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Chemical Sciences Division

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Membrane Osmometers and Osmotic Pressures of Aqueous Bovine Serum Albumin (BSA) Solutions

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Abstract

Using osmometers similar to that described by Vilker et al (1981), osmotic pressures of aqueous bovine serum albumin (BSA) solutions were measured at three pHs (4.5, 5.4, 7.4) and at different sodium chloride concentrations (from 1 to 5 M). Experimental data were compared with results calculated from two van-der-Waals-type models using a potential of mean force including hard-sphere and double-layer repulsions, and van der Waals attraction. In both models, the Carnahan-Starling equation of state represents the contribution of the hard-core repulsion to the osmotic pressure. Van-der-Waals attraction and double-layer repulsion are represented by their contributions to the osmotic second virial coefficient in the first model, or in the second, to the random-phase-approximation (RPA). While both models give a semi-quantitative description of the osmotic pressures of BSA solutions at various conditions, the first model represents the data slightly better than the second, using the same number of adjustable parameters. Better representation of experimental data requires a more accurate potential of mean force for protein molecules in salt solution.

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Introduction

Industrial-scale production of biological products often requires a selective, non-denaturing purification method. Salt-induced precipitation has been successfully applied for separating proteins in downstream processing (Belter et al, 1988; Rothstein, 1994). For efficient design of a protein separation process, good understanding of the phase behavior of proteins in aqueous electrolyte solutions is helpful. A reliable molecular thermodynamic model for this purpose relies on an accurate potential of mean force between protein molecules at various solution conditions. However, current understanding is limited to how proteins interact in aqueous electrolyte solutions, not only because of the complicated molecular structure of proteins, but also because of the complex many-body effects of small ions and water molecules.

Interactions between proteins in an aqueous solution can be indirectly measured by light-scattering (low-angle laser light scattering and dynamic light scattering), by osmotic-pressure, and by hydrodynamic methods (diffusion, sedimentation and viscosity) (George, 1993). Compared with the others, the osmotic-pressure method is more popular because of its low cost and broad applicability. Osmotic-pressure data at low protein concentrations can be used to determine the protein molecular weight and the second virial coefficient that is closely related to the potential of mean force. At high protein concentrations, osmotic-pressure data may provide valuable information to test the theoretical models for describing the properties of aqueous protein solutions. Two disadvantages of osmometry are poor accuracy at very low protein concentrations (where low-angle light scattering is better) and slow data acquisition.

This work discusses construction and use of two membrane osmometers similar to those reported by Vilker et al. (1981), and by Amos et al. (1998). Both osmometers were tested by repeating some of Vilker's osmotic-pressure results for aqueous Bovine Serum Albumin (BSA) solutions at low sodium chloride concentration. Osmotic pressures of BSA solutions at high sodium chloride concentrations were measured at three pH (4.5, 5.4 and 7.4). High salt-concentration conditions are of interest because they are used in industrial protein-separation processes. Previously published osmotic-pressure measurements of BSA solutions were restricted to low salt concentrations; the sodium chloride concentrations in this work range from 1.0 to 5.0 M.

The experimental results were compared with those calculated from two van-der-Waals type models for protein solutions. In each model, the potential of mean force between protein molecules in an electrolyte solution contains contributions from hard-core repulsion, dispersion and electrostatic interactions. The last two contributions are approximated using the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory (Hiemenz and Rajagopalan, 1997). The Carnahan-Starling equation of state is used to calculate the contribution of hard-core repulsive interaction to the osmotic pressure (McQuarrie, 1976). In the first model, the DLVO potential is used through its contribution to the osmotic second virial coefficient. The second model uses the random-phase-approximation theory (RPA) as discussed, for example, by Vlachy et al (1993). Both models provide a semi-quantitative description of the osmotic pressure of BSA solutions at low salt concentrations but not at high salt and protein concentrations. At these conditions, when electrostatic shielding is high, it appears that the potential of mean force

should be more repulsive than that given by the DLVO theory. As a result, both models underpredict the osmotic pressure at high salt and protein concentrations.

Membrane Osmometer

Figure 1 shows schematically the experimental apparatus for osmotic-pressure measurements. The osmometer cell consists of two chambers separated by a membrane permeable to solvent molecules and small ions but impermeable to proteins. One chamber contains a protein solution and the other contains a protein-free solvent, i.e., a solution of salt and buffer ions. External pressure is applied to the protein side such that, at equilibrium, no net mass transfer occurs between the two chambers. The applied pressure is controlled by one of the two precision pressure regulators, one for low-pressures (<1000 mmH_2O) and the other for higher pressures. The osmotic pressure, equal to the applied external pressure at equilibrium, is read either from a home-made water manometer or from a high-precision pressure gauge. Resolution of the volume change in the capillaries is about 0.002 ml. The osmometer is kept isothermal by immersion in a water bath controlled at 25 ± 0.1 °C. External pressure was supplied by a high-pressure nitrogen tank. Attainment of equilibrium may require several days for high protein concentrations. To accelerate data acquisition, two identical osmometer systems were constructed.

Experimental Procedure

Materials

Bovine Serum Albumin (BSA) (>98% albumin, heat-shock fraction) was purchased from Sigma Chemical Company (St. Louis, MO) and stored at about 5°C. Analytical-grade monobasic and dibasic sodium phosphates, sodium hydroxide pellets, hydrochloric acid (0.1 N) and crystal sodium chloride were purchased from Aldrich (Milwaukee, WI). Sodium azide was from Eastman Kodak Company (Rochester, NY). All chemicals were used as received and all solutions were prepared using deionized water. Cellulose membranes with 10,000 molecular-weight cut-off were purchased from Millipore Corporation (Bedford, MA). The molecular weight of BSA is 66,000.

Procedures

Sodium chloride aqueous solutions of 1.0, 3.0 and 5.0 M in Na^+ at pH =7.4, 5.4 and 4.5 were prepared using 0.1 N mono-basic/dibasic sodium phosphate buffer solution and crystal sodium chloride. All solutions contained sodium azide (0.020 g/L) as an anti-bacterial agent. High-concentration BSA-stock solutions (about 100 g/L) were prepared by dissolving albumin crystals in a given sodium chloride solution. Each stock solution was then diluted to about 20, 40, 60 and 80% of its original protein concentration with the corresponding sodium-chloride-buffer solution. pH was measured using a Sargent-Welch Model 8400 Ion/pH meter with a Fisher Scientific Model SN 13-620-286 electrode. Final solution pH was adjusted to the desired value by addition of non-buffered aqueous solution of 0.1 N sodium hydroxide or hydrochloric acid. Vigorous vortex mixing was applied during acid/base addition process to prevent local protein denaturation. The slight

difference in Na^+ concentration induced by pH adjustment was neglected. All solutions were kept at 5 °C overnight before osmotic-pressure measurement.

