolds number corresponding to this flow rate was estimated to be about 8.7 [2] and is still considered to be in the laminar regime (Re < 10), although the decrease in the friction factor begins to level off in this range [15].

11.3.2 Peak broadening (plate height) according to modified van Deemter equation

The scanning electron micrographs of Fig. 11.5(a)-(b) show relatively large interfiber spaces (on the order of micrometers). Convection is likely to occur through both interyarn and interfiber channels, as illustrated schematically in Fig. 11.5(c). The interfiber channels have a small cross-sectional area while the interyarn channels are large. Just as flow in parallel pipes of equal lengths distribute according to cross-sectional areas, flow through these channels are envisioned to distribute in proportion to their cross-sectional areas. Consequently, pore or interfiber velocity, $v_p$, is a small fraction of the interyarn velocity, and therefore of the chromatographic velocity,
Fig. 11.4. Schematic representation of stationary phase rolling, packing, and the column apparatus used to minimize dead volume at inlet and outlet (Reprinted with permission from ref. [2], copyright 1998 American Chemical Society and American Institute of Chemical Engineers).

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v_{chrom}. As the eluent flow rate increases, both the pore and the chromatographic velocity will increase proportionately while their ratio will remain constant, independent of flow rate. At higher flow rates, the pore velocity could become high enough to make convective transport through the interfiber space appreciable relative to diffusive transport. This would in turn affect the overall plate height expression. In order to estimate the contribution of convective intrafiber transport to band spreading, a simplifying assumption of plug flow is made. The effects of convection and diffusion can be added in parallel after Guttmann and DiMarzio [16]. The convective contri-
buton of plate height, $H$ for piston flow is independent of velocity. Thus, the total plate height due to the "intraparticulate pore" space at high velocity $v_{\text{chorm}}$ is given by equations 11.1 and 11.2:

\[
\frac{1}{H_{\text{por}, \text{total}}} = \frac{1}{H_{\text{lam}}} + \frac{1}{H_{\text{diff}}} = \frac{1}{D} + \frac{1}{Cv_{\text{pore}}} \quad (11.1)
\]

\[
H_{\text{total}} = A + \frac{B}{v_{\text{chorm}}} + \frac{D}{D + C} \frac{Cv_{\text{chorm}}}{v_{\text{chorm}}} \quad (11.2)
\]

The total plate height equals $A + D$ when $v_{\text{chorm}}$ is large.

11.3.3 Effect of characteristic dimensions on performance

Extensive modeling has been done on the effect of flow rate on pressure drop and plate height for rolled stationary phase columns, but there is relatively little information available on the effect of different fabric treatments on column separation performance. This drove the preliminary work by Keim et al. [17] which consisted of treating each fabric swatch with the cellulose enzyme Spezyme® CP, secreted by Trichoderma longibrachiatum. The enzyme treatment of cotton print cloth decreased

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(c)

Fig. 11.5 (continued from previous page). Scanning electron micrographs of flat fabric (a), cross-section of rolled fabric (b), and schematic of the parallel flow patterns available, representing flow through the interyarn and interfiber channels (c). There are numerous interfiber channels for every interyarn channel, but for simplicity, the diagram only shows one channel of each kind. Scale bar indicated at bottom of micrographs equals 100 µm (Reprinted with permission from ref. [1], copyright 1992 Elsevier).

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the water-accessible (total) pore volume by almost 20% (Table 11.1). Data of Table 11.1 originate from the simple logistic model explained in the next section.

Since enzyme treatment significantly affects the number of ionizable groups that can be attached to the cellulose surface, enzyme treatment may also affect resolution if the column is further derivatized with a ionizable group.

11.3.4 Models

One of the first models to estimate void fraction in rolled stationary phase columns is based on Lin et al. [18]. The model, which has four curve-fit parameters, is:

\[ I = V_e \cdot V_s = \frac{\alpha}{1 + e^{(\beta - \gamma)}} \]  

(11.3)

where \( I \) is the inaccessible pore volume, \( V_e \) is the elution volume of the probe of interest, \( V_i \) is the total accessible pore volume, \( \alpha \), \( \beta \), and \( \gamma \) are logistic curve fit parameters, and \( x \) is the log of the pore diameter, \( D \). Both \( V_i \) and \( V_s \) are divided by the mass of the rolled stationary phase to normalize the data on a weight basis.

