# Characterization of Gel-Filled Membranes for Plasma Protein Fractionation

*David R. Latulippe, Department of Chemical Engineering, McMaster University*
*Carlos D. M. Filipe, Department of Chemical Engineering, McMaster University*
*Raja Ghosh, Department of Chemical Engineering, McMaster University*
*Ron F. Childs, Department of Chemistry, McMaster University*
*Alicja M. Mika, Department of Chemistry, McMaster University*

Prepared for Presentation at AIChE 2004 Annual Meeting, November 8th 2004, Advances in Bioseparations: Membrane Separations

Unpublished


---

## Introduction

Protein fractionation (i.e. protein-protein separation) is a challenging application for ultrafiltration (UF). However, the advantages over competing separation technologies (high product throughput and ease of scale-up) make UF an attractive process for such applications. This study examines the use of Gel-filled (GF) membranes for the separation of human plasma proteins human serum albumin (HSA) and human immunoglobulin G (HIgG). GF membranes consist of microporous/macroporous supports filled with cross-linked, charged, water swollen polyelectrolyte gels. Stirred cell UF experiments were conducted to characterize the GF membranes in terms of their permeate flux and plasma protein rejection properties. These membranes could be operated at significantly higher permeate fluxes than commercially available UF membranes while achieving equivalent protein-protein separation efficiencies. Thus the product throughputs obtained were significantly higher.

GF membranes are environmentally responsive to solution conditions due to charge repulsion effects within the immobilized gel. The conformation of the gel within the support is predominantly determined by the weak polyelectrolyte degree of dissociation which is dependent on the pH and salt concentration of the bulk solution. The changes in gel conformation modify the effective pore size of the membrane and produce a reversible 'valve-effect' as seen by changes in pure water flux. Mika et al. (2002) incorporated a cross-linked poly(4-vinylpyridine) gel in the pores of a polyethylene microporous membrane that exhibited an order of magnitude reversible decrease in pure water flux with a decrease in pH.

GF membranes are significantly different from commercial UF membranes. The majority of protein fractionation studies have used commercial membranes while little research has been done with GF membranes for similar applications. Gudeman & Peppas (1995) examined the effect of solution pH on the diffusion coefficients of a range of solutes through polyacrylic acid (PAA) and poly vinyl alcohol (PVA) hydrogel membranes. Masawaki et al. (1993) investigated the effect of solution pH on protein rejection (trypsin, protein A, & IgG) by vinylpyridine-acrylonitrile copolymer pH responsive UF membranes. They found that higher rejection values were obtained at pH 4 compared to pH 10 however IgG rejection was consistently high (>90%) and insensitive to pH changes.

## Experimental

Experiments were conducted using an AKTA Prime liquid chromatography system (Amersham Biosciences) in combination with a stirred cell (Ghosh & Cui, 2000) with a working volume of 19.6 mL. The cell was fitted with a GF membrane of carboxylic acid functionality with an effective diameter of 24 mm. Permeate flux and plasma protein experiments were conducted at constant flux rates of $9.21 \times 10^{-5}$ m/s and $3.68 \times 10^{-5}$ m/s respectively. The transmembrane pressure was monitored continuously to evaluate the GF membrane response and as an indicator of membrane fouling in protein experiments. The permeate stream was continuously monitored using a flow through UV monitor (280 nm), pH electrode, and conductivity cell from which the outputs were continuously logged into a computer for storage and analysis.

Plasma protein experiments were carried out in the pulsed sample injection ultrafiltration mode (Ghosh & Cui, 2000). Solution buffer was fed continuously to the system at a constant flow rate. Protein solutions were injected into the system in the form of a pulse using a 500 µL sample loop. Permeate protein concentrations were determined using UV absorbance data and calibration curves of known protein concentrations. The apparent sieving coefficient was calculated using the permeate protein concentration data and the method of Ghosh & Cui (2000).

## Results

The response of the GF membrane to changes in solution pH and salt concentration was determined. The required transmembrane pressures at constant flux are presented in Figure 1 as a function of the solution pH for fixed sodium chloride concentrations.

