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Shear separation: a promising method for protein fractionation

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Abstract— Shear Separation is a method of separating macromolecules suspended in a fluid by inducing hydrodynamic forces that depend on size. The forces are produced in the laminar sub-layer of a turbulent shear boundary layer adjacent to a porous membrane. The suspended particles are lifted away from the porous membrane, against the drag of the permeate flow, by the viscous part of the shear stress. We have found that the transmission rate of a protein through the membrane depends on the shear rate, the permeate rate, the protein size, and the protein shape, even when the pore size of the membrane is substantially larger than the largest of the proteins. As a result, this is a size and shape dependent separation of the macromolecules. The membrane functions as a surface upon which an extremely high shear laminar sub-layer at the membrane surface may be generated. The effect has been demonstrated on the bench scale with clean mixtures of cytochrome C and bovine serum albumin. The separation can be observed with membranes rated approximately 300 kg·mol^{-1} molecular weight cut off (MWCO) or less. Using this technique, milk serum containing BSA and smaller proteins without fats, caseins or immunoglobulins has been produced from skimmed milk. In addition, the milk serum has been fractionated with transmission fractions of 18% for BSA, 33% for β-lactoglobulin, and 62% for α-lactalbumin.

shear separation / fractionation / dairy / ultra-filtration / protein

1. INTRODUCTION

The proteins contained in milk are well recognized as valuable additives in food and beverages, as well as having medical uses [1]. Also, use of transgenic animals to produce milk containing human proteins may become an economical method of manufacturing proteins with direct medical and pharmaceutical benefit. The principal methods available for isolation of proteins from biological fluids at commercial scales are chromatography and filtration. The high concentrations of fats and caseins in milk

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make the use of both of these methods difficult.

In this paper we present preliminary evidence that proteins can be isolated from milk by shear separation in which size dependent hydrodynamic forces lift the proteins away from the membrane surface while permeate drag (also size dependent) tends to move the proteins towards the membrane. In the Pall LAB6 DMF^{TM} device sufficiently large shear forces can be generated to use this method for extracting proteins from a variety of fluids. We believe that this is the first time this approach to separation of proteins from milk has been presented. The forces on the suspended proteins are produced in a shear flow adjacent to a porous membrane. If the membrane is sufficiently smooth, as is the case with UF membranes, and the permeate flow rate is not too large, the turbulent boundary layer, generated on the membrane by the shear flow, contains a viscous sublayer at the membrane surface [4]. Proteins immersed in this sub-layer experience a lift force [2, 3] which counteracts the drag of the permeate flow. These are hydrodynamic forces that are applied to any body suspended in a shear flow near a wall. The balance of lift and drag forces depends on the size and shape of the body.

2. MATERIALS AND METHODS

2.1. The LAB6 DMF^{TM} device, membranes, and chromatography column

The experimental investigation reported in this paper was accomplished using a LAB6 DMF^{TM} filtration device. This is an experimental device (available in limited quantities by arrangement with Pall Corporation) in which extremely high shear rates are produced on the upstream surface of a single sheet of membrane by a rotor disc spinning in a plane parallel to the filter surface and separated from that surface by approximately 1 cm. The surface area of the membrane installed in the device is 0.0137 m^2. Feed flow is introduced into the gap between the rotor and the membrane. The portion that passes through the membrane is conducted to the permeate outlet while the portion that does not pass through the membrane is conducted to the retentate outlet. Although, like a cross-flow device, the LAB6 has a retentate flow, the gap between the rotor and the filter membrane is too large (approximately 0.6 cm) for this flow to provide any significant reduction of fouling or gel layer formation. Instead, this is accomplished by the high shear generated by the spinning rotor.