Although the four-parameter logistic function fits the data fairly well, three of the parameters do not have any significant physical meaning and only two of the terms (\( V_i \) and \( \alpha \)) can be estimated à priori. The curve-fitting procedure requires reasonable initial guesses for two parameters (\( \beta \) and \( \gamma \)) that cannot be easily estimated from the data. Thus, comparing the fit parameters from different rolled stationary phase columns does not provide much useful scientific information.

Another model (logistic) has been suggested that applies to rolled stationary phase columns is given by [17]:

\[ V_s = \frac{\alpha}{1 + \beta \cdot e^{-\gamma D}} \]  

(11.4)

Equation (11.4) differs from equation (11.3) because it is based on accessible void volume (instead of inaccessible void volume) and uses the pore diameter, \( D \), in the exponential term in the denominator instead of \( \log D \). The model also uses \( \beta \) as a pre-exponential term in the denominator instead of \( e^\beta \) in equation (11.3). For equation (11.4), \( \alpha \) is approximately equal to the mass-normalized external void volume, \( V_0 \), and \( \beta \) can be calculated from equation:

\[ \beta = \frac{(V_0)^2}{V_i} \]  

(11.5)
<table>
<thead>
<tr>
<th>Specimen</th>
<th>3.9%</th>
<th>4.3%</th>
<th>2.8%</th>
<th>2.0%</th>
<th>1.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (µm)</td>
<td>0.316</td>
<td>0.34</td>
<td>0.39</td>
<td>0.69</td>
<td>0.34</td>
</tr>
<tr>
<td>CPC with 0.82%</td>
<td>0.313</td>
<td>0.327</td>
<td>0.359</td>
<td>0.56</td>
<td>0.37</td>
</tr>
<tr>
<td>CPC with 4.1%</td>
<td>0.304</td>
<td>0.34</td>
<td>0.42</td>
<td>0.44</td>
<td>0.51</td>
</tr>
<tr>
<td>CPC with 8.2%</td>
<td>0.295</td>
<td>0.30</td>
<td>0.41</td>
<td>0.41</td>
<td>0.39</td>
</tr>
<tr>
<td>CPC with 16%</td>
<td>0.29</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Cotton spun cloth (CPC): 0.649

---

**Table 11.**

<table>
<thead>
<tr>
<th>Type</th>
<th>% Error</th>
<th>Predicted</th>
<th>Measured</th>
<th>Total Porosity, %</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>f</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

The predicted and measured weight accessible pore volume for each fabric is shown and labeled as total pore volume. The parameters for the pore size distribution using the logistic function model given in Equation (14).
This logistic model has fewer curve-fit parameters than the model of Lin et al. [18]. Two of the three curve fit parameters can be easily estimated from the data.

11.4 APPLICATIONS

11.4.1 Separations

11.4.1.1 Desalting

Rolled stationary phase columns have many applications. One of these applications is desalting. Hamaker et al. [2,12] showed that a DEAE derivatized 60/40 cotton/polyester blend fabric afforded size exclusion separations of bovine serum albumin (BSA) from sodium chloride which was added to the mobile phase to suppress the charges on the surface of the stationary phase. Li et al. [19], was able to show that complete size exclusion separations of BSA from NaCl and BSA from glucose were achieved in less than 8 minutes using cotton print cloth (Fig. 11.6).

Fig. 11.6. Separation of BSA from NaCl, and from glucose using cotton print cloth rolled stationary phases (Reprinted with permission from ref. [19], copyright 2002 American Chemical Society and American Institute of Chemical Engineers).
11.4.1.2 Protein refolding

Another application for the rolled stationary phase involves refolding of a denatured protein, namely recombinant secretory leukocyte protease inhibitor (rSLPI) as described by Hamaker et al. [12]. rSLPI accumulates intracellularly in the form of inclusion bodies in an inactive form. After cell disruption, rSLPI is only partially soluble and to obtain full stability and activity, the protein must be solubilized and then refolded into the proper conformation. Previous work by Seely and Young [20] showed that the protein would refold at a 10-fold dilution factor. This was done because it was thought that if less denaturants were present, the protein would be more susceptible to refold correctly. Hamaker et al. [12] showed that the highest yield of refolded protein was obtained for a 3 mL fraction collected 3 min after the 2 mL sample had been injected (Fig. 11.7). This fraction contained 96% of the initial rSLPI injected into the column at a concentration of 1.28 mg/mL. This represents a 1.56x dilution of the protein, compared to a 10-fold dilution used by Seely and Young [20]. Hamaker et al. [12] also showed that this approach, if scaled up, would significantly reduce process volumes and therefore improve viability of downstream processing of large amounts of proteins.