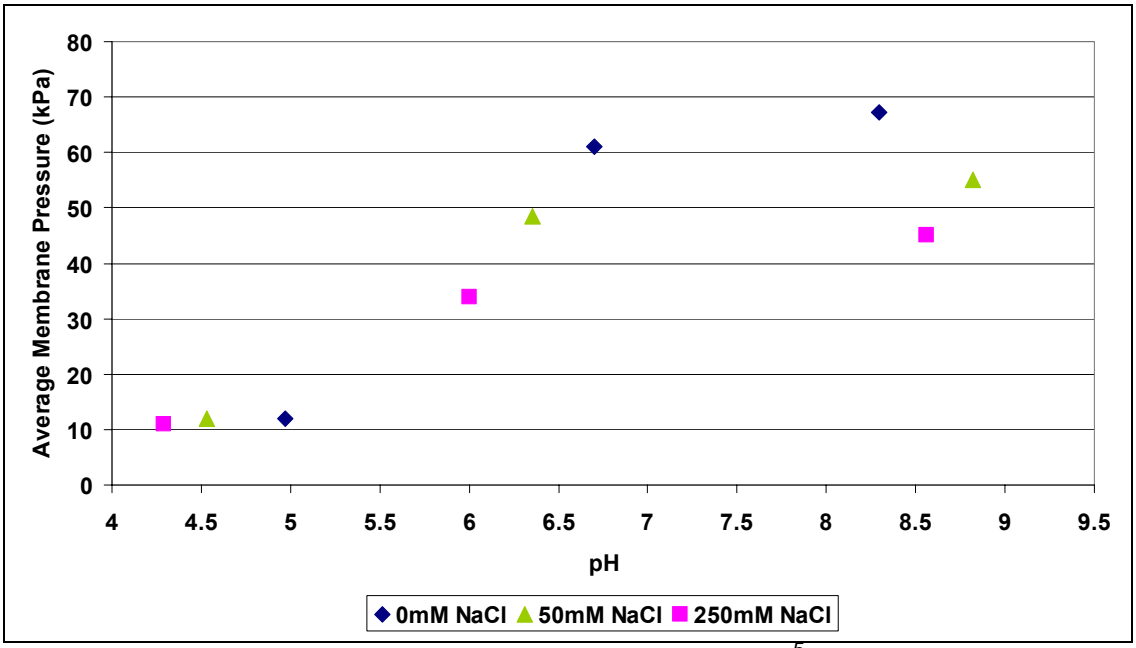


Figure 1: Gel-filled transmembrane pressure at flux = $9.21 \times 10^{-5}$ m/s for fixed NaCl concentrations.

As the solution pH increases, the required transmembrane pressure increases due to changes in the gel conformation within the support. Potentiometric titration results gave an apparent pKa value of 6.5 for the polyelectrolyte gel. At high pH the polyelectrolyte gel is negatively charged and adopts a 'swollen' conformation due to electrostatic repulsions. This expanded conformation results in a smaller effective pore size. Increasing salt concentration shields the electrostatic repulsions and allows the gel to relax within the support thereby reducing the required transmembrane pressure.
As the solution pH decreases below the polyelectrolyte gel pKa, the majority of the membrane species are non-ionized such that charge repulsion effects are insignificant. At this condition, the gel adopts a compact conformation resulting in lower transmembrane pressures and no salt concentration effects due to the neutral-charge of the gel.

Human plasma protein UF experiments were conducted at a range of solution pH and salt concentration conditions. Single protein experiments were carried out to determine apparent sieving coefficients and potential fractionation conditions. The results for HSA and HIgG are presented in Tables 1 and 2 respectively.

**Table 1:** HSA Apparent Sieving Coefficient at different pH and salt concentrations for GF membrane (flux = $9.21 \times 10^{-5}$ m/s)

| pH | NaCl Concentration (mM) | Maximum Apparent Sieving Coefficient ($C_p/C_b$) |
|---|---|---|
| 4.9 | 10 | 0.23 |
| 5.1 | 250 | 0.75 |
| 7.1 | 0 | 0.89 |
| 8.5 | 10 | 1.00 |
| 8.5 | 250 | 1.06 |

**Table 2:** HIgG Apparent Sieving Coefficient at different pH and salt concentrations for GF membrane (flux = $9.21 \times 10^{-5}$ m/s)

| pH | NaCl Concentration (mM) | Maximum Apparent Sieving Coefficient ($C_p/C_b$) |
|---|---|---|
| 4.5 | 10 | 0.02 |
| 4.6 | 250 | 0.29 |
| 6.7 | 0 | 0.14 |
| 8.5 | 10 | 0.95 |
| 8.5 | 250 | 0.97 |

From the results in Tables 1 and 2, it is apparent that the sieving coefficients of both proteins respond in a similar manner to changes in the solution pH and salt concentration. At fixed pH, the sieving coefficient increases with increasing salt concentration due to screening of the electrostatic interactions between the protein and membrane.

At fixed salt concentrations, the sieving coefficient increases with increasing solution pH. This trend is different than the conventional UF fractionation strategy that generalizes the optimum pH value is at the isoelectric point of one protein which is allowed to permeate the membrane while the other protein is retained due to charge repulsion. This deviation suggests a unique protein rejection mechanism as both proteins are preferentially transmitted through

the GF membrane at high pH conditions where the protein is charged and the GF membrane exhibits a smaller effective pore size due to charge repulsion effects within the polyelectrolyte gel. Based on these experiments, conditions likely to be favourable for HSA-HIgG fractionation could potentially be identified.

The performance of the GF membranes was significantly different from commercial UF membranes in three important respects:

1. The GF membrane results showed a considerably greater change in apparent sieving coefficients of the proteins with change in pH, i.e. the separation was significantly more pH responsive.

2. For the same transmembrane pressure, hydrodynamic conditions and feed protein concentrations, these GF membranes gave permeate fluxes higher by a factor of five than those obtained with conventional UF membranes.

3. The amount of membrane fouling was significantly lower. Even when operated at a constant permeate flux value of $3.68 \times 10^{-5}$ m/s there was no evidence of fouling as would have been indicated by an increasing transmembrane pressure.

The results obtained would suggest a unique protein rejection mechanism and improved operating conditions are obtainable when using GF membranes compared to commercial UF membranes in protein fractionation applications.

References

Ghosh, R. & Z. Cui, Analysis of protein transport and polarization through membranes using pulsed sample injection technique, J. Mem. Sci. 175 (2000) 75-84

Gudeman, L. & N. Peppas, pH-sensitive membranes from poly(vinyl alcohol)/poly(acrylic acid) interpenetrating networks, J. Mem. Sci. 107 (1995) 239-248

Masawaki, T., H. Sato, M. Taya, & S. Tone, Molecular weight cut-off characteristics of pH-responsive ultrafiltration membranes against macromolecule solution, Kagaku Kogaku Ronbunshu 19 (1993) 620-625

Mika, A., R. F. Childs, J. M. Dickson, Salt separation and hydrodynamic permeability of a porous membrane filled with pH-sensitive gel, J. Mem. Sci. 206 (2002) 19-30