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ULTRATHIN MEMBRANE FOULING MECHANISM TRANSITIONS IN DEAD-END FILTRATION OF PROTEIN

Karl J. P. Smith
Department of Biomedical Engineering
University of Rochester, 252 Elmwood Ave Rochester, NY 14627
(412) 915-3591, karl_smith@urmc.rochester.edu

Joshua Winans[1], **James McGrath**[2]
[1]: Process Development Engineer, Enovix
[2]: Department of Biomedical Engineering
University of Rochester, 252 Elmwood Ave Rochester, NY 14627

ABSTRACT

Ultrathin membranes will likely see great utility in future membrane-based separations, but key aspects of the performance of these membranes, especially when they are used to filter protein, remain poorly understood. In this work we perform protein filtrations using new nanoporous silicon nitride (NPN) membranes. Several concentrations of protein are filtered using dead end filtration in a benchtop centrifuge, and we track fouling based on the amount of filtrate passed over time. A modification of the classic fouling model that includes the effects of using a centrifuge and allow for the visualization of a transition between pore constriction and cake filtration demonstrate that for a range of protein concentrations, cake filtration supersedes pore constriction after about 30 seconds at 690 *g*.

INTRODUCTION

Typical laboratory ultrafiltration steps, including sample concentration, buffer exchanges, and the separation of colloids of different sizes are often performed in benchtop centrifuges. Samples containing protein [**?**, **?**], nanoparticles [**?**, **?**, **?**], and pathogens [**?**, **?**, **?**] have been prepared this way. In industrial applications, stirred cells or tangential flow devices are used to sweep stuck particles off the surface and prevent fouling, but these systems are typically more complex and expensive, and may result in dilution of small-volume samples. The downside of benchtop centrifuge separations is that without a stirring action of some kind colloids that are rejected by the membrane can build up behind and clog membranes quickly.

Nanoporous Nitride (NPN) membranes are a commercially available nanoporous material only 50 nm thick [**?**], which is 100-1000x thinner than the ultrafiltration membranes currently used for such separations (Figure 1a). These membranes have pore sizes that are tuneable during manufacture from 20-80 nm (Figure 1b) and have porosities as high as 35% [**?**]. Because of the thinness of the material and the high pore density, these membranes have extremely high hydraulic permeabilities (40 mL/(cm^2 min bar)) [**?**] and minimal internal surface area. Simple pore geometry and tight pore size distributions mean that the membranes have ~~size cutoff of~~ 10 nm [**?**]. If we can take advantage of these unique material properties, we expect a significant breakthrough in separations science, enabling low-pressure, high-flux, high-resolution separations, which will in turn dramatically drive down the cost of therapeutic biotech products.

We have previously shown [**?**] that NPN membranes used to filter polystyrene nanoparticles exhibit fouling behavior that is well characterized by the classic fouling model summarized by Zydney [**?**]. In particular, we found that for nanoparticles larger than the pores, fouling occurred primarily through the formation of a thick deposit of particles, called a cake, on the backside of the membrane. This cake filtration mode was observed across a range of particle concentrations. For particles smaller than the pores, we still saw cake filtration, but only for particles at a concentration above 10^{12} part/mL. Below 10^{12} part/mL the data was best fit by particles adsorbing to the interior of the pores, a filtration mode called pore constriction. For particles at 10^{12} part/mL, we found that a clear transition between the two fouling modes was visible, indicating that in the beginning of the separation the membrane