11.4.1.3 Gradient chromatography

Rolled stationary phases also have the capability for gradient chromatography. Yang et al. [1] was able to separate a four protein mixture of BSA, anti-human IgG, insulin, and -galactosidase using a rolled stationary phase consisting of 95% Nomex (poly(m-phenylene isophthalamide)) / 5% Kevlar (poly(p-phenylene terephthalamide)) (Fig. 11.8). The effect of both ionic and hydrophobic interactions between these aramid fibers and proteins can be explained through Table 11.2 that summarizes retention characteristics for the four proteins in five different eluents.

Yang [3] was also able to separate the four-protein mixture stated above using a DEAE (anion exchanger) and sulfated (cation exchanger) cotton stationary phase using stepwise desorption conditions as shown in Tables 11.3 and 11.4.

11.4.1.4 Affinity chromatography of binding domains

Cellulosic affinity chromatography stationary phases must match binding preferences of the binding domain protein and give good flow properties. When using cotton cellulose, the material needs to be pretreated in some manner to remove adsorbed particles first. Otherwise, the cellulose-binding-domain protein could cause release of cellulose particles, which in turn would migrate through the column to the retaining

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Fig. 11.7. Elution profile of rSLPI/denaturant mixture (Reprinted with permission from ref. [12], Fig. 4, copyright 1999, American Chemical Society). Conditions: flow rate 2 mL/min; buffer of 50 mmol/L Tris-HCl, pH 8.0, 500 mmol/L NaCl; protein concentration of 2 mg/mL dissolved in mobile phase, sample size 2 mL; UV detection at 280 nm.

frit at the outlet and plug it [9]. A suitable matrix is suggested by the work of Yang et al. [1] in which packed beds of woven cotton and ramie textiles have the desired porosity characteristics and mechanical flow stability when inserted in a chromatography column.
Fig. 11.8. Chemical structures of Nomex and Kevlar aramids.

**TABLE 11.2**

RETENTION CHARACTERISTICS OF ROLED ARAMID STATIONARY PHASE [1]

<table>
<thead>
<tr>
<th>Elution step</th>
<th>Protein a</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>BSA</td>
<td>Insulin</td>
<td>β-Gal</td>
</tr>
<tr>
<td>DI water (pH 5.5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 mmol/L Na2B4O7 (pH 9.2)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 mol/L (NH4)2SO4 (pH 4.5)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>80 mmol/L NaAc+80 mmol/L HAc (pH 4.7)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0.1 mol/L NaCl (pH 5.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a Retention of protein is marked with +, lack of retention with -

**TABLE 11.3**

EFFECT OF ELUENT PROPERTIES ON ADSORPTION OF PROTEINS ON ROLED DEAE COTTON COLUMN [3]

<table>
<thead>
<tr>
<th>Eluent</th>
<th>Protein a</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>BSA</td>
<td>Insulin</td>
<td>β-Gal</td>
</tr>
<tr>
<td>DI water (pH 5.5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25 mmol/L Trizma buffer (pH 7.2)</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>50 mmol/L Trizma buffer (pH 7.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2 mol/L NaCl</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Retention of protein is marked with +, lack of retention with -

References p. 253
TABLE 11.4

EFFECT OF ELUENT PROPERTIES ON ADSORPTION OF PROTEINS ON ROLLED SULFATED COTTON COLUMN [3]

<table>
<thead>
<tr>
<th>Eluent</th>
<th>Protein a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA</td>
</tr>
<tr>
<td>DI water (pH 5.5)</td>
<td>+</td>
</tr>
<tr>
<td>80 mmol/L NaAc·HAc (pH 4.7)</td>
<td>-</td>
</tr>
<tr>
<td>2.0 mmol/L NH₄Cl (pH 4.5)</td>
<td>-</td>
</tr>
<tr>
<td>5 mmol/L Na₂HPO₄·2KH₂PO₄ (pH 5.9)</td>
<td>-</td>
</tr>
<tr>
<td>10 mmol/L Na₂B₄O₇ (pH 9.2)</td>
<td>-</td>
</tr>
</tbody>
</table>

a Retention of protein is marked with +, lack of retention with –

11.4.2 Textile properties

There are many properties associated with certain textiles that make them great candidates for separations. Napping, a mechanical textile finishing process that lifts the fibers from yarns near the surface of the fabric to produce a hairy or fuzzy surface [21] was shown to decrease the HETP of BSA and NaCl [19]. Plain weave fabrics have a pronounced wale on the surface, which forms parallel diagonal ridges [22]. When twill fabrics are rolled into a stationary phase column, the curves between the ridges may cause molecules to channel and thus result in a 400–500% increase in HETP (Table 11.5).

