Surface-modified Nonwoven Membranes with High Capacity for Protein Binding

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Nonwoven Fabrics

- **High-speed, low cost processes**
  - 300 - 1,000 meters/minute, 5-6 meters wide

- **Inexpensive materials** (PP, PET, PBT, Nylon)

- **Engineered fiber diameters, basis weight, thickness**
  - Spunbonding: $d_f > 200 \text{ nm}$; 150-300 Kg/m/hour
  - Meltblowing: $d_f > 500 \text{ nm}$; 100-200 Kg/m/hour
  - Electrospinning: $d_f \text{50 to 200 nm}$; 50-200 gm/m/hr
  - Bicomponent fibers, particle-nonwoven composites
  - Fiber splitting for smaller diameters

- **Large number of 3-D formation options**
  - Hydroentanglement, calendering
  - Multilayer, corrugated structures
Nonwoven Fabrics in Bioseparations

Controlled fiber diameters
- Variable surface areas for protein adsorption

Low cost
- Disposables, single use

Surfaces can be modified
- Grafting, surface coatings
- Filtration, ion exchange, affinity ligand attachment

Large porosities
- Low pressure drops

Controlled pore sizes
- Can allow passage of debris, including cells
- Large molecule capture

Interconnected pores
- Convective mass transfer
- Negligible diffusion limitations

ProMetic Biosciences
Fiber Diameter and Surface Area for Adsorption

**Intrinsic Surface Area**

\[ \text{Area/Mass} = \frac{4}{d_f \rho_p} \]

<table>
<thead>
<tr>
<th>Fiber Diameter</th>
<th>Area/Mass (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 µm</td>
<td>0.57</td>
</tr>
<tr>
<td>1 µm</td>
<td>2.9</td>
</tr>
<tr>
<td>100 nm</td>
<td>29</td>
</tr>
<tr>
<td>50 nm</td>
<td>57</td>
</tr>
</tbody>
</table>
Objectives

• Modify hydrophobic nonwoven fabrics (PP, PBT) to make them suitable for bioseparation applications
  • Graft uniform, conformal coating of functional hydrophilic polymer on fiber surfaces
  • Hydrophilization to reduce nonspecific protein adsorption
  • Conversion of modified surface with DEA for anion exchange applications

• Measure protein adsorption, transport properties of hydrophilized membrane, and an anion exchange membrane
## Nonwoven Properties

<table>
<thead>
<tr>
<th>Property</th>
<th>PP</th>
<th>PBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber diameter (µm)</td>
<td>8 ± 4</td>
<td>3 ± 0.7</td>
</tr>
<tr>
<td>Pore diameter (µm)</td>
<td>11 ± 4</td>
<td>8 ± 3</td>
</tr>
<tr>
<td>Mat thickness (µm)</td>
<td>250</td>
<td>300</td>
</tr>
<tr>
<td>Basis weight (g/m²)</td>
<td>40</td>
<td>52</td>
</tr>
<tr>
<td>Apparent density (g/cm³)</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td>Porosity</td>
<td>0.80</td>
<td>0.85</td>
</tr>
<tr>
<td>Specific area (m²/g)</td>
<td>0.5</td>
<td>0.8</td>
</tr>
</tbody>
</table>
UV-Induced GMA Grafts on PBT Nonwovens

Blank PBT

Grafting Solution
GMA in 1-butanol with BP (benzophenone) as initiator, UV wavelength (365 nm)

Epoxy groups

Grafted Polymer Brush

PBT-GMA

UV Polymerization

UV

Polymer Brush
GMA Grafting Controlled by Reaction Time

Degree of GMA grafting (%)

\[ \frac{W_1 - W_0}{W_0} \times 100 \]

BP:GMA 2:100
Grafting Confirmed by FTIR Analysis

(a) blank PBT; (b) PBT exposed to UV without GMA and BP in 1-butanol (c) PBT after GMA grafting; (d) polyGMA
Uniform, Conformal GMA Grafts