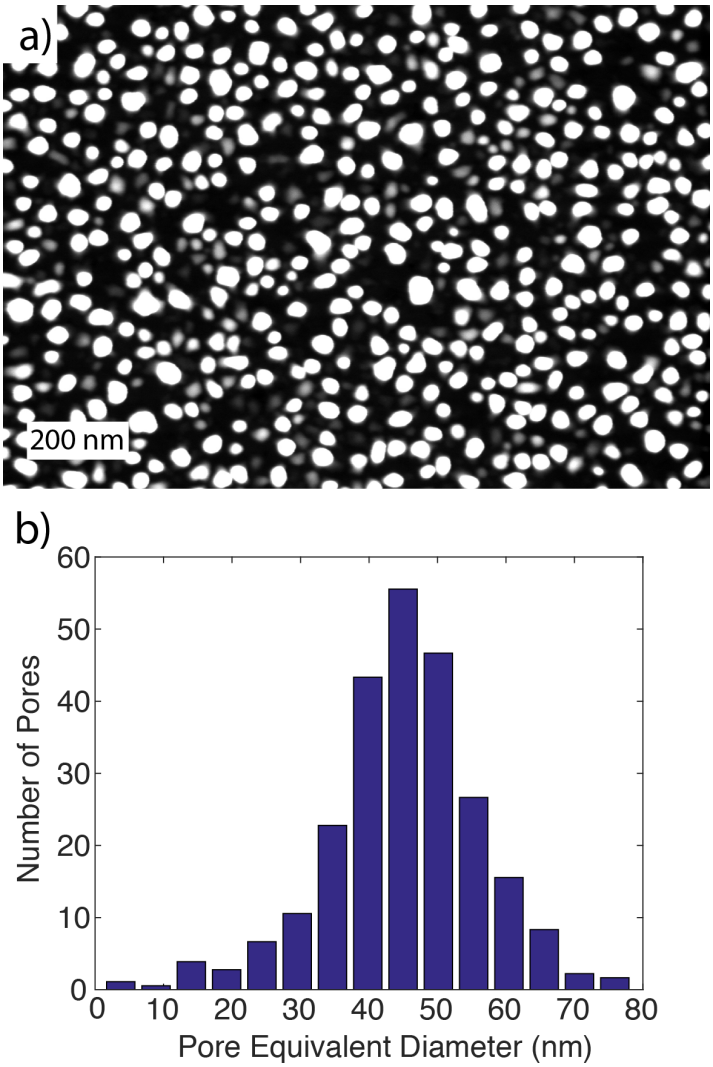

FIGURE 1: a) A TEM image of the NPN membrane. Light grey areas are partial pores that do not make it through the membrane and which do not contribute to flow. b) A pore size histogram generated using Figure 1a and custom image processing software.

clogs by pore clogging, but by the end a cake is formed and cake filtration behavior dominates the process. This is not unique to NPN, instead a transition from blockage models to cake filtration is observed after some time in variety of membrane types [**?**, **?**].

In this work, we extend this analysis of fouling modes from the filtering of polystyrene beads to the filtering of protein solutions. Using a version of the Zydney protein fouling model that incorporates the head-height-dependent pressure in a centrifuge, we found that proteins exhibit this transition behavior across a range of concentrations, suggesting that practical laboratory separations of protein from nanoparticles that avoids the high hydraulic resistance associated with cake filtration will need to be carefully optimized.

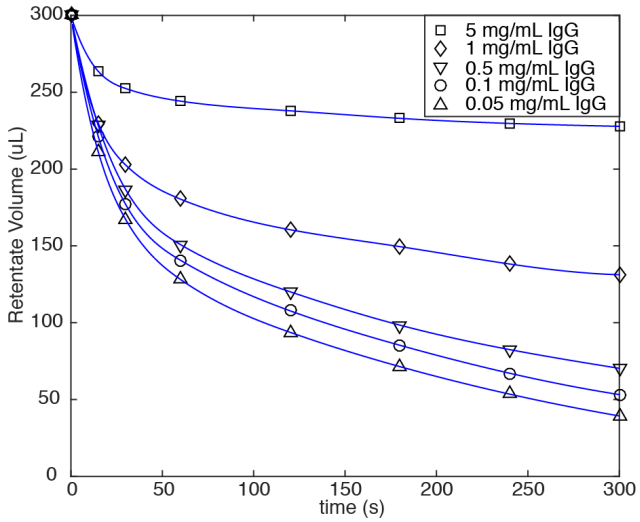

FIGURE 2: Total retentate volume as a function of time for various concentrations of IgG. All five protein solutions start with high fluxes that decline significantly around 30 seconds and afterwards hold relatively constant.

EXPERIMENTAL

NANOMEMBRANE FABRICATION

Complete details regarding membrane fabrication can be found in DesOrmeaux et. Al. [**?**]. Three different layers - SixNy, amorphous silicon (a-Si), and silicon dioxide (SiO2) - are deposited onto a 300 μm thick double-side-polished Si wafer. This wafer is rapid thermal annealed, which crystalizes the a-Si and forms a pnc-Si film. Afterwards, the SiO2 is removed and reactive ion etching is used to transfer the pores into the Si_xN_y film. XeF_2 is used to removed the pnc-Si mask. Surface features such as the freestanding membrane areas are defined with standard lithography processes on the back of the wafer and etched using ethylene diamine pyrocatechol. A transmission electron microscope (TEM) is used to image the membranes (Figure 1a) and a pore size histogram is generated using image processing software developed in our lab (Figure 1b).