The regenerated cellulose membrane was soaked in deionized water for 1-2 hours and then in the desired sodium-chloride-buffer solution overnight before use. The osmometer cell was assembled by sandwiching the pre-prepared membrane between two Plexiglas chambers. The solvent (i.e., aqueous sodium chloride and buffer solution) was injected into both chambers using syringes, and the osmometer was set in the water bath overnight before osmotic-pressure measurement. Cell leakage and zero-point error were prevented by checking the solvent level at each capillary.

Before each measurement, both solution and solvent chambers of the osmometer were flushed by injecting and removing excess amount of desired solvent; the solution chamber was rinsed two or three times with the sample solution. The protein solution and the solvent were then simultaneously injected into the corresponding chambers to the liquid levels in both capillaries reaching about 2/3 of their full length. Pressure was applied gradually to the solution side such that there was no net mass flow between the two chambers. Equilibrium was attained when there was no liquid-level change in both capillaries for about three hours at a given applied pressure. At the conclusion of each measurement, solvent and solution samples were taken simultaneously using different syringes while the solution was still under pressure. The protein concentrations in both chambers were measured using a Shimadzu Model UV-160 spectrophotometer. Concentration measurement on the solvent side was conducted to monitor the working status of the membrane osmometer because leakage could occasionally occur due to

inappropriate assembling of the osmometer. The extinction coefficient for BSA at 278 nm is 1.52 in aqueous solutions, independent of pH and salt concentration.

Experimental Results

Table 1 shows the measured osmotic pressures of BSA solutions at different pH and salt concentrations. To display the trend of measured osmotic pressure as a function of pH, Figure 2 shows reduced osmotic pressure (osmotic pressure/protein concentration) versus concentration for pH=7.4 and 4.5 in 1 M sodium chloride solution. Because of higher protein charge as discussed in the next paragraph, the osmotic pressure at pH =7.4 is much higher than that at pH=4.5.

According to Vilker, the BSA charge numbers in 0.15 M sodium chloride aqueous solution are -20.4, -9.1 and +4.5 at pH 7.5, 5.4 and 4.5, respectively. We assume that these charge numbers are independent of salt concentration. At pH=4.5, attractive forces between proteins dominate over repulsion interactions; the former leads to negative second virial coefficients. (A negative second virial coefficient reduces osmotic pressure relative to that given by the van't Hoff equation). Association between BSA molecules has been reported by Kanal et al (1994) in the range 4<pH<6. Figure 3 shows the effect of salt concentration on the osmotic pressure of BSA solution at pH 7.4. In general, the osmotic pressure decreases as salt concentration rises because of increased screening of the electrostatic repulsion between protein molecules. Figure 4 shows the osmotic pressure of BSA aqueous solutions at high salt concentrations at pH 5.4. At these conditions, the osmotic second virial coefficients are negative, indicating that the overall force between BSA molecules is attractive. As expected, because screening declines with

falling ionic strength, the interaction between proteins is more repulsive at lower salt concentrations.

Theoretical Analysis of Osmotic-Pressure Data

Potential of Mean Force Between Proteins in Aqueous Electrolyte Solutions

To represent the osmotic pressure of BSA solutions at different pH and salt concentrations, we assume that proteins in solution can be represented by charged hard spheres of diameter σ immersed in an aqueous solvent containing salt ions. The total potential of mean force between protein molecules includes contributions from hard-sphere repulsion $W^{hs}(r)$, from dispersion attraction $W^{dis}(r)$, and from double-layer electrostatic repulsive interaction, $W^{dl}(r)$ (Hiemenz and Rajagopalan, 1997),

$$W^{total}(r) = W^{hs}(r) + W^{dis}(r) + W^{dl}(r) \tag{1}$$

where r is center-to-center distance between two protein molecules. The last two terms are known as the Derjaguin-Landau-Verwey-Overbeek (DLVO) potential, $W^{DLVO}(r)$.

The dispersion potential between two protein molecules is given by (Russel et al., 1989)

$$W^{dis}(r) = -\frac{H}{12}\left(\frac{\sigma^2}{r^2-\sigma^2} + \frac{\sigma^2}{r^2} + 2\ln\frac{r^2-\sigma^2}{r^2}\right) \qquad r > \sigma\ . \tag{2}$$

where H is the Hamaker constant of proteins in water. Because Eq. (2) diverges when two protein molecules are in contact, we assume that there is a minimum distance between two protein surfaces, denoted as δ. A possible interpretation of δ is to regard it as the average diameter of atoms on the protein surface. However, because δ is obtained by adjusting experimental osmotic-pressure data to our calculations, it is essentially an

empirical fitting parameter. The strength of dispersion interaction is sensitive to the minimum surface-to-surface distance.

The double-layer repulsive interaction $W^{dl}(r)$ is approximated by

$$W^{dl}(r) = \frac{z_p^2 e^2}{4\pi\varepsilon_0\varepsilon r} \frac{e^{-\kappa(r-\sigma)}}{(1+\kappa\sigma/2)^2}, \tag{3}$$

where z_p is protein charge, e is the charge of an electron, ε is the dielectric constant of water and e_0 is the permittivity constant. κ is the Debye screening parameter related to the small-ion concentration ρ_i, valence z_i and temperature T by

$$\kappa^2 = \sum_i \frac{\rho_i e^2 z_i^2}{\varepsilon_0 \varepsilon kT}, \tag{4}$$

where k is the Boltzmann constant. Here subscript i refers to all small ions. Equation (3) is derived using the linear approximation of the Poisson-Boltzmann equation (Hiemenz and Rajagopalan, 1997)

van-der-Waals Type Equations of State for Protein Solutions

We use two van-der-Waals type equations of state to correlate experimental osmotic-pressure data of BSA solutions measured in this work and those reported by Vilker et al. (1981). Both equations contain the Carnahan-Starling equation of state to take into account short-range repulsive interactions (Carnahan and Starling, 1969). In the first model, the DLVO potential is considered through its contribution to the perturbation part of the second virial coefficient, denoted by B. The osmotic compressibility factor Z is given by,

$$Z = \frac{\Pi}{\rho_p kT} = \frac{2}{\rho_p}\left\{[(z_p\rho_p/2)^2 + \rho_s^2]^{1/2} - \rho_s\right\} + + \frac{1+\eta+\eta^2-\eta^3}{(1-\eta)^3} + B\cdot\rho_p \tag{5}$$

where Π is osmotic pressure; ρ_P is the number density of protein; η is packing fraction, $\eta = \frac{\pi}{6}\rho_P\sigma^3$; ρ_s is the number density of salt in the "solvent" side. Because the volume of the solvent side is large compared to that of the protein side, ρ_s is identical to the original number density of salt. The perturbation part of the second virial coefficient B is given by

$$B = 2\pi\int_{\sigma+\delta}^{\infty}\{1-\exp[-\frac{W^{DLVO}(r)}{kT}]\}r^2dr. \tag{6}$$

The first term in Eq. (5) comes from the ideal Donnan effect for a 1:1 electrolyte solution. It takes into account the uneven distribution of small ions between the two chambers of the osmometer (Vilker et al, 1981). When the McMillan-Mayer theory is applied to calculate the osmotic pressure of protein solutions or other solutions of charged solutes, consideration must be given to the difference in "solvents" ; i.e., the "solvent" (in this case, a salt solution) on the protein side of the osmometer is slightly different from that on the other side because of Donnan equilibrium. For the solutions considered here, the ideal Donnan effect provides a simple first-order correction due to the difference in solvents. [Another approach to calculate the osmotic pressure of a protein solution containing electrolytes is to consider explicitly interactions between proteins and small ions, as discussed by Amos et al (1998).] For convenience, we call Eq(5) the B model.