Grafting conditions:
2.0 M GMA - 15min

BP:GMA = 0.5:100
BP:GMA = 1:100
BP:GMA = 2:100

Blank

PBT
Average Grafted Layer Thickness

For a 12% weight change

\[
\frac{(R + \delta)^2 - R^2}{R^2} \approx 0.12
\]

\[
\frac{\delta}{R} \approx \frac{0.12}{2} = 0.06
\]

\[R = 3 \mu m; \delta = 180nm = 1800Å\]

Consistent with SEM (100 – 200 nm)
Conversion of GMA to Hydroxyl Groups

1. GMA grafting with UV-induced polymerization
2. DEG (diethylene glycol) attachment or diol attachment
# Contact Angle and Hydrostatic Penetration Pressure

<table>
<thead>
<tr>
<th>12 wt% GMA + hydrophilization</th>
<th>Blank PBT</th>
<th>After modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact angle (degree)</td>
<td>108 degrees</td>
<td>0</td>
</tr>
<tr>
<td>Hydrostatic penetration pressure (Pa)</td>
<td>2450 Pa</td>
<td>0</td>
</tr>
<tr>
<td>Mean flow pore size (μm)</td>
<td>~ 8</td>
<td>~ 8</td>
</tr>
</tbody>
</table>

Hydrostatic penetration pressure measured with DI water,
Mean flow pore size measured with Capillary Flow Porometer, PMI
Flow Permeability Unchanged by Grafting

Darcy’s law:

\[ u_0 = k \cdot \frac{\Delta p}{L \cdot \mu} \]

- \( u_0 \): superficial velocity
- \( k \): permeability
- \( \Delta p \): pressure drop
- \( L \): column length
- \( \mu \): dynamic viscosity

\[ \frac{\Delta P}{L \cdot \mu} \text{ (cm}^{-1}\text{·s}^{-1}) \]

\( k = 2 \times 10^{-8} \text{ cm}^2 \)

Bed density: 0.47 g/ml
Bed porosity = 0.67
Effect of Grafting on Non-specific Adsorption of BSA

BSA conc. 0.5 mg/ml
PBS buffer at pH 7.4

\[ \% \text{Grafting} = \frac{W_1 - W_0}{W_0} \]
Non-specific Binding of BSA Reduced by Diol or DEG Attachment

12% GMA weight gain
Monolayer adsorption on fiber surface
Adsorption much less than a monolayer
Reduction of Non-specific Binding of Other Proteins

12% GMA weight gain
protein conc. 2 mg/ml
Anion Exchange Membrane from GMA-grafted PBT

Step 1. Uniform GMA grafts
Step 2. Conversion of epoxy to DEA (diethylamine)
Step 3. Conversion of the remaining epoxy to diol
Reaction Time for Conversion of Epoxy to DEA

50% (v/v) DEA solution at 30°C

DEA conversion =

\[
\frac{(W_2 - W_1)}{73.14} \div \frac{(W_1 - W_0)}{142.15}
\]

- \(W_0\) = weight of blank PBT
- \(W_1\) = weight of PBT-GMA
- \(W_2\) = weight of PBT-GMA-DEA
- 73.14 = DEA molecular weight
- 142.15 = GMA molecular weight
Confirmation of DEA Attachment by FTIR

- Epoxy peaks: 907 cm\(^{-1}\), 847 cm\(^{-1}\)
- Esters: 1149 cm\(^{-1}\)
- No epoxy peaks
- OH group: 3400 cm\(^{-1}\)
- Tertiary amine: 1065 cm\(^{-1}\)
Nitrogen Detection by XPS

Blank PBT

PBT-GMA

PBT-GMA-DEA

N was found only after DEA treatment
Pore Structure Maintained after DEA Conversion

- Blank PBT
- PBT-GMA
- PBT-GMA-DEA
BSA Adsorption Increases with DEA Density

DEA density = \frac{(W_2 - W_1)}{73.14} (\text{mol/g})

BSA binding capacity (mg/g)

85 x Monolayer adsorption

Static measurements
BSA ~ 1 mg/ml
Enhanced BSA Adsorption to DEA Membrane by Electrostatic Interactions

