Model of Osmotic Pressure for High Concentrated Binary Protein Solutions

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The high concentration of rejected solutes, known as concentration polarization, is inevitable in multicomponent protein ultrafiltration. These rejected proteins at the membrane surface exhibit very high osmotic pressures. Surprisingly however, little research has been done to quantify the contribution of the osmotic pressure to the concentrations of the various species to further our understanding of these highly concentrated multicomponent systems.

Recently, we have shown that hydration and ion binding could account for the nonidealities for single, highly concentrated, protein solutions of moderate ionic strengths (Yousef et al., 1998a,b). In these cases, the osmotic pressure can be represented as

$$\pi \approx \frac{RT}{\bar{V}_1}\ln\left\{\frac{\left(N_I^{\mathrm{II}}+(1-\nu_{12}-\nu_{23})N_2^{\mathrm{II}}+N_3^{\mathrm{II}}\right)N_1^{\mathrm{I}}}{\left(N_1^{\mathrm{II}}-\nu_{12}N_2^{\mathrm{II}}\right)N^{\mathrm{I}}}\right\} \quad (1)$$

where, the subscripts 1–3 represent the solvent, proteins, and ion species, respectively. N^j is the initial total mols of solvent in compartment j, ν_{ij} is the net number of mols of solvent component i that are interacting with protein j, and N_i^j is the initial total mols of solvent species i in compartment j. This model suggested that the solvent-solute contributions are substantially more significant in describing osmotic pressure for concentrated protein solutions than that assumed by virial expansion models based on McMillan-Mayer (1945) theory. This research investigates the potential for the free-solvent model to represent the osmotic pressure for a binary protein solution at moderate ionic strength.

Theory

Sum of individual osmotic pressure contributions model

For relatively low concentrations, a solution with mixed solutes can be approximated as ideal and the total osmotic pressure can be written as (Tombs and Peacocke, 1974)

$$\pi = \sum_i \pi_i \quad (2)$$

where π_i is the osmotic pressure contribution due to species i. However, at high protein concentrations found in the polarization boundary layer, this approximation is not valid.

Virial expansion model

For multicomponent systems, the osmotic pressure can be related to the protein mass fraction by (Casassa and Eisenberg, 1964)

$$\pi = \frac{RT}{\bar{V}_1}w_s\left[\frac{1}{M_n}+A_s w_s+\ldots\right] \quad (3)$$

where $w_s=\sum_j w_j$ is the total concentration of proteins in the solutions, M_n is the number average molecular weight, and the virial coefficient A_s is given by

$$A_s = \sum_J \sum_K \alpha_{JK} y_J y_K \quad (4)$$

where $y_J = w_J/w_s$ the weight fraction of component J and α_{JK} is a function of the species activity coefficients, molecular weights, and interaction.

A common modification for the virial expansion used to consider the interaction of the proteins is written as

$$\pi = \pi_2 + \pi_4 + RTB_{24}w_2 w_4 \quad (5)$$

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The subscripts 2 and 4 here refer to protein 2 and protein 4, respectively. Subsequently, π_2 and π_4 are the osmotic pressure of the two proteins, respectively, and B_{24} is the virial coefficient that represents the protein-protein interactions, usually referred to as the mixed virial coefficient. For dilute solutions, the osmotic pressure of BSA and ovalbumin can be represented in terms of the virial equation truncated after the second virial equation, hence

$$\frac{\pi}{RT} = \frac{1}{M_2} w_2 + B_{22} w_2^2 + \frac{1}{M_4} w_4 + B_{44} w_4^2 + B_{24} w_2 w_4 \quad (6)$$

where M_2 and M_4 are the molecular weights of the two respective proteins. The parameters B_{22} and B_{44} are the second virial coefficients for the two respective proteins obtained from the single protein information. For a fixed ratio between the proteins concentrations in solution, we can define $\beta = w_2/w_4$. If total protein concentration is defined as $w_t = w_2 + w_4$, then, in terms of β and w_t, $w_2 = \beta w_t/(1+\beta)$ and $w_4 = w_t/(1+\beta)$. Equation 6 becomes (McCartly and Adams, 1987)

$$\frac{\pi}{RTw_t} = \frac{1}{M_n} + B_* w_t \quad (7)$$

where M_n is the average molecular weight of the mixture and is defined by

$$M_n = \frac{w_2 + w_4}{w_2/M_2 + w_4/M_4} \quad (8)$$

or in terms of β

$$\frac{1}{M_n} = \frac{\beta}{(1+\beta)M_2} + \frac{1}{(1+\beta)M_4} \quad (9)$$

Here, B is

$$B_* = \frac{1}{(1+\beta)^2}\left[\beta^2 B_{22} + B_{44} + \beta B_{24}\right] \quad (10)$$

If B_{22} and B_{44} are known from single protein data, then B_{24} can be determined from B_*.