In the experiments described below, the retentate flow rate was set between 0.5 and 1.0 $L \cdot min^{-1}$ to prevent the heat generated by the spinning disk from raising the temperature of the process fluid more than 4 °C. To remove this heat and maintain a steady state in the first two experiments, the retentate line passed through an ice bath before being recycled to the feed tank. In the third experiment (milk separation) the retentate was not recycled. At a retentate flow rate of 1.0 $L \cdot min^{-1}$, the average time the fluid was subjected to the high shear of the DMF^{TM} device was approximately 12 s. Transmembrane pressure (TMP) was less than 2 bar in all cases.

In the experiment described in Section 2.3, a Pall 0.2 μm nylon membrane was used as a prefilter. In all other cases, the membrane was either a Pall Filtron Omega 100K PESO membrane, with 100 $kg \cdot mol^{-1}$ MWCO, or a Pall Filtron Omega 300K PESO membrane, with 300 $kg \cdot mol^{-1}$ MWCO. Replications were not run in any of these experiments. The high performance liquid chromatography (HPLC) results described below were all determined using Zorbax SE-250 size exclusion column obtained from Dionex Corporation. In the first two experiments, the HPLC work was done at a contract laboratory. Accuracy was reported as linearity of UV absorption with protein concentration (correlation coefficient of at least 0.997 in all cases.) For the last experiment (milk), the

HPLC work was done in house. Five calibration samples of each protein were tested at approximately 0.1 $g \cdot L^{-1}$. The standard deviation of concentration to peak area was 14% of the mean value for Bovine Serum Albumin (BSA) and 6% for α-lactalbumin and β-lactoglobulin.

2.2. Bovine Serum Albumin and Cytochrome C

In this experiment, the feed to the DMF^{TM} device was a phosphate buffered (pH 7.4) aqueous suspension of 0.228 $g \cdot L^{-1}$ Bovine Serum Albumin (BSA), approximately 67 $kg \cdot mol^{-1}$ molecular weight, and 0.0949 $g \cdot L^{-1}$ Cytochrome C (12 $kg \cdot mol^{-1}$). The surfactant Tween 20 was added to the suspension at a concentration of 0.1% by volume to reduce agglomeration of the BSA. Tests were conducted at several different spin rates and permeate fluxes, using a single 100 $kg \cdot mol^{-1}$ MWCO membrane. The membrane was neither replaced nor cleaned during the experiment. Transmission results were quantified by HPLC. The temperature of the solution was maintained between 8 °C and 12 °C throughout the experiment.

A permeate sample was collected first at zero RPM with TMP at 0.16 bar. The spin rate was then changed to 1 000 RPM (shear rates of 1.67×10^4 sec^{-1} and 5.0×10^4 sec^{-1} at the inner and outer radii, respectively) and permeate samples were collected at monotonically increasing permeate fluxes. A permeate volume at least equal to the hold up volume of the DMF^{TM} device was collected at each permeate flux before the sample for analysis was collected. Once all samples had been collected at 1 000 RPM, the spin rate was returned to zero and another permeate sample was collected at approximately 0.16 bar TMP. This procedure was repeated at 2 000 RPM (5.8×10^4 sec^{-1} and 1.8×10^5 sec^{-1} at the inner and outer radii, respectively) and 3 000 RPM (1.2×10^5 sec^{-1} and 3.6×10^5 sec^{-1} at the inner and outer radii, respectively).

2.3. Cysteinylated BSA and Gamma Globulin

Two batches of 4 L of phosphate buffered saline solution (pH 7.4) were prepared. S-cysteinylated BSA (Sigma A0161) was added to one batch at a concentration of 0.2 $g \cdot L^{-1}$ of solution and the batch was filtered through an Omega 300 $kg \cdot mol^{-1}$ MWCO membrane to remove any large impurities. HPLC analysis was performed on samples collected before and after filtration.

Human gamma globulin (IgG) of 150 $kg \cdot mol^{-1}$ molecular weight, was added to the second batch of saline solution at a concentration of 0.2 $g \cdot L^{-1}$. To avoid agglomeration, 0.4% by volume of the surfactant Tween 20 was added. This solution was filtered through a 0.2 μm nylon membrane. The two solutions were mixed together and the mixture was filtered using a DMF^{TM} device and Omega 300 $kg \cdot mol^{-1}$ MWCO membrane. The series of tests were as described above for the BSA and cytochrome C solution except that 600 RPM (6.6×10^3 sec^{-1} and 2.0×10^4 sec^{-1} at the inner and outer radii, respectively) was added to the tested spin rates.