An alternate way to include the DLVO potential is to use the random-phase approximation (RPA) (See, for example, Vlachy et al, 1993) that gives the equation of state

$$Z = \frac{\Pi}{\rho_p kT} = \frac{2}{\rho_p}\left\{[(z_p\rho_p/2)^2+\rho_s^2]^{1/2}-\rho_s\right\}+\frac{1+\eta+\eta^2-\eta^3}{(1-\eta)^3}+\rho_p\cdot\frac{U_1}{2kT} \tag{7}$$

where $U_1 = 4\pi \int_{\sigma+\delta}^{\infty} [W^{dis}(r) + W^{dl}(r)] r^2 dr$. The RPA is a perturbation theory that approximates the direct correlation function of the solution by that of the reference system. U_1 is analogous to constant *a* in the van der Waals equation of state with dimension energy-volume.

At high salt concentrations or at low protein charge, the ideal Donnan-effect term in Eq.(5) or Eq.(7) is negligible because the "solvents" in both chambers of the osmometer are essentially identical. However, this term can be significant for the osmotic pressure of highly charged proteins in dilute salt solutions. For example, at pH=7.4 and C_s=0.15 M, it accounts for about 20% of the total osmotic pressure for the protein concentrations studied here.

In both models, molecular parameters δ and H are assumed independent of solution pH, protein concentration and salt concentration. These parameters are obtained by regression of experimental osmotic-pressure data measured in this work and those reported by Vilker et al (1981). The BSA diameter (σ=62.6 Å) and charge numbers (valence) at various pH are also from Vilker et al (1981). Table 2 gives the two adjustable parameters for both models. The Hamaker constants obtained from the two models do not agree due to different approximations introduced in each model.

Figures 5-8 compare experimental osmotic pressures for BSA solutions with calculated results using the van-der-Waals type equations of state. The experimental data for C_s =0.15 M are from Vilker et al (1981). While both models can semi-quantitatively describe the osmotic pressure of BSA solutions, the B model is slightly better than the RPA model. Both models predict osmotic pressures that are too small at high protein concentrations, perhaps because the DLVO theory is based on the linear approximation

of the Poisson-Boltzmann equation; this approximation gives less repulsion when compared with the correct interaction potential between macroions at small separations (Israelachvili, 1991). At pH 4.5 and 5.4, deviations at low protein concentrations may be related to association of proteins.

To represent the data, we have tried using other, more sophisticated models including the Barker-Henderson perturbation theory with the macro-compressibility approximation (McQuarrie, 1976) and the Percus-Yevick solution of the sticky hard-sphere model (Baxter, 1968). Regrettably, these models do not improve agreement with experiment. It appears that the main weakness of the two van-der-Waals models follows from the over-simplified potential of mean force used here for proteins in electrolyte solutions. Progress in understanding the properties of saline protein solutions is not likely to come from more sophisticated statistical mechanics but from an improved potential of mean force.

Conclusions

Two membrane-osmometer systems have been constructed for protein solutions at low and high protein concentrations. The reliability of these systems was tested by measuring the osmotic pressure of BSA solutions at various pHs and salt concentrations; good agreement with Vilker's (1981) data was obtained. While the osmometers described here are inexpensive and easy to operate, osmotic-pressure measurements are time consuming, especially at high protein concentrations.

New and previously published osmotic data show the effects of pH and salt concentration on the osmotic pressures of BSA solutions. The osmotic pressure falls as

protein charge decreases or as salt concentration increases. The experimental data can be semi-quantitatively interpreted using either a second-virial-coefficient model, or a model based on RPA theory. However, neither model is successful at high salt concentrations. For more accurate description of the properties of protein solutions, we require a better potential of mean force. Toward meeting that requirement, molecular-simulation studies are in progress.

Acknowledgement

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Literature Cited

Amos D. A., Radke C. J. and Lynn S., "Osmotic pressure and inter-particle interactions in ionic micellar surfactant solutions", *J. Phys. Chem. B*, **102**:2739-2753 (1998).

Baxter R. J., "Percus-Yevick equation for hard spheres with surface adhesion", *J. Chem. Phys.*, **49**:2770-2774(1968).

Belter P.A., Cussler E. L. and Hu W. S., "*Bioseparations: Downstream Processing Operations*", John Wiley & Sons, New York(1988).

Carnahan, N. F. and Starling, K. E., "Equation of state for nonattracting rigid spheres", *J. Chem. Phys.*, **51**:635-636(1969).

Chapman W. G., Gubbins K. E., Jackson G. and Radosz M., "New reference equation of state for associating liquids". *Ind. Eng. Chem. Res.*, **29**:1709-1721(1990).

George J. L., "Osmotic pressure measurement of α-Chymotrypsin association in aqueous electrolyte solutions", Master Thesis, University of California, Berkeley (1992).

Hiemenz P. C. and Rajagopalan R. "*Principles of Colloid and Surface Chemistry*", 3rd Edition, Marcel Dekker, Inc., New York(1997).

Israelachvili J., *Intermolecular & Surface Forces*, Academic Press, New York(1991).

Kanal K. M., Fullerton G. D. and Cameron I. L., "A study of the molecular sources of nonideal osmotic pressure of Bovine Serum Albumin solutions as function of pH", *Biophysical J.*, **66**, 163-160(1994).

McQuarrie D. A., "*Statistical Mechanics*", Harper & Row publishers, New York(1976).

Rothstein F., "Differential precipitation of proteins", in "*Protein Purification Process Engineering*", Harrison R. G., ed., Marcel Dekker, New York(1994).

Russel W. B., Saville D. A. and Schowalter W. R., *Colloidal Dispersions*, Cambridge University Press, Cambridge; New York(1989).

Vilker V. L., Colton C. K. and Smith K. A., "The osmotic pressure of concentrated protein solutions: Effect of concentration and pH in saline solutions of Bovine Serum Albumin", *J. Colloid and Interface Sci.*, **79**:548-566(1981).

Vlachy V., Blanch H. W. and Prausnitz J. M., "Liquid-liquid phase separations in aqueous solutions of globular proteins", *AIChE J*, **39**:215-223 (1993).

Table 1 Measured osmotic pressures Π for solutions of BSA in aqueous sodium chloride.