Free solvent model

Let a two-compartment osmometer have each side separated by a semi-permeable membrane, and designate I and II as the solvent and solution chambers, respectively. Then, for a solution made up of n distinct species, species 1 is the solvent, species 2 through p are the proteins, and species $p+1$ through n are the remaining diffusible solvent components. The initial total mols of the solution in compartment II are expressed as

$$N^{II} = \sum_{i=1}^{n} N_i^{\mathrm{II}} \quad (11)$$

where i is representative of each of the n species. The final total mols of free-solvent (diffusible salts and water) in compartment II is then

$$N_*^{\mathrm{II}} = N^{\mathrm{II}} - \sum_{\substack{i=1 \\ i \neq 2,\, p}}^{n} \sum_{j=2}^{p} \nu_{ij} N_j^{\mathrm{II}} - \sum_{j=2}^{p} N_j^{\mathrm{II}} \quad (12)$$

where N_j^{II} denotes the mols of protein j in solution and ν_{ij} is the net number of mols of solution component i that is interacting with protein j to make up the new solvent-interacting protein. Then, the mol fraction of free-solvent 1 in solution compartment II is

$$\chi_1^{\mathrm{II}} = \frac{N_1^{\mathrm{II}} - \sum_{j=2}^{p} \nu_{1j} N_j^{\mathrm{II}}}{N_*^{\mathrm{II}} + \sum_{j=2}^{p} N_j^{\mathrm{II}}} \quad (13)$$

and

$$x_1^{\mathrm{I}} = \frac{N_1^{\mathrm{I}}}{N^{\mathrm{I}}} \quad (14)$$

in the solvent compartment I.

The free-solvent model for binary protein solution can be written by taking into account the hydration and the salt binding of each protein. The mol fraction of water in the protein solution is then

$$x_1^{\mathrm{II}} = \frac{N_1^{\mathrm{II}} - \nu_{12} N_2 - \nu_{14} N_4}{N_1^{\mathrm{II}} + (1 - \nu_{12} - \nu_{32}) N_2 + (1 - \nu_{14} - \nu_{34}) N_4 + N_3^{\mathrm{II}}} \quad (15)$$

and that of water in the solvent is

$$x_1^{\mathrm{I}} = \frac{N_1^{\mathrm{I}}}{N_1^{\mathrm{I}} + N_3^{\mathrm{I}}} \quad (16)$$

where the subscripts 1 and 3 refer to water and salt (NaCl), respectively. The subscripts 2 and 4 refer to two proteins, respectively. The subscript definitions were modified to be consistent with the virial expansion species labeling in Eqs. 5–10. The osmotic pressure is then estimated using the van Laar equation

$$\pi = -\frac{RT}{\bar{V}_1} \ln\left(\frac{x_1^{\mathrm{II}}}{x_1^{\mathrm{I}}}\right) \quad (17)$$

Note that the free solvent model in this form does not consider protein-protein interaction. The model is structured so that only hydration and salt binding information from the single component studies for each protein are needed to provide predictions of the binary protein osmotic pressure.

Experimental Studies

The binary protein mixture used to test these models was BSA/ovalbumin. The osmotic pressure was measured in the osmometer which is a modification of the device used by Vilker (1976), and Yousef et al. (1998a). The osmometer consisted of two chambers, a solution chamber and a solvent chamber, separated by a semi-permeable membrane (5000 MWCO, cellulose ester, Molecular/Por, Type C, Spectrum, Laguna Hills, CA). The volume of the solution chamber is approximately 2.5 mL while the volume of the solvent chamber is approximately 25 mL. The solution side of the osmometer is fitted with one of two different strain gauge pressure transducers depending on pressure range (PX726, range 0-40 kPa and 0-170 kPa, Omega, Stamford, CT). The 1 in. flush diaphragm is positioned at the very end of the sensor and forms the rear wall for the solution chamber. The overall distension of the diaphragm under pressure results in a negligible volume change during operation. A digital readout was used. After calibration, the transducers gave an accuracy of measurement between ± 0.41 kPa and ± 1.7 kPa, respectively.

The solution chamber was first charged with the protein solution and the ports were closed. The solvent chamber was then filled with the buffer or saline solution and remained open to atmosphere.