SEPARATIONS

All separations were performed using a custom plastic 'SepCon' housing designed to hold the membranes [**?**]. The SepCons were first loaded with 10 uL of 1x phosphate

buffered saline (pbs) buffer on both sides of the membrane as a means to bypass surface tension effects that can prevent fluid flow. A Beckman Coulter Microfuge 18 centrifuge equipped with a F241.5P angled rotor and spun at 3,000 rpm (690 *g*) was used to drive the separations. Filtrate volumes were determined by comparing the dry weight of the 1.5 mL conical centrifuge tube to the weight after spinning.

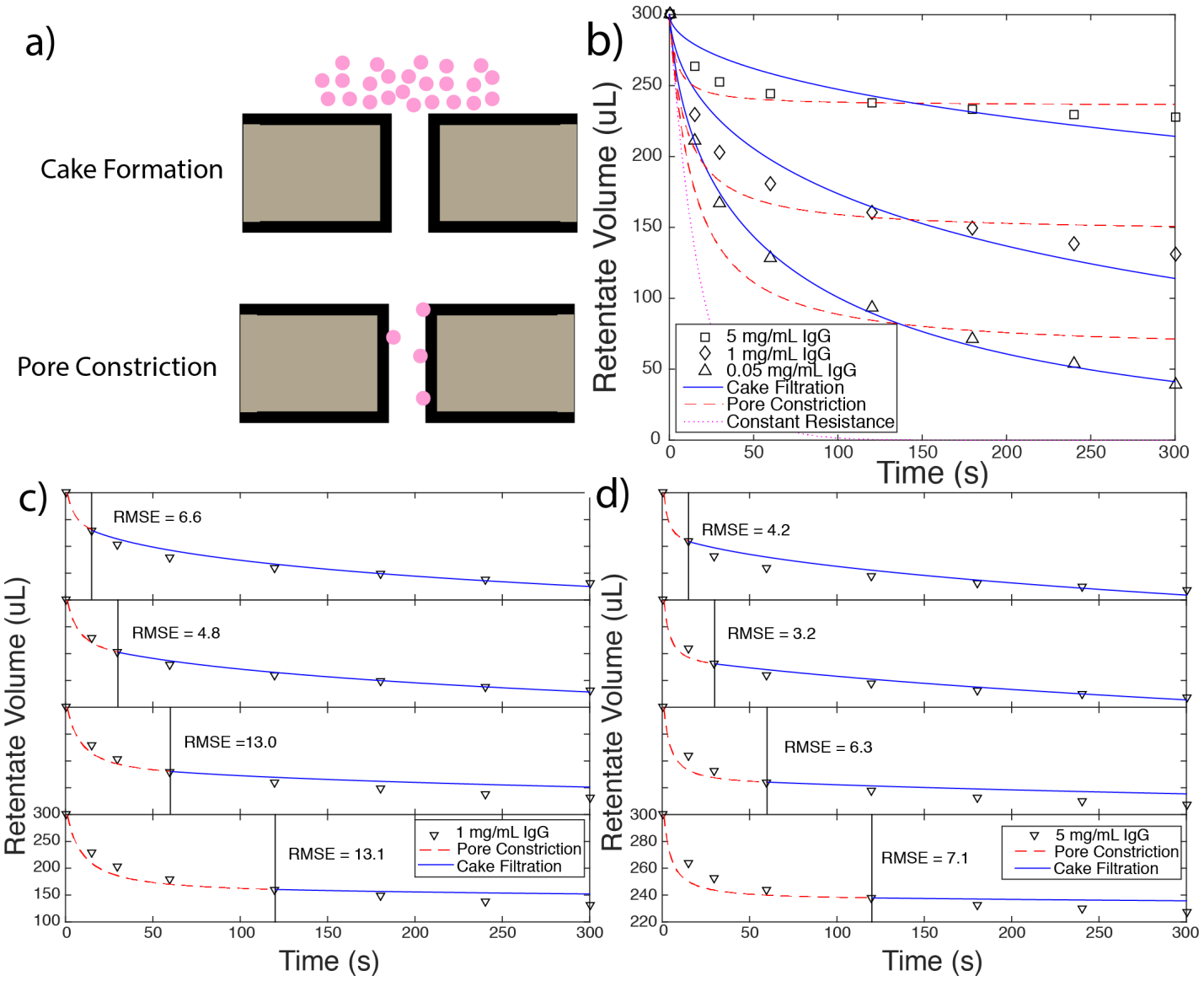

FIGURE 3: a) The two fouling mechanisms we model. Pore constriction results when colloids bind to the interior of the membrane pores, while cake filtration happens when colloids just above the membrane reach a sufficient concentration that they begin to act like a second membrane. b) Pore constriction underestimates retentate volume at early times, and overestimates volumes at later times, while cake filtration does the opposite. c) A hybrid model, that uses pore constriction for early times, and, after an instantaneous transition, a cake filtration for late times, has a local minimum in RMSE when the transition point is fixed at 30 s for a 5 mg/mL IgG solution. d) a similar analysis on a 1 mg/mL IgG solution also reveals a transition between the two models occuring at 30 seconds. Data for 0.5, 0.1, and 0.05 mg/mL IgG show the same local minimum in RMSE and are contained in the supplement.

MATERIALS

UltraPure Distilled Water was used for dilutions, and was purchased from Invitrogen life technologies. The pbs buffer solution was diluted from a 10x, pH 7.2 solution purchased from Gibco by life technologies. A combination of both unlabeled and fluorescently labeled IgG were used in this work. The unlabeled IgG was from human serum, reagent grade, 95% (SDS-PAGE), essentially salt-free, lyophilized powder, purchased from Sigma-Aldrich. The fluorescently labeled goat anti-rabbit IgG (H+L) Antibody, Fluorescein (FITC) Conjugate, affinity purified, was purchased from Novex life technologies. The final solution was composed of 2.5 % fluorescently labeled IgG.

RESULTS