C_s =1.0 M			
pH 7.4		pH 4.5	
C_p (g/L)	Π (mmH_2O)	C_p (g/L)	Π (mmH_2O)
19.5	85	12.2	30
44.6	224	25.5	61
55.8	282	38.6	114
84.5	515	44.3	157
101.1	731	61.7	240
C_s =3.0 M			
pH 7.4		pH 5.4	
C_p (g/L)	Π (mmH_2O)	C_p (g/L)	Π (mmH_2O)
15	50	34.5	93
25	89	55.2	175
38.4	143	73.5	295
50.3	202	89.8	393.5
65.3	275	93.5	405
		122.8	692
C_s =5.0 M			
pH 7.4		pH 5.4	
C_p (g/L)	Π (mmH_2O)	C_p (g/L)	Π (mmH_2O)
33.4	127	30.1	67
68.9	283	49.5	100
100.1	435	58.9	154
133.5	615	77.3	240
150	892	99.1	430

C_s= molar concentration of salt (sodium chloride)
C_p= mass concentration of protein (BSA)

Table 2 Molecular Parameters Obtained from Data Regression

	H/kT	δ (Å)
B model	0.31	0.12
RPA model	2.48	0.05

H= Hamaker constant
δ= empirical parameter reflecting minimum surface-to-surface distance

Figure Captions

Figure 1 Schematic diagram of the osmometer system

Figure 2 Reduced osmotic pressure (Π/C_P) vs. BSA concentration at 25 °C in 1 M sodium chloride solution at pH 7.4 and 4.5 (Lines are to guide the eye)

Figure 3 Reduced osmotic pressure (Π/C_P) vs. BSA concentration at 25 °C and pH 7.4 in 1 and 5 M sodium chloride solutions (Lines are to guide the eye)

Figure 4 Reduced osmotic pressure (Π/C_P) vs. BSA concentration at 25 °C and pH 5.4 in 3 and 5 M sodium chloride solutions (Lines are to guide the eye)

Figure 5 Calculated and measured osmotic pressures of BSA solutions at C_s=5 M. Solid lines are calculated from the B model; the dashed lines are from RPA theory.

Figure 6 Calculated and measured osmotic pressures of BSA solutions at C_s=3 M and pH=7.4 , 5.4. Notation is similar to that in Figure 5.

Figure 7 Calculated and measured osmotic pressures of BSA solutions at C_s=1 M. Notation is similar to that in Figure 5.

Figure 8 Calculated and measured osmotic pressures of BSA solutions at C_s=0.15 M. Notation is similar to that in Figure 5. Experimental data from Vilker et al., 1981.

Figure 1

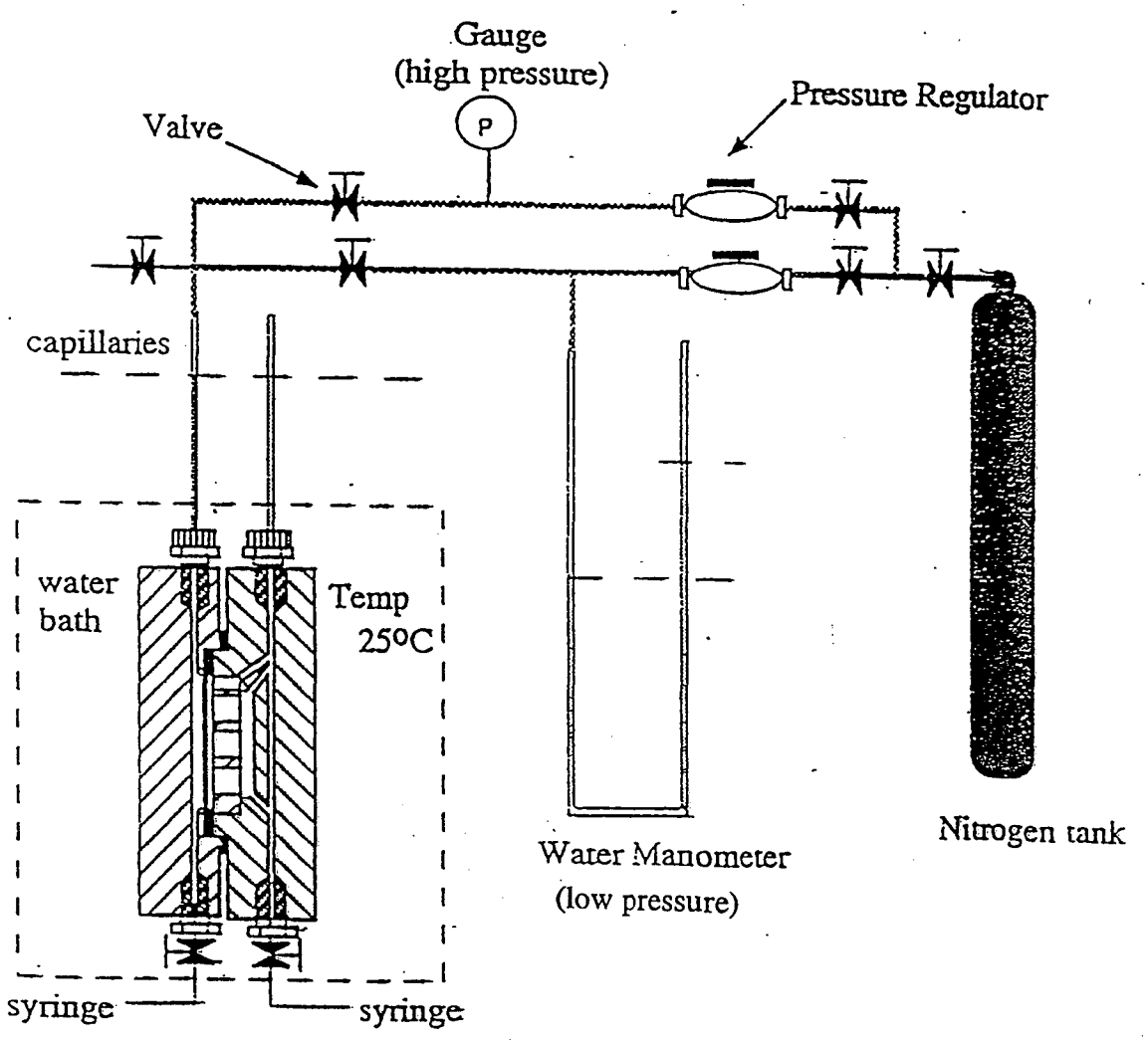

Gauge
(high pressure)
P
Valve
Pressure Regulator
capillaries
water
bath
Temp
25°C
Water Manometer
(low pressure)
Nitrogen tank
syringe
syringe