2.4. Milk fractionation

In this experiment, non-homogenized, non-pasteurized, skim milk (pH 6.7) was obtained from a local dairy and filtered using an Omega 300 $kg \cdot mol^{-1}$ MWCO membrane. The milk was held at 4 °C and allowed to rise to room temperature (approximately 20 °C) before filtration. The maximum hold time from collection at the dairy to filtration was three days. The objective was to try to produce a milk serum as permeate by retaining fats and casein at one spin rate, and then produce a BSA enriched retentate in a second filtration of the serum. Several short runs were made at 1 500 RPM (3.5×10^4 sec^{-1} and 1.0×10^5 sec^{-1} at the inner and outer radii, respectively) and 3 000 RPM

spin rates and several different permeate fluxes. At each permeate flux, a 120 ml sample was collected and sent to a contract laboratory for analysis. Total solids was measured by oven drying. Casein and serum proteins were measured by the Kjeldahl method, and fat was measured by the Mojounier method. Measurement accuracy was not reported.

From the results of these runs, 1 500 RPM and 45 $L \cdot h^{-1} \cdot m^{-2}$ were chosen as reasonable process conditions. At these conditions, 10 L of milk serum were produced during a continuous filtration run of approximately 5 h duration using two DMF^{TM} devices in parallel. The resulting permeate was filtered again through a second Omega 300K membrane at 2 000 RPM and 88 $L \cdot h^{-1} \cdot m^{-2}$ permeate flux. Feed and permeate samples collected at 21, 109, and 203 min into the run were analyzed by HPLC.

3. RESULTS AND DISCUSSION

3.1. BSA and Cytochrome C

The results of the experiment, summarized as transmission percentages, are shown in Figure 1. At zero spin rate, BSA (67 $kg \cdot mol^{-1}$) or Cytochrome C (12 $kg \cdot mol^{-1}$) transmissions are both greater than 85%, as expected with a membrane of 100 $kg \cdot mol^{-1}$ MWCO. Clearly, at all nonzero spin rates, transmission of Cytochrome C is significantly greater than transmission of BSA. Also, the transmission of both proteins increases with permeate flux, which is equivalent to increasing drag force, and decreases with spin rate which is equivalent to increasing lift force. This experiment clearly demonstrates separation of proteins of two distinct sizes by a balance of shear lift and permeate drag forces alone.

Because the transmission at zero spin rate remained large throughout the experiment, membrane fouling or concentration polarization do not appear to play a role in the reduction in transmission.

3.2. Cysteinylated BSA and Gamma Globulin

UV absorption and HPLC analysis performed on samples of the IgG solution, before and after the addition of Tween 20, showed that the addition of Tween 20

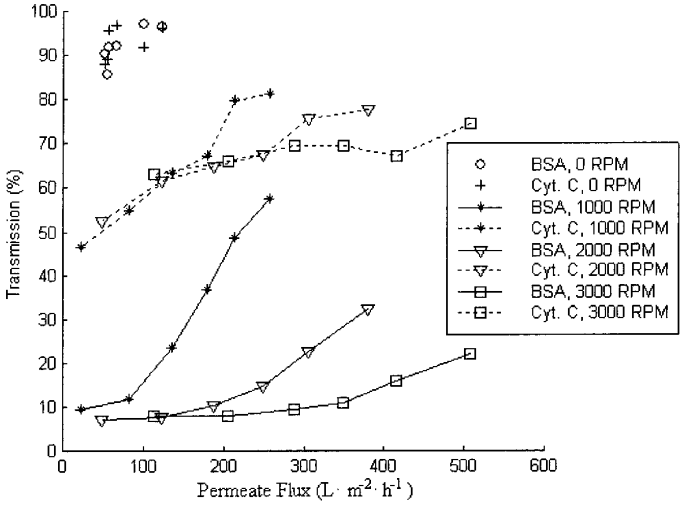

Figure 1. BSA and cytochrome C transmission percentages (100 times the ratios of permeate to feed concentrations) versus permeate flux, using a LAB6 DMF^{TM} device and Pall Filtron Omega 100KD VR ultrafiltration membrane.