To simulate a practical laboratory separation, we passed protein through NPN membranes using a desktop centrifuge. 300 μL aliquots of IgG at concentrations from 0.5 mg/mL to 5 mg/mL were loaded into Sepcon vials that had been pre-wetted. All samples were spun at 690 *g*. Filtration volumes were collected throughout the separation and are plotted against time in Figure 2. Note that the high flux in the beginning of the separations is at least partially due to the fact that the fluid head height behind the membrane is greatest at the start of a separation, and gradually decreases, which causes the effective pressure at the membrane to decrease. At around 30 seconds, though, the flux of all samples drops and remains relatively constant for the remainder of the separation, indicating that the hydraulic resistance of the membrane has changed due to fouling. To determine which mode of fouling (pore constriction or cake filtration, demonstrated in Figure 3a) was driving this change in hydraulic resistance, we adapted the classic model of fouling behavior [**?**] to include the variable pressure of the centrifuge [**?**]. In Figure 3b, these models are plotted against the data from Figure 2. Note that neither of the fits matches the data well, and that pore constriction overestimates filtrate volumes in the first half of the dataset and underestimates in the latter half, and that cake filtration does the opposite. We assumed that what we were seeing was the transition between these two modes, and so in Figure 3c and 3d, we fit the date to a hybrid model that switches between pore constriction and cake filtration ~~at several discrete time points~~. We calculated the RMSE for each of these curves and found for all five concentrations of IgG (analysis for 0.05 mg/mL, 0.1 mg/mL, and 0.5mg/mL are contained in the supplement) there was a local minimum in the RMSE when the transition point was fixed at 30 seconds.

DISCUSSION

Ultrafiltration membranes used as dead-end filtration devices have been shown to be useful for several types of nanoparticle separations. The need for efficient size-based separations of these types of particles is real, but the greater utility of these membranes will come from separations involving proteins. Proteins are prone to cake layer formation, and tend to aggregate and stick to surfaces even in the absence of flow. Understanding the fouling behavior, and in particular the transitions between the fouling modes, is a critical first step in enabling NPN to be used for efficient benchtop protein separations.

ACKNOWLEDGEMENTS

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