Figure 2

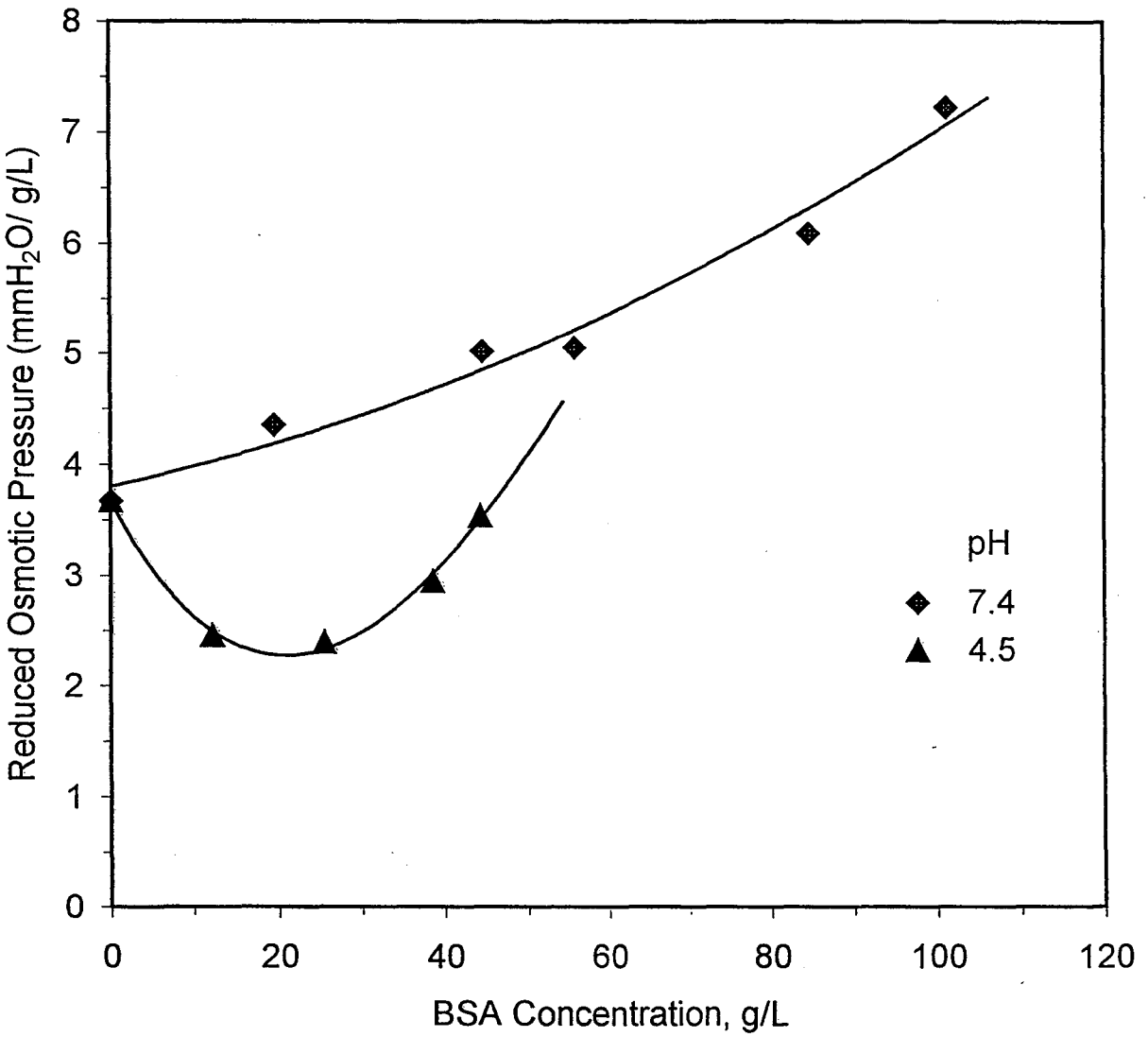

Reduced Osmotic Pressure (mmH2O/ g/L)
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
BSA Concentration, g/L
pH
7.4
4.5

Figure 3

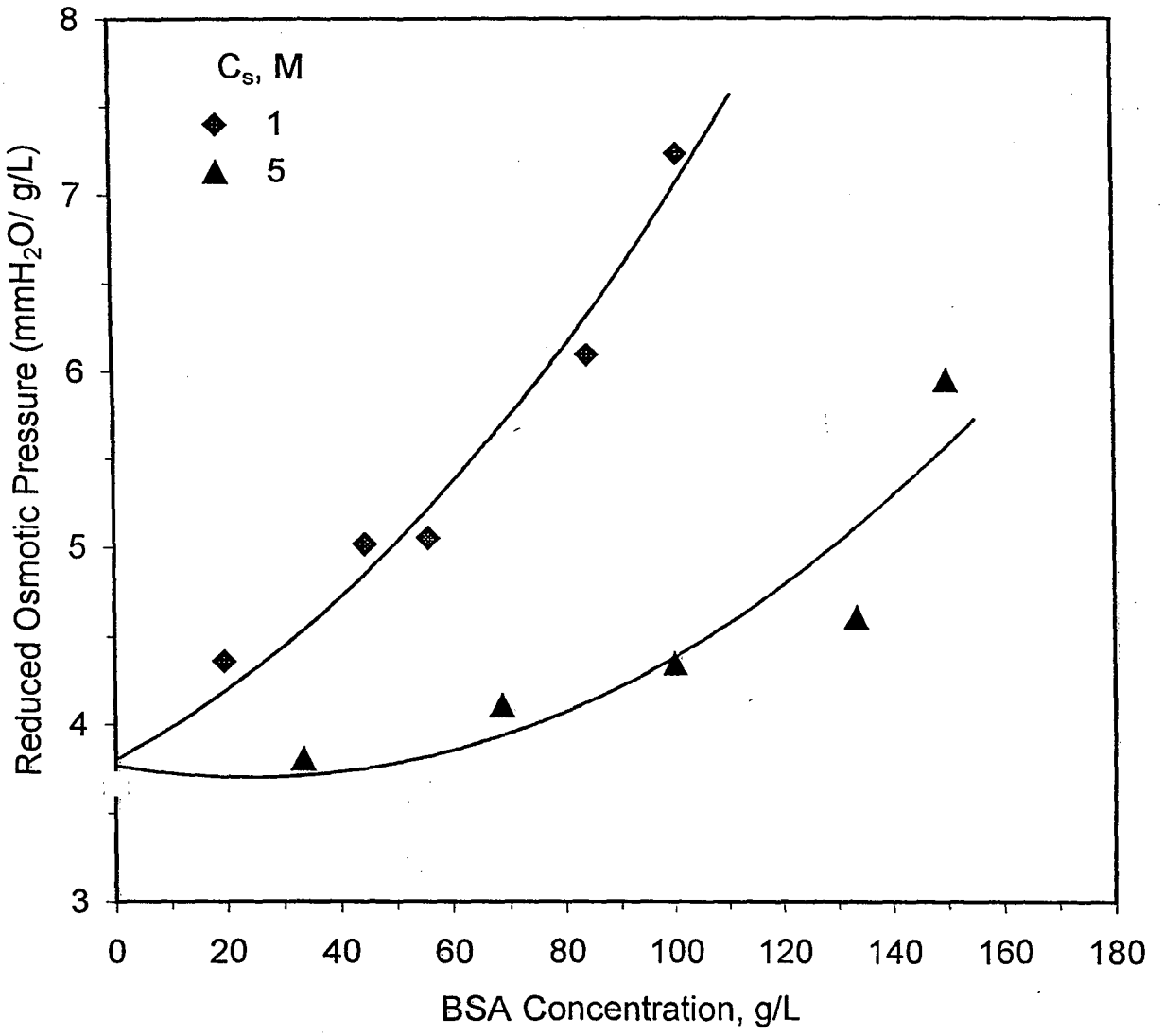

C_s, M
1
5
Reduced Osmotic Pressure (mmH_2O/ g/L)
8
7
6
5
4
3
0
20
40
60
80
100
120
140
160
180
BSA Concentration, g/L

