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Ultrafiltration of Immune Serum Globulin and Human Serum Albumin: Regression Analysis Studies

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NOTE

Ultrafiltration of Immune Serum Globulin and Human Serum Albumin: Regression Analysis Studies

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Abstract

Experimental values for ultrafiltration fluxes at various bulk protein concentrations and bulk fluid velocities are presented for immune serum globulin (ISG) and human serum albumin (HSA) at neutral pH prepared from human serum plasma by Cohn's Cold Ethanol process. Protein concentration at the membrane surface and the exponent on the bulk fluid velocity are calculated from a concentration polarization model to be 19.14 g/100 ml and 0.67 for ISG; 23.85 g/100 ml and 0.61 for HSA.

Freeze drying is utilized as the standard method for removal of organic solvents from plasma proteins. Recently, ultrafiltration has been investigated (*1–3*) as a practical alternative for final purification and concentration steps. We have experimentally determined the effect of bulk protein concentration and bulk fluid velocity on ultrafiltration flux for human serum albumin (HSA) and immune serum globulin (ISG) prepared by the Cohn Cold Ethanol process (*4*). ISG solutions were prepared from Fr II paste of Cohn's Cold Ethanol (*4*) process. Pastes were dissolved in water-for-injection ($0 \pm 1\,^{\circ}C$) at pH 6.5 ± 0.2 units and Diafiltered against 0.3 *M* glycine for five volume replacements in the Amicon DC 30 Hollow Fiber Unit at $+5\,^{\circ}C$. The retentate was the feed material for

flux measurements in the ultrafiltration experiments. HSA solutions were prepared from Fr V pastes of Cohn's Cold Ethanol process (*4*) by dissolving the paste at pH 6.9 ± 0.2 units in water for injection followed by five volume replacement with sterile distilled water at +5°C in the Amicon DC 30 unit. Sodium chloride was added to the retentate solution to a concentration of 0.85%.

All ultrafiltration experiments were carried out with the Amicon TCF 10 Thin Channel Unit with a PM 10 membrane (nominal molecular weight cut off 10,000 daltons). Effective filtration area was 40 cm^2 and the spiral channel dimension 9.5 × 0.38 × 760 mm. Operating temperature was ambient (+25°C) at an operating pressure of 25 psig.

TABLE 1

Ultrafiltration Flux vs Recirculation Velocity and Bulk Protein Concentration for ISG

Observation no.	U (cm/sec)	C_b (g/100 ml)	J (cc/cm^2 sec)
1	71.56	3.72	2.92×10^{-4}
2	71.56	4.58	2.50×10^{-4}
3	71.56	5.73	2.50×10^{-4}
4	71.56	6.44	2.21×10^{-4}
5	71.56	7.68	1.79×10^{-4}
6	71.56	8.79	1.58×10^{-4}
7	105.26	6.19	2.71×10^{-4}
8	105.26	6.82	2.50×10^{-4}
9	105.26	8.16	1.96×10^{-4}
10	105.26	9.81	1.58×10^{-4}
11	105.26	11.79	1.04×10^{-4}
12	147.28	4.66	3.75×10^{-4}
13	147.28	5.68	3.25×10^{-4}
14	147.28	7.17	2.29×10^{-4}
15	147.28	8.53	1.88×10^{-4}
16	147.28	9.52	1.79×10^{-4}
17	179.13	5.75	4.08×10^{-4}
18	179.13	6.97	3.54×10^{-4}
19	179.13	8.16	3.13×10^{-4}
20	179.13	9.75	2.50×10^{-4}
21	290.40	6.81	4.79×10^{-4}
22	290.40	8.68	3.96×10^{-4}
23	290.40	11.71	2.21×10^{-4}
24	290.40	12.65	1.88×10^{-4}
25	290.40	13.84	1.46×10^{-4}
26	290.40	14.84	1.04×10^{-4}

Experimental results of ultrafiltration fluxes for ISG and HSA at various fluid velocities and bulk protein concentrations are shown in Tables 1 and 2. Under the concentration polarization model (pressure-independent flux), assuming neglibible protein leakage through the membrane, the solvent flux, J, is related to the bulk protein concentration, C_b, by the following relation (*5*):

$$J = K \ln (C_w/C_b) \tag{1}$$

where C_w = concentration at the membrane surface and K = mass transfer coefficient.