increased the measured concentration of IgG by a factor of approximately 20%. Subsequent HPLC analysis indicated that addition of Tween 20 to a solution of Cysteinylated BSA shows no effect on the measured concentration of BSA. We suspect that the surfactant either forms micelles of approximately 150 kg·mol^{-1} molecular weight, or attaches to the IgG causing an increase in UV absorption of the IgG/surfactant complex. IgG concentrations reported here are adjusted for the effect of the Tween 20. No adjustment is made to the measured concentration of BSA.

Transmission ratio percentages of IgG and BSA at all the tested permeate fluxes and rotor spin rates are given in Figure 2. At zero spin rate, the measured transmission ratio is approximately 50% for both proteins. It is likely that at zero spin rate, the IgG forms a fouling layer that reduces transmission of both proteins.

At all nonzero spin rates tested, the transmission fraction of both proteins depends strongly on permeate flux as would be expected if transmission depends on the balance of hydrodynamic lift and drag. However, although IgG is a little more than twice the molecular weight of BSA, it behaves as if it experiences less lift, or more drag than the smaller BSA molecule. One possible explanation of this is that BSA is a globular protein while IgG has a three-lobed plate structure. It is likely that the preferred orientation of the IgG in the shear flow is such that the plane of the plates is parallel to the membrane surface. This presents the maximum surface area to the permeate flow, thus maximizing the drag, and possibly minimizing the lift.

Finally, we note that at 600 and 1 000 RPM spin rates, the transmission percentages of both proteins show a distinct maximum. One possible explanation of this observation is that for permeate fluxes higher than that at which the transmission maximum is achieved, the drag force from the permeate flow is so much larger than the lift force from the shear flow that the proteins are carried towards the membrane much as though the lift did not have any effect. This would result in a fouling layer on the membrane and a reduction in transmission of both proteins. Further experiments at higher permeate fluxes would be useful to verify this explanation.

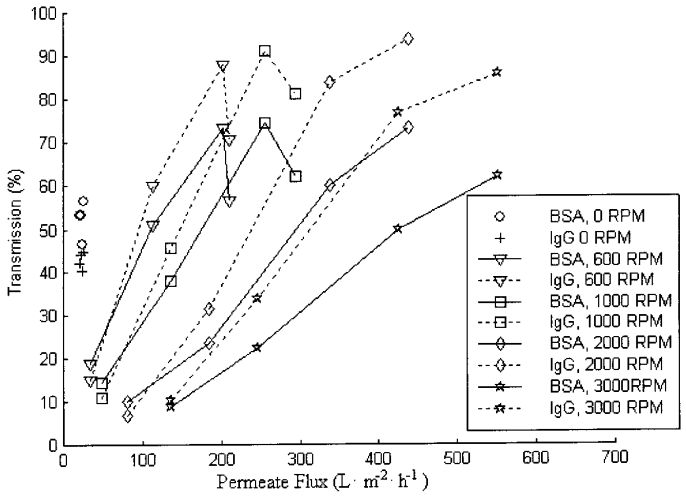

Figure 2. Cysteinylated BSA and human IgG transmission percentages (100 times the ratios of permeate to feed concentrations) vs. permeate flux using a LAB6 DMF^{TM} device and Pall Filtron Omega 300k ultrafiltration membrane.

Table I. Average transmission percentages (100 times the ratios of permeate to feed concentrations) of total solids, fats, caseins and serum proteins for skim milk through a Pall Filtron Omega 300K PESO membrane (300 kg·mol^{-1} MWCO) in a Pall LAB6 DMF^{TM} filtration device at various spin rates and permeate fluxes.

