

## 1. Background

Risks associated with transmission of pathogenic infectivity through blood transfusions remain high despite efforts in the development of sensitive assays to screen potential donors. These risks can be curtailed through the removal of the pathogenic contaminants via adsorption to affinity ligands immobilized on chromatographic resins.

However, the small interstitial hydraulic radii of typical packed-bed columns can preclude separations from sources containing large particles, such as red blood cells. One approach to circumvent this limitation is to entrap chromatographic resin particles in a nonwoven membrane support, allowing larger particles to pass unimpeded while maintaining high surface contact with the chromatographic resin.

## 2. Aims

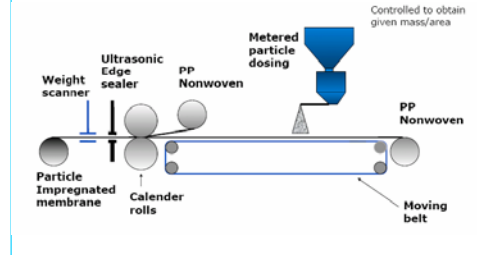
In this study, a hybrid particle-nonwoven membrane medium is investigated in which a polymeric chromatographic resin is entrapped between layers of a nonwoven polypropylene membrane. The membrane-supported resin offers the advantage of increased interstitial pore diameter and porosity while maintaining suitable flow properties.

## 3. Methods

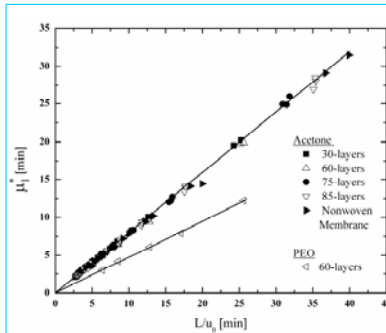
Particle-impregnated membranes were produced by spreading an even layer of a polymeric resin between two layers of nonwoven polypropylene membrane. The material was bonded together by calendaring, and cut into circles, which were then stacked together and packed into a column. Challenge solutions (protein solutions and human red blood cell concentrate (RBC)) were passed through the columns at different flow rates. Interstitial porosity was determined using first absolute moment analysis using pulse experiments.

## 4. PIM Production

Particle-impregnated membrane (PIM) is produced as below, resulting in a layer of resin between two sheets of nonwoven membrane



## 5. Porosity



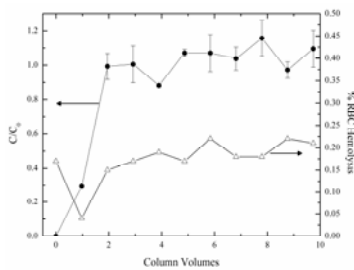
Pulse injections of acetone to column packed with 30-85 layers of PIM and pulse injection of PEO to a column packed with 60 layers of PIM

Interstitial bed porosities and permeability coefficients measured for a column packed with PIM, resin, and nonwoven membrane:

| Material          | Porosity | $\kappa$ [cm <sup>2</sup> ] |
|-------------------|----------|-----------------------------|
| Nonwoven membrane | 0.75     | $2.9 \times 10^{-7}$        |
| PIM               | 0.49     | $2.1 \times 10^{-8}$        |
| Resin             | 0.32     | $8.79 \times 10^{-9}$       |

**Particle-Impregnated Membrane has higher interstitial bed porosity than resin packed bed**

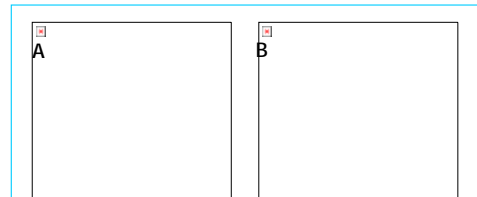
## 6. Hemolysis



Breakthrough (solid symbols) and resulting hemolysis (open symbols) of RBC fed to a PIM packed column at a linear flow velocity of 0.014 cm/s

**Particle-Impregnated Membrane does not damage red blood cells**

## 7. Binding of BSA to PIM and Packed Columns



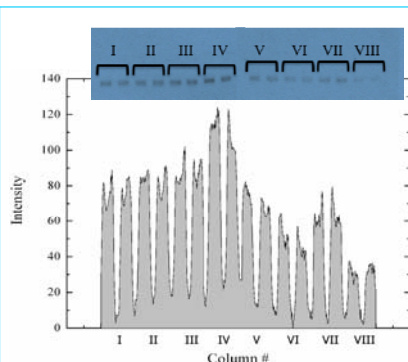
Experimental (symbols) vs. GR model simulated (lines) breakthrough curves at flow rates of 5.0, 10.0, and 20.0 ml/min from a (A) PIM column and (B) resin packed column with BSA concentration of 1.0 mg/ml

$$\frac{\partial c_b}{\partial \tau} - \frac{1}{Pe} \frac{\partial^2 c_b}{\partial z^2} + \frac{\partial c_b}{\partial z} + \xi(c_b - c_{p|r=1}) = 0$$

$$(1 - \epsilon_p) \frac{\partial q}{\partial \tau} + \epsilon_p \frac{\partial c_p}{\partial \tau} - \eta \left( \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial c_p}{\partial r} \right) \right) = 0$$

**Dynamic adsorption of BSA is similar in Particle-Impregnated Membrane and packed bed**

## 8. Prion Removal



- ❖ Eight PIM columns in series challenged with 0.05% normal hamster brain homogenate spiked into human leucoreduced red blood cell concentrate
- ❖ First 4 columns were saturated
- ❖ Spike used is 5 to 6 orders of magnitude larger than expected contamination in blood products
- ❖ Western blot shows bound fraction

**Particle-Impregnated Membrane removes prion protein from red blood cell concentrate**