Figure 4

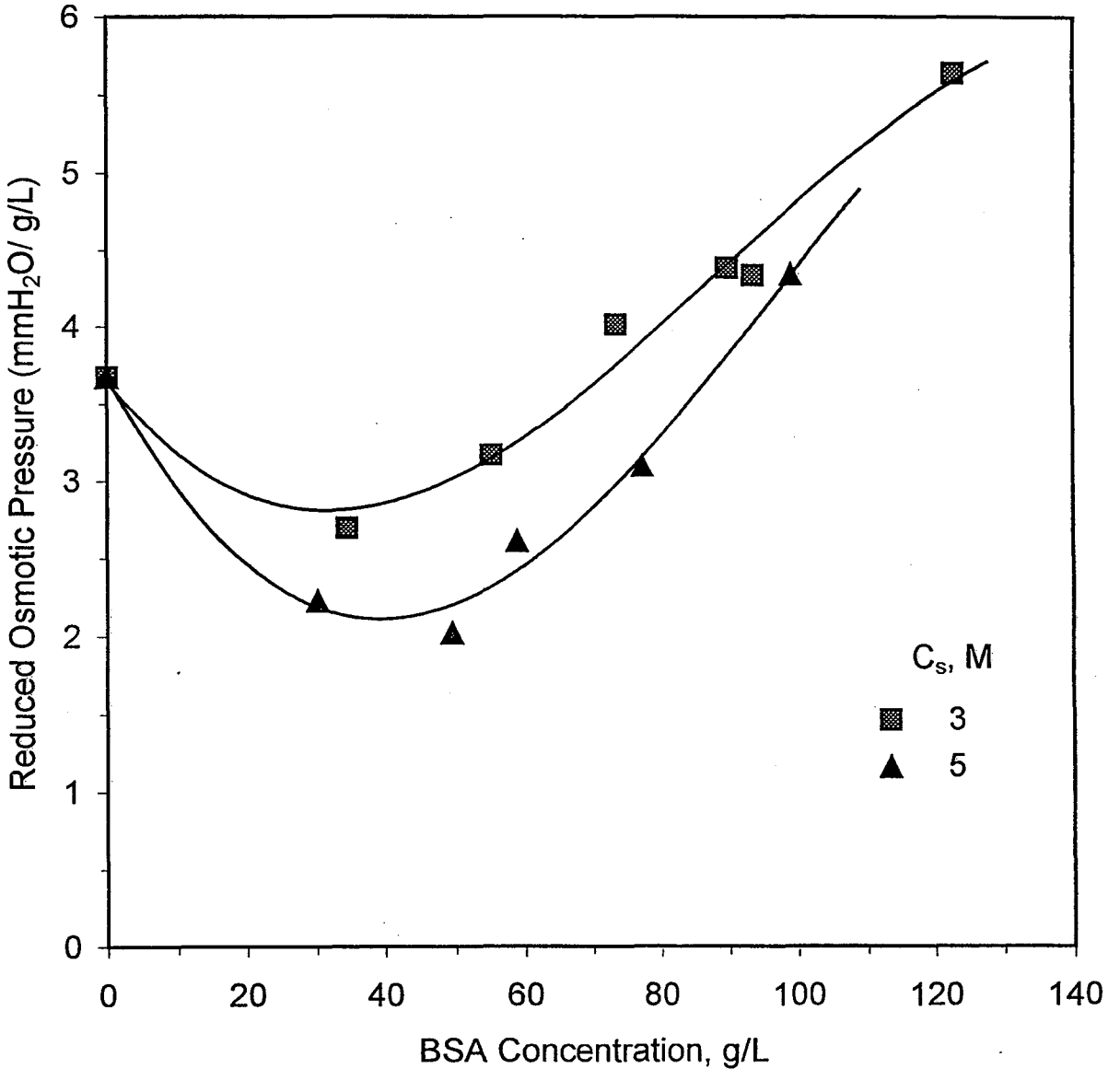

6
5
4
3
2
1
0
Reduced Osmotic Pressure (mmH2O/ g/L)
0
20
40
60
80
100
120
140
BSA Concentration, g/L
Cs, M
3
5