TABLE 2

Ultrafiltration Flux vs Recirculation Velocity and Bulk Protein Concentration for HSA

Observation no.	U (cm/sec)	C_b (g/100 ml)	J (cc/cm^2 sec)
1	33.70	3.83	6.25×10^{-4}
2	33.70	4.65	5.63×10^{-4}
3	33.70	6.05	5.00×10^{-4}
4	33.70	7.83	4.17×10^{-4}
5	33.70	9.43	3.54×10^{-4}
6	49.86	3.25	8.25×10^{-4}
7	49.86	4.66	7.29×10^{-4}
8	49.86	6.75	5.00×10^{-4}
9	49.86	8.66	4.58×10^{-4}
10	49.86	10.63	2.92×10^{-4}
11	49.86	13.41	2.92×10^{-4}
12	65.56	3.34	9.38×10^{-4}
13	65.56	4.09	8.33×10^{-4}
14	65.56	7.12	6.46×10^{-4}
15	65.56	9.25	4.79×10^{-4}
16	65.56	11.23	3.33×10^{-4}
17	65.56	13.68	3.75×10^{-4}
18	108.96	1.80	12.50×10^{-4}
19	108.96	5.01	8.67×10^{-4}
20	108.96	7.50	6.67×10^{-4}
21	108.96	9.20	4.79×10^{-4}
22	108.96	11.82	2.42×10^{-4}
23	290.40	1.80	29.38×10^{-4}
24	290.40	5.00	19.33×10^{-4}
25	290.40	7.50	14.46×10^{-4}
26	290.40	9.20	10.00×10^{-4}
27	290.40	11.80	5.83×10^{-4}

Mass transfer coefficient K is dependent upon diffusivity of the protein molecule and the boundary layer thickness over which the solute concentration gradient exists. For the purpose of this analysis a constant diffusivity is assumed for the range of protein concentrations studied (1.80 to 14.84 g/100 ml) and the following model is assumed for nonlinear regression analysis:

$$J = AU^B \ln (C_w/C_b) \tag{2}$$

where U = bulk fluid velocity, and A and B are constants.

Kozinski and Lightfoot (*6*) in their analysis calculated the correction factor for the variation of diffusivity and viscosity with protein concentration and found this factor not to depart a great deal from unity. Anderson and Rauh have shown (*7*) the mutual diffusion coefficient of bovine serum albumin to be dependent upon protein concentration only below a solution ionic strength of 10^{-1} M. Since the total salts concentration in these experiments were greater than 10^{-1} M, the constant diffusion coefficient is most likely a reasonable assumption for the regression analysis to follow. Also, C_w, the concentration at the membrane surface, is assumed to be constant which is the osmotic equivalent of the applied pressure.

Regression analysis results are shown in Tables 3 and 4. Table 3 shows parameters B and C_w are quite accurate while A is not. To judge the accuracy of the models we calculated the mean absolute error (MAE) or the average deviation of the predicted values from the observed values with the sign discarded. Both models appear acceptable and the fit is slightly better for ISG. Errors for both models are approximately normally distributed according to the Kolmogorov-Smirnov test at 5% significance level (0.10 and 0.15 for ISG and HSA, respectively, with critical level at 0.27).

The exponent on the bulk fluid velocity is 0.61 for HSA and 0.67 for ISG. For the laminar flow region the Lévêque solution gives a value of

TABLE 3

Accuracy of the Proposed Models

	HSA	ISG
Mean absolute error	92.0×10^{-6}	18.0×10^{-6}
Mean absolute error as % of the mean of J	12.0	7.3
Standard deviation of mean absolute error	80.0×10^{-6}	14.9×10^{-6}

TABLE 3

Parameter Estimation by Nonlinear Regression

	HSA			ISG		
Parameter	Value	Standard deviation	SD as % of parameter value	Value	Standard deviation	SD as % of parameter value
A	3.5×10^{-5}	0.758×10^{-5}	21.7	1×10^{-5}	0.209×10^{-5}	20.9
B	0.613	0.039	6.4	0.6711	0.0434	6.5
C_w	23.854	2.278	9.5	19.137	0.798	4.2

0.33 for the exponent (*8*) and for the turbulent flow region the Dittus-Boelter solution (*9*) predicts an exponent of 0.80. Although the operating Reynold's number for all the recirculation rates was <500, the model predicts an exponent larger than the theoretically predicted value for laminar flow situations. Porter (*10*) calculated an exponent of 0.52 for albumin in the laminar flow region. In general, deviations from theoretical predictions have been explained by tubular pinch effects (*11*). Protein concentration at the wall, C_w, is predicted to be 23.85 g/100 ml for HSA and 19.14 g/100 ml for ISG. Ng et al. (*1*) had previously calculated this value to lie between 19.11 and 21.84 g/100 ml for HSA. It is interesting to note that C_w for HSA (MW = 67,000) is greater than that for ISG (MW = 160,000). This surface concentration is generally regarded as the osmotic pressure equivalent for the protein. Although HSA concentration is about 55% of total plasma proteins, it contributes to >80% of plasma colloidal osmotic pressure due to its lower molecular weight compared to the other plasma proteins. At neutral pH, HSA has a high net negative charge which also results in increased osmotic pressure due to Donnan equilibrium. ISG has a considerably higher molecular weight and lower net charge compared to HSA, which results in decreased osmotic pressure for ISG solutions. The difference in C_w values predicted by the models are thus consistent with physical properties of the individual protein species.

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