BSA concentration = 0.78 mg/ml
Binding buffer: 20 mM Tris buffer at pH 7.0
Adsorption of Other Proteins

![Bar chart showing static protein binding ability (mg/g membrane) for different proteins at pH 11. The proteins are transferrin, BSA, β-casein, β-lactoglobulin, ovalbumin, and lysozyme. The y-axis represents the binding ability ranging from 0 to 350 mg/ml, and the x-axis lists the proteins. The chart indicates that transferrin has the highest binding ability, followed by β-casein, β-lactoglobulin, ovalbumin, BSA, and lysozyme. The pH is noted as 11.]
Electrostatic Adsorption Is Reversible

- **Binding conditions:**
  20 mM Tris-HCl at pH 7.0

- **Elution conditions:**
  20 mM Tris +1 M NaCl at pH 7.0

**Graph: BSA Binding Capacity (mg/g)**

- Column 1
- Column 2
- Column 3
- Column 4

**Legend:**
- Binding-washing-elution
- same membrane
BSA Binding Isotherm

BSA binding capacity

\[
= \frac{(C_0 - C_1) \times V}{W}
\]

270 mg/g
Large Binding Capacity Enabled by Polyelectrolyte Brush Structure

- Intrinsic surface area = 0.8 m$^2$/g
- Protein adsorbed/area = 312 mg/m$^2$
- Approximately 85 monolayers (3.6 mg/m$^2$)
- Thickness of GMA layer ~ 1000 - 2000 Å
- Thickness of expanded GMA-DEA layer ~ 2000 – 4000 Å
  - Dimensions of BSA: 14 nm x 4 nm x 4 nm
  - Number of BSA molecules/layer = 50-100

High DEA density in grafted layer (5.8 mmoles/cm$^3$)
BSA Binding under Flow Conditions

5 mg/ml BSA, 5ml
Different flow rates
Elution after 60 min,
1 M NaCl

Elution

<table>
<thead>
<tr>
<th>Flow Rate (ml/min)</th>
<th>Velocity (cm/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>6.4</td>
</tr>
<tr>
<td>0.2</td>
<td>12.8</td>
</tr>
<tr>
<td>0.3</td>
<td>19.2</td>
</tr>
<tr>
<td>0.4</td>
<td>25.6</td>
</tr>
<tr>
<td>0.5</td>
<td>32</td>
</tr>
</tbody>
</table>
Equilibrium Adsorption under Flow Conditions – No Effect of Fluid Velocity

30% lower binding capacity under flow conditions

170 mg/g or 80 mg/ml
Direct Application – Prion Removal

• Grafted membrane was further derivatized to add a ligand specific for prions
• Ligand density was at full capacity
• Membranes were tested in 30-layer stacks in tandem
• Membranes were challenged with an excess of infectivity to observe breakthrough, and better evaluate column capacity
Binding of Infectious Prion from Infectious HaBH

• Tested with 0.01% Infectious hamster brain homogenate in plasma.
• Total load of about $10^7$ ID
• Derivatized membrane containing a ligand bound infectious prion protein
• Four columns of membrane removed all detectable infectivity
• About 0.4 grams of membrane were used (0.1 g membrane/column)
Conclusions (1)

• Nonwoven fabrics have unique properties that make them interesting alternative materials for bioseparations
• pGMA grafting and hydrophilization highly effective in reducing nonspecific adsorption
• Equilibrium DEA anion exchange binding capacity of 270 mg/g or 127 mg/ml under static conditions (12% weight gain)
Conclusions (2)

• DEA anion exchange equilibrium binding capacities of 170 mg/g or 80 mg/ml under flow conditions (12% weight gain)
  • Flow by-passing in very short column lengths
• Low pressure drops, high permeability, independent of grafting level (below 12%)
• After further derivatization (ligand attachment) grafted membranes were able to bind infectious prions ($10^7$ ID in 0.4 g membrane)
Future Work

• Hydrodynamic dispersion analysis – Membrane packing
• Yield, purity, product concentration, contaminant removal in real applications
• Effects of grafting level, fiber diameter, pore size distribution