Figure 5

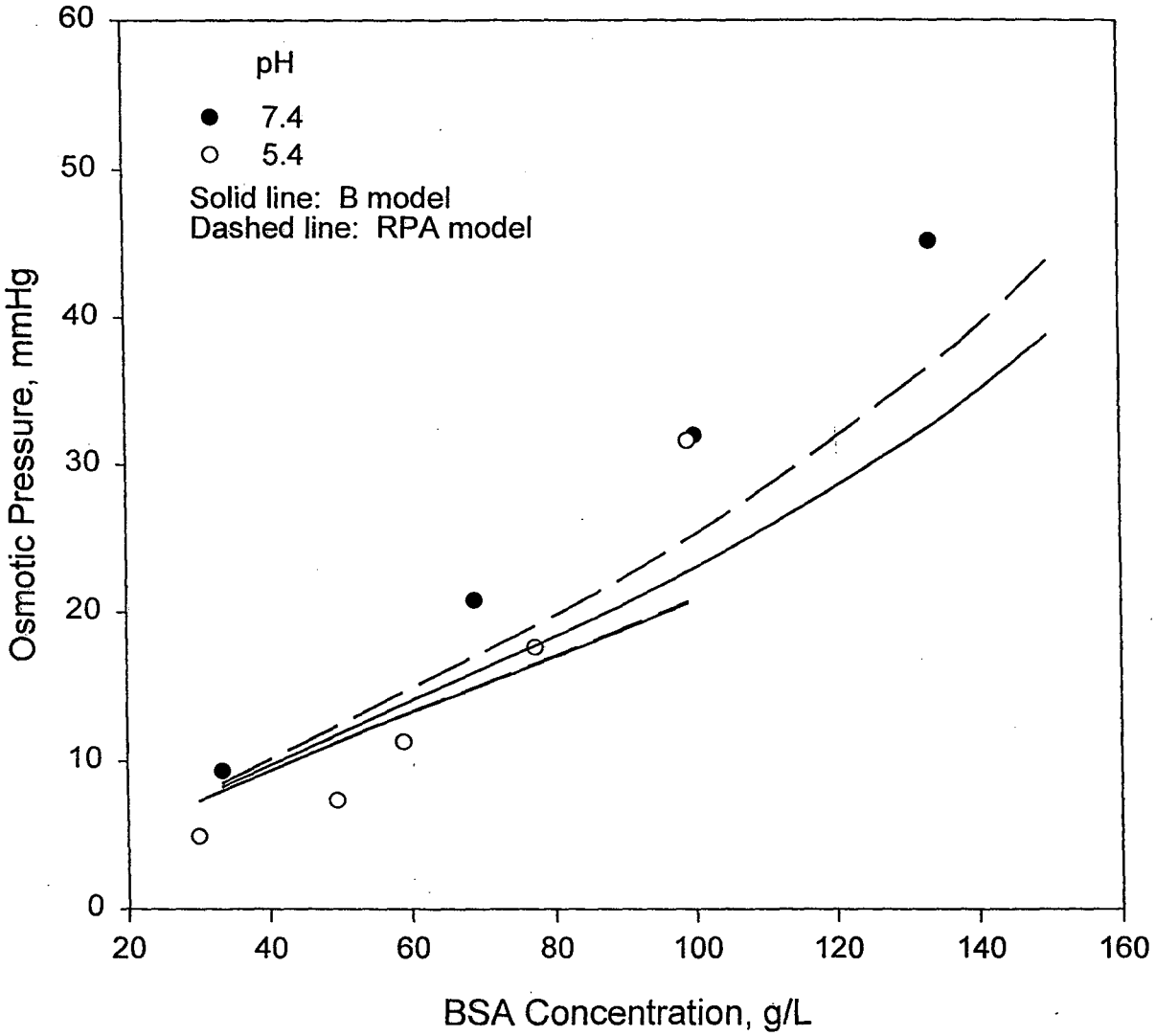

pH
7.4
5.4
Solid line: B model
Dashed line: RPA model
Osmotic Pressure, mmHg
BSA Concentration, g/L
0
10
20
30
40
50
60
20
40
60
80
100
120
140
160

Figure 6

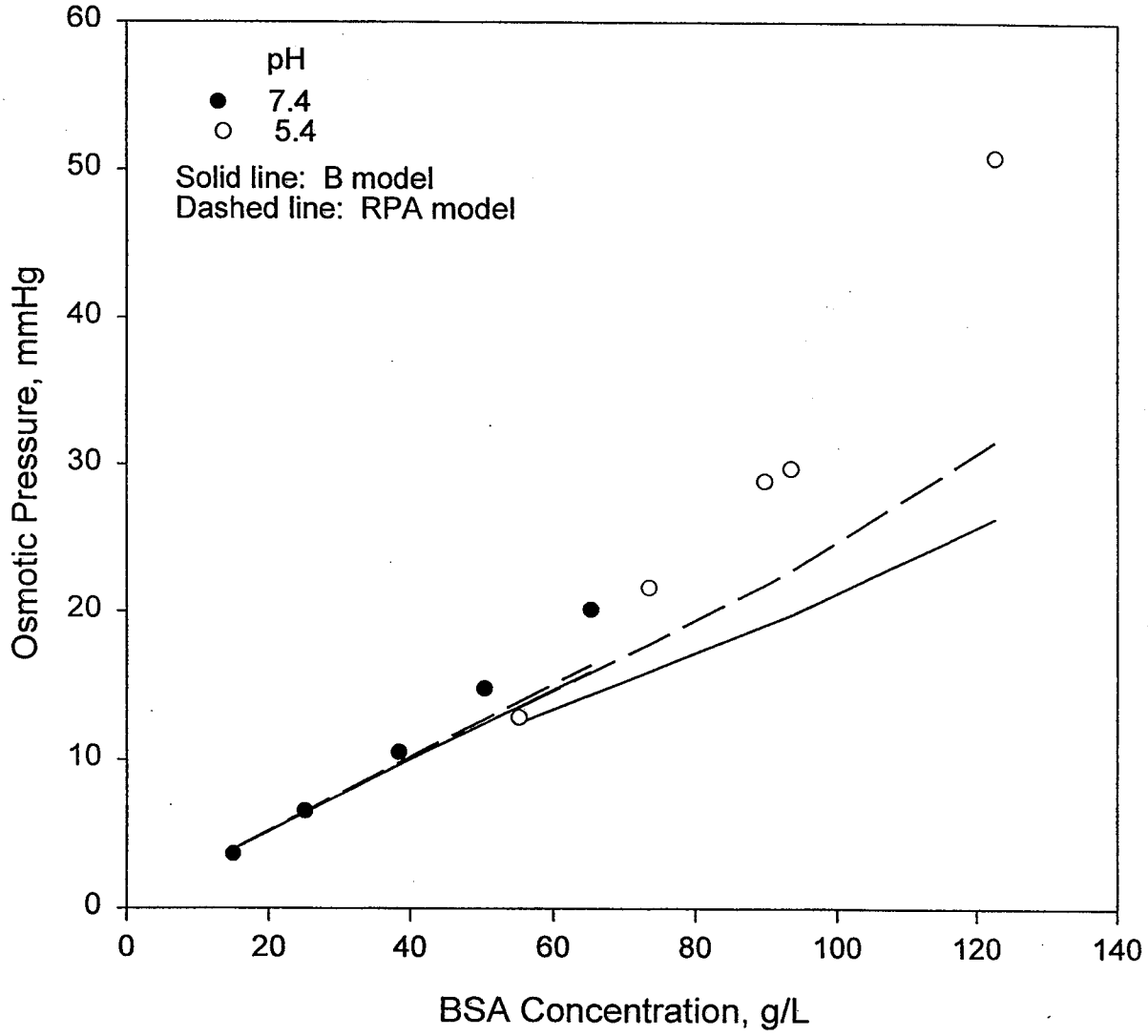

pH
7.4
5.4
Solid line: B model
Dashed line: RPA model
Osmotic Pressure, mmHg
BSA Concentration, g/L
0
10
20
30
40
50
60
0
20
40
60
80
100
120
140