The saline solution was prepared by dissolving 8.766 grams of NaCl (catalog No. S-7653, Sigma, St. Louis, MO) in 1 L of deionized water to produce 0.15 M NaCl solution. The appropriate weighted amounts of ovalbumin powder (albumin, chicken egg, cat. No. A-5503, Sigma) and BSA (bovine serum albumin, catalog No. A-4503, Sigma) were dissolved in a known amount of saline solution. The solution was then stirred and stored in the refrigerator at 4°C for one day or until it completely dissolved. The solution was used within 48 h.

The pH of both the protein solution and the solvent was adjusted before each run to 7.0 by adding aliquots of 0.1 N NaOH or 0.1 N HCl. The protein solution was mixed using a vortex mixer (Vortex Genie, model No. G-560, Fisher Scientific, St. Louis, MO) during the pH adjustment to prevent local denaturation of the protein. The pH measurements were conducted using pH Ag/AgCl combination electrode with a glass body (Model 300474.1, Denver Instruments, Arvada, CO) and pH/Ion/Conductivity meter (Accument Model 50, Fisher Scientific). After each run, the pH of both the protein solution and the solvent were checked and found to be within the error of the pH electrode (± 0.2 pH units).

The amount of acid or base used to adjust the pH was between 10 and 100 μL, and was considered part of the solvent when the concentration of the protein solution was determined.

The concentration of either BSA or ovalbumin in grams per kg solvent was calculated directly from the known amounts of either protein and the solvent required to make up the solution. The total protein concentration was the algebraic sum of both concentrations. The concentration of either protein (g/L solution), w_{BSA} or w_{OVL} was calculated by dividing the weight of each protein wt_i by the volume of solution in liters. The volume of the protein solution (mL) was calculated using the specific volume of the proteins ($\bar{\nu}_{BSA} = 0.736$ cm^3/g and $\bar{\nu}_{OVL} = 0.75$ cm^3/g) and the specific volume of the solvent which was approximated as $\bar{\nu}_{H_2O} = 1.0$ cm^3/g. The volume of solution was then $V = wt_{BSA}\bar{\nu}_{BSA} + wt_{OVL}\bar{\nu}_{OVL} + wt_{H_2O}\bar{\nu}_{H_2O}$. The total protein concentration in grams per liter w_t was taken to the sum of both concentrations.

Table 1. Osmotic Pressure of Equimolar Mixtures of BSA and Ovalbumin in 0.15 M NaCl and pH 7.0 at 25°C and Model Predictions for Osmotic Pressure

Solution Protein Conc. (g/l)			Measured		Predicted π (kPa)	
BSA w_2	Ovalbumin w_4	Total w_t	π (kPa)	$\Sigma_i \pi_i$	Virial Eq.	Free Solvent
30.3	19.9	50.2	3.6	11.4	3.2	7.1
72.5	46.8	119.3	10.5	12.6	10.9	21.8
92.3	60.3	152.6	16.0	12.7	16.0	30.6
125.2	81.3	206.5	26.3	16.4	26.2	48.4
156.3	101.7	258	48.3	25.8	38.2	71.5
179.6	117.1	296.7	60.5	37.0	48.6	94.7
184.9	120.6	305.5	61.9	40.0	51.2	101.0
187.7	122.3	310	79.6	41.6	52.5	104.4
218.8	142.6	361.4	121.0	62.6	68.8	154.0
245.7	160.3	406	182.9	84.3	84.7	223.9
			RMS*	**$\pm$40.3**	**$\pm$36.7**	**$\pm$27.3**

*Root mean square of the error between the measured pressure and that predicted.

Results

The measured osmotic pressures up to a total protein concentration of 400 g/L are tabulated in Table 1 and plotted in Figure 1.

Sum of individual osmotic pressure contributions model

For this model, the osmotic pressures of the single protein solutions of BSA in 0.15 M NaCl and pH 7.4 were taken from

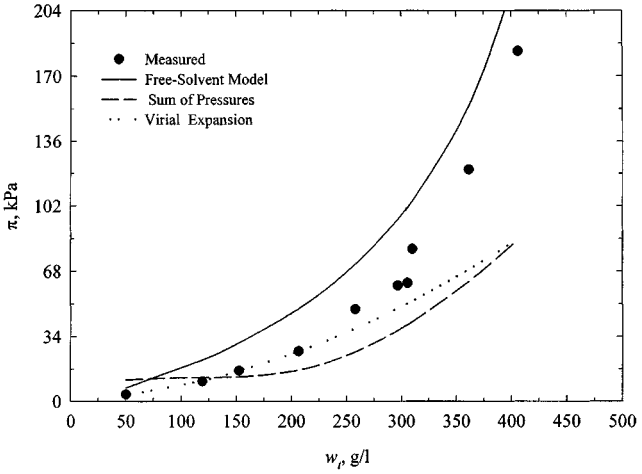

Figure 1. Measured and predicted osmotic pressure of BSA-ovalbumin mixtures.