Mercerization is a textile wet processing treatment that improves the porosity of cotton fabrics in the textile industry [23,24]. Li et al. [19] showed that mercerization improved porosity of cotton fabric columns by increasing the internal void fraction by 80% and decreasing the external void fraction by 36%. A side effect of mercerization is that mercerized fabrics are not as compressible as unmercerized ones, which causes an increase in HETP of BSA and NaCl by 170% and 270%, respectively. Even though the unfavorable percent changes in HETP are larger than the favorable changes in void fractions, the overall resolution was still improved by 35%. This indicates that the void fractions have a greater effect on resolution than does the plate count. In summation, mercerized, napped, plain weave fabrics with large yarn diameter, low fabric count, and high compressibility are desired attributes for a good rolled chromatography column for carrying out size-exclusion chromatography. Of the ten fabrics
TABLE 11.5

PERCENT CHANGES IN THE COLUMN PERFORMANCE PARAMETERS BY NAPPING, TWILL, AND MERCERIZATION [19]

<table>
<thead>
<tr>
<th>Change in column performance, %</th>
<th>Napping a</th>
<th>Twill b</th>
<th>Mercerization c</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_{BSA}$</td>
<td>-49.9</td>
<td>+392.7</td>
<td>+171.8</td>
</tr>
<tr>
<td>$H_{NaCl}$</td>
<td>-31.5</td>
<td>+500.8</td>
<td>+274.6</td>
</tr>
<tr>
<td>$\epsilon_p$</td>
<td>+1</td>
<td>+35.9</td>
<td>+81.6</td>
</tr>
<tr>
<td>$\epsilon_b$</td>
<td>+11.6</td>
<td>-49.9</td>
<td>-36.0</td>
</tr>
<tr>
<td>$R_s$</td>
<td>+28.2</td>
<td>-100.0</td>
<td>+35.2</td>
</tr>
</tbody>
</table>

a Changes from unnapped fabric columns (sea island and cotton print cloth) to napped fabric columns (cotton flannel, cotton velveteen, and double napped cotton blanket) measured at 2 mL/min eluent flow rate.

b Changes from plain fabric columns (sea island cotton, cotton print cloth, cotton/polyester blends) to twill fabric columns (microdenier viscose rayon fabric) at 8 mL/min flow rate.

c Changes from cotton print cloth columns to mercerized cotton print cloth columns measured at 2 mL/min eluent flow rate.

tested, mercerized cotton flannel meets all the requirements and is the best candidate according to the value of resolution, as indicated in Fig. 11.9.

11.5 PERSPECTIVES AND FUTURE DIRECTIONS

Cellulose based stationary phases could be very useful at the process scale because cellulose is a natural, nontoxic, and renewable hydrophilic polymer. The work that has previously been done with the rolled stationary phase gives excellent preliminary results for its use at the process scale. One consideration on future applications involves work by Keim et al. [17]. Since enzyme treatment significantly affects the number of ionizable groups that can be attached to the cellulose surface, this treatment may affect separation resolution differently if the column is further derivatized with ionizable groups.

Li et al. [19] showed that cotton flannel met all the requirements for fabrics to make a good separation and is the best candidate based on resolution. This work also indicated that the mass of the stationary phase in the column affects HETP data for different fabric swatches inserted into a 10 mm × 200 mm glass tube. According to

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Fig. 11.9. Comparison of resolution in different types of cotton based rolled stationary phases packed in 10 mm × 200 mm liquid chromatography columns (Reprinted with permission from ref. [19], copyright 2002 American Chemical Society and American Institute of Chemical Engineers).

In this work, 8.0 g in a 10 mm × 180 mm column gives the best resolution. Hence, further scale-up studies are needed.

11.6 CONCLUSIONS

We have demonstrated the reproducibility and utility of rolled continuous monolithic columns. Scale-up for industrial applications offers the promise of enabling rapid separations of large volumes of bioproducts, specifically for size exclusive separations. Other applications are envisioned for ion exchange and hydrophobic types of separations, as well. The biological origins of many textiles, and their application to separations of bioproducts, leads to the definition of a new type of stationary phase called biotextiles.

11.7 ACKNOWLEDGEMENTS

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11.8 REFERENCES