Figure 7

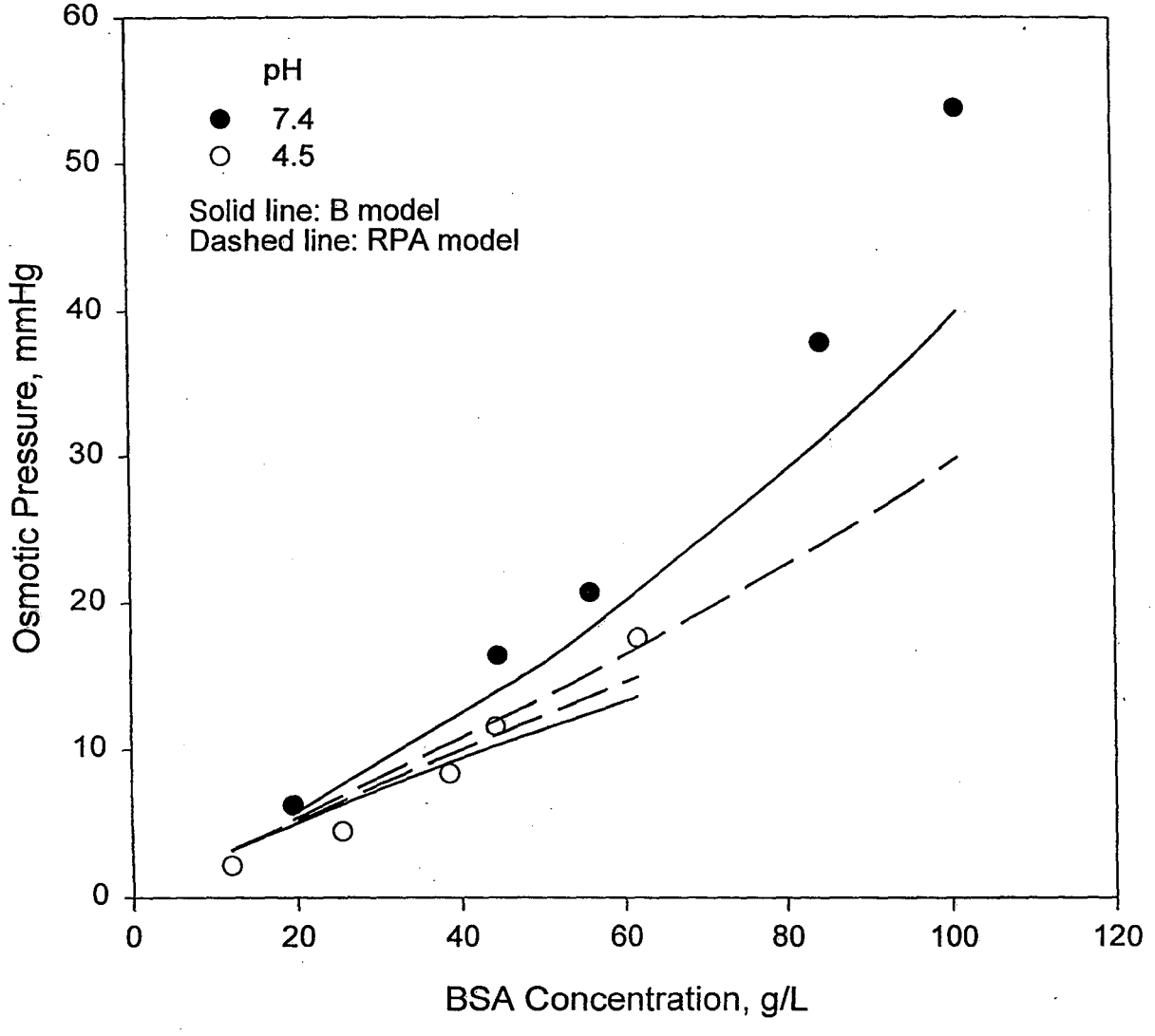
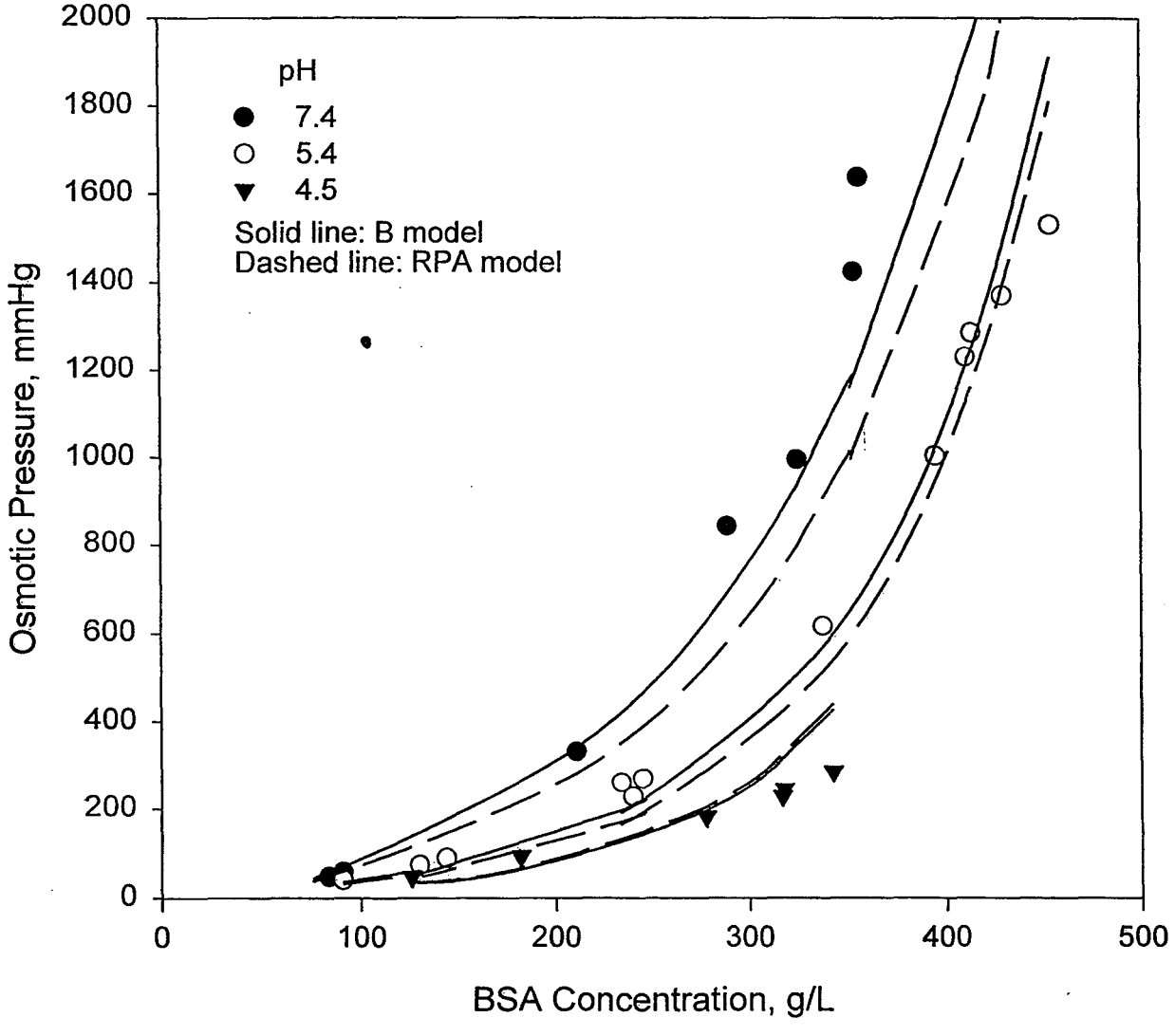

pH
7.4
4.5
Solid line: B model
Dashed line: RPA model
Osmotic Pressure, mmHg
BSA Concentration, g/L
0
10
20
30
40
50
60
0
20
40
60
80
100
120

Figure 8


pH
7.4
5.4
4.5
Solid line: B model
Dashed line: RPA model
Osmotic Pressure, mmHg
BSA Concentration, g/L
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0
100
200
300
400
500

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