Measured values, •; predicted models, free-solvent model (____), virial equation (· · · ·), and direct sum of individual osmotic pressures (— — — —). Parameters used in the free-solvent model and virial equations are in Table 2.

Vilker et al. (1981) and the osmotic pressures of ovalbumin were measured in this article.

Virial expansion model

The subscripts 2 and 4 for Eqs. 5–10 refer to BSA and ovalbumin, respectively. In order to obtain B_{24} from the experimental data, the analysis of McCartly and Adams (1987) was followed. In this work, β has a fixed value of 1.534. The second virial coefficient of ovalbumin was determined to be 1.79×10^{-7}L·mol/g^2. The second virial coefficient of BSA was obtained from fitting the relatively dilute data of Vilker et al. (1981) and was determined to be 3.1×10^{-7} L·mol/g^2. Vilker et al. (1981) also calculated the second virial coefficient for BSA, but used DLVO theory. Their model did not reasonably correlate to their data except for results at pH 5.4. Nevertheless, the value of B_{24} by Vilker et al. (1981), (1.34×10^{-7} L·mol/g^2), is in relatively good agreement with the value 3.1×10^{-7}·L·mol/g^2 which was used here. Values of these second virial coefficients, B_{22} and B_{44}, are listed in Table 2.

Fitting the relatively dilute osmotic pressure data for the binary protein mixture ($w_t < 250$ g/L) to Eq. 7 resulted in values of 48,310 ± 5,600 g/mol and $1.47\pm0.17\times10^{-7}$ L·mol/g^2, for M_n and B_*, respectively. This value of M_n is about 15% lower than the calculated value of M_n (57,003 g/mol) using Eq. 9 and the reported molecular weights of BSA and ovalbumin of 69,000 and 45,000 g/mol, respectively, with $\beta = 1.534$. The difference in M_n might be due to the use of an extended range for concentration since, usually, very dilute solutions, w between 10 to 50 g/L or smaller are used to make this calculation. Therefore, the estimate of B_*, and hence B_{24}, the reported values of the molecular weights, and consequently, will be used in Eq. (7), reducing it to requiring only one fitted parameter, B_*.

The resulting value of B_* is then $1.67\pm0.07\times10^{-7}$ L·mol/g^2. There is about 13% difference from the value obtained from letting M_n vary in Eq. 7. The value of B_{24} calculated from Eq. 10 using the values of B_{22} and B_{44} in Table 2 and $\beta = 1.534$ is $1.07\pm0.5\times10^{-7}$ L·mol/g^2. This result is shown in Table 2. Figure 1 shows the predicted osmotic pressure of the protein mixture using Eq. 7 along with the parameters in Table 2.

Free solvent model

For the free solvent model, all of the parameters used in the model were independent of the binary protein osmotic pressure data. For BSA, the salt binding ν_{32} was obtained independently from the data of Scatchard et al. (1946a,b) and it was found to equal to 8.89 mol NaCl/mol BSA. The hydration of BSA ν_{12}, was found to 1.177 g H_2O/g BSA from previous analysis of the single protein data (Yousef et al., 1998b). Similarly, the osmotic pressure of ovalbumin was used to determine the hydration of ovalbumin ν_{14}, which was found to be 0.86 g H_2O/g ovalbumin (Yousef et al., 2002). The salt binding ν_{34} was also determined to be 4.08 mol-NaCl/mol ovalbumin. These values are listed in Table 2. Using these values, the osmotic pressure of the binary protein mixture was calculated using Eqs. 15–17. The free solvent model are shown in Table 1 and plotted in Figure 1.

Discussion

The estimated osmotic pressure using the free solvent model is in relatively good agreement with experimental data and captures both the physical rate of change and magnitude of the osmotic pressure with respect to concentration. It provides a better estimate than the sum of the individual osmotic pressure or the virial expansion models, particularly at high concentrations. This is despite the fact the value of B_{24} used in the virial expansion model is obtained directly from the binary experimental data fitted to Eq. 10, and that the free solvent model used no fitted parameters from the binary protein data.