Spin rate (RPM)	Permeate flux (L·h^{-1}·m^{-2})	Total solids	Fat	Casein	Serum protein
1500	18	61	0	0.4	28
1500	43	63	6	1.2	29
1500	61	65	0	0.8	32
3000	61	63	0	1.9	14
3000	88	61	0	1.3	15
3000	105	64	0	1.3	16

3.3. Milk Fractionation

The results of filtering non-pasteurized and non-homogenized skim milk in the LAB6 are summarized in Table I. Because the time required to take each 120 ml sample was several minutes, these results must be considered as averages rather than instantaneous. At 1 500 RPM, it was found that TMP increased with time when the permeate flux was held at any value greater than 61 L·h^{-1}·m^{-2}. We take this continual increase in TMP as evidence of membrane fouling. At 3 000 RPM, similar fouling occurred when the permeate flux exceeded 105 L·h^{-1}·m^{-2}. At all lower permeate fluxes, for these spin rates, no fouling was observed. The nonzero transmission of fat at 1 500 RPM and 44 L·h^{-1}·m^{-2} is due to a single measurement, which we assume is an experimental error.

As shown in Table I, increasing the spin rate from 1 500 to 3 000 RPM nearly doubles the permeate flux that can be achieved without fouling. The same change in spin rate also cuts the transmission of serum proteins in half. To allow a large fraction of serum proteins to be transmitted without risking fouling of the membrane, a spin rate of 1 500 RPM and a permeate flux of 45 L·h^{-1}·m^{-2} was chosen to produce a 10 L batch of milk serum, using two LAB6s in parallel. Permeate flow was maintained for more than 4 h with very little evidence of fouling. At the end of the run, the permeate rate was raised to 65 L·h^{-1}·m^{-2}. This resulted in a dramatic increase in the fouling rate.

To separate BSA from the smaller proteins in the milk serum, four liters of serum produced in the test described above were filtered, again using an Omega 300K membrane. In this run, the spin rate was increased to 2 000 RPM to improve the retention of the larger serum proteins and the permeate flux was increased to 88 L·h^{-1}·m^{-2}, in hopes of increasing the transmission of the smaller serum proteins.

The transmission percentages of BSA, β-lactoglobulin (18 kg·mol^{-1}), and α-lactalbumin (14 kg·mol^{-1}) at various times during the run are shown in Table II. As expected, transmission of the smallest protein, α-lactalbumin, was largest and transmission of the largest protein, BSA, was smallest at each time. The transmission at 203 minutes is nearly double that at the earlier times. Further work is needed to determine why this is the case.

4. CONCLUSION

For a variety of solutions, we have found that the transmission rate of proteins through an ultra-filtration membrane in a LAB6

Table II. Transmission percentages (100 times the ratios of permeate to feed concentrations), at various times during the run, of BSA, β-lactoglobulin, and α-lactalbumin for milk serum through a Pall Filtron Omega 300K PESO membrane (300 $kg \cdot mol^{-1}$ MWCO) in a Pall LAB6 DMF^{TM} filtration device at a spin rate of 2000 RPM and permeate flux of 88 $L \cdot h^{-1} \cdot m^{-2}$.

Run time (min)	Transmission Percentage		
	BSA	β-lactoglobulin	α-lactalbumin
21	12	25	57
109	14	24	46
203	27	51	82
Average	18	33	62

depends strongly on the shear rate and the permeate flux. This dependence appears to be mediated by both the size and the shape of the proteins, which allows the transmission characteristics of a membrane to be controlled by the shear rate and permeate flux.

In particular, we have shown that milk serum can be produced by filtration through a 300K membrane and that the serum can be again fractionated with a 300K membrane at a different molecular weight cut off by changing the shear and permeate fluxes used. From these preliminary results, it appears plausible that further development of shear separation equipment and methods could lead to very finely controlled separations of proteins from milk and other biological fluids.

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