The predicted osmotic pressure for the free solvent model consistently overestimates the experimental data by about 4 psi. This might be due to the presence of interactions between the hydrated proteins not taken into account in the application of the free-solvent model. A factor containing either ν_{24} or ν_{42} could be introduced in Eq. 13 to account for such interaction. This interaction parameter represents the reduction in the total number of mols of the proteins due to interactions between the protein molecules, such as aggregation. However, further research is required to determine whether this deviation in osmotic pressure is due to protein-protein interaction accounted for by terms containing ν_{24} or ν_{42}, or other factors. This research implies that the physics described by the free solvent model is an appropriate starting point for analyzing the osmotic pressure of concentrated binary protein solutions.

Acknowledgments

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Table 2. Values of the Second Virial Coefficients and Protein Hydration and Salt Binding Parameters

	BSA		Ovalbumin		BSA-Ovalbumin
Second Virial Coeff. L·mol/g^2·	B_{22} 3.1×10^{-7}		B_{44} 1.79×10^{-7}		B_{24} 1.07×10^{-7}
Hydration and salt binding*	ν_{12} 1.177	ν_{32} 8.86	ν_{14} 0.86	ν_{34} 4.08	

* Hydration values (ν_{12} and ν_{14}) are in grams H_2O per gram protein. Salt binding units (ν_{32} and ν_{34}) are in mols NaCl per mol protein.

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Errata

In the article titled "Horizontal Drying Fronts During Solvent Evaporation from Latex Films" by Alexander F. Routh and William B. Russel (pp. 2088-2098, September 1998), the authors require the following errata: We examine the drying of latex films and show how a front of close packed particles propagates through a drying film starting at an edge. Through this packed portion of the film, the the fluid-phase pressure decreases from zero at the position of the particle front, to a minimum value at the edge of the film.

A finite maximum capillary pressure shows the propagation of the front by allowing the solvent to recede into the film, once the maximum negative pressure is reached at the edge of the film. To quantify this, we assume that the receding water front is vertical. For this to be true, the pressure at the position of the water front must equal the maximum capillary pressure. Although, for it to move, the pressure gradient must be negative, implying that the pressure at some point from the front passes through a minimum, with a value lower than the minimum value allowed. This leads to the conclusion that the water front cannot be vertical. Instead, a stagnant region exists at the edge of the film, within which the water level recedes into the film, due to continued evaporation, while the pressure remains at the maximum capillary pressure. This is in contradiction to our previous analysis from Eq. 49 onward. We signify the position of this stagnant region by $\bar{x}_s$, as shown in Figure 1a.

Equation 49 is no longer useful since no horizontal flow exists in the stagnant region, and the height of the water layer merely decreases due to evaporation. Equations 50 and 51 still apply in the packed region where the pressure is no longer at its minimum value. Equation 52 for the pressure distribution in the packed region should read

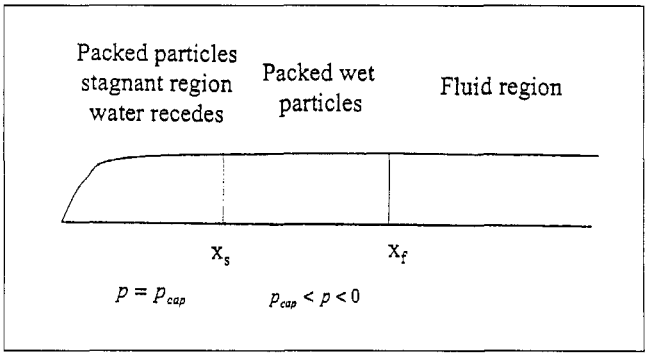

Figure 1. Three regions in drying film.

$$\bar{p} = \bar{p}_{cap} + \frac{1}{(1-\phi_m)} \int_{\bar{x}_s}^{\bar{x}} \frac{\bar{x}' - \bar{x}_s}{\bar{h}} d\bar{x}' \qquad (52)$$

with the position $\bar{x}_s$ determined such that $\bar{p}(\bar{x}_f) = 0$. Equation 53 no longer applies and the expression for the water velocity at the position of the particle front (Eq. 54) should read

$$\bar{u}_x(\bar{x}_f) = -\frac{\bar{x}_f - \bar{x}_s}{\bar{h}(\bar{x}_f)(1-\phi_m)} \qquad (54)$$

With these new equations, we examine the effect of the maximum capillary pressure, as before. Figure 6 is re-plotted, and we see the effect of the error is small. For Figure 7, the water front no longer applies, and the progression of the par-