Thermodynamics of the Partitioning of Biomaterials in Two-Phase Aqueous Polymer Systems: Comparison of Lattice Model to Experimental Data

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the early stages of the process can be attributed to the adsorption of micelles. Taking the size of the micelles into account, as well as the protective layer formed by adsorbed molecules, we expect the probability that a micelle will reach the surface and adsorb to decrease with time. Moreover, the strong attraction between the PVP block in the core of the micelle and the wall is screened by the polystyrene shell. This screening also acts on the unimers but is presumably weaker. We conjecture that the slow adsorption at long times can be attributed essentially to unimers. A modeling of the kinetics of adsorption which would involve micelles at short times, then unimers, is currently under progress. We can note that such a mechanism of formation of the adsorbed layer would lead to a nonhomogeneous layer, since we expect adsorption of micelles to create regions with a high polymer density.

At the present time, we do not possess an interpretation of the S-shaped kinetics where adsorption goes through a plateau for intermediate times. The possibility of further modification of the conformation of adsorbed molecules has been raised.[31] It is interesting to note that a similar behavior has been observed for the adsorption of poly(methyl methacrylate) on sapphire.[11]

Conclusion

In this paper we have shown that the surface plasmon technique could be successfully used to study the kinetics of adsorption of polymer chains on a metal surface. The existence of even a small molecular weight PVP block induces an adsorption roughly 10 times higher than polystyrene of the same molecular weight. Our results clearly indicate that the adsorption rate as well as the equilibrium adsorbance are strongly dependent on the length of each block.

The kinetics of adsorption does not follow any simple first order reaction nor any Langmuir-type model. The role of micelles in the adsorption process has been demonstrated. The experimental results lead to the conclusion that the first stages of the adsorption process correspond to the adsorption of micelles, whereas longer times involve adsorption of unimers.

By determining the coverage of the metal surface by the PVP blocks and extracting the thickness of the adsorbed layer, we have provided further information on the structure of the interface. The dimension of the adsorbed layer corroborates the modeling of an adsorbed chain assuming a flattened configuration of the molten PVP block at the surface and a strong stretching of the PS tails toward the solution.

Acknowledgment. This work has benefited from many helpful discussions with Dr. D. Ausserre, whom it is a pleasure to thank. Margaret Best has provided much assistance with the laboratory automation software. Engineering design of the sample and prism holder was done by Robert Mizrahi. J.F.T. also gratefully acknowledges I.B.M. for providing him financial support during this stay at the Almaden Research Center.

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Thermodynamics of the Partitioning of Biomaterials in Two-Phase Aqueous Polymer Systems: Comparison of Lattice Model to Experimental Data

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The partitionings of five proteins—lysozyme, chymotrypsinogen, ovalbumin, human serum albumin, and bovine serum albumin—in two-phase systems made from poly(vinyl methyl ether) (PVME) and poly(ethylene oxide) (PEO) were examined as functions of the compositions of the systems. Chymotrypsinogen partitioning was also examined as a function of PEO molecular weight. Experimental data for the partition coefficients were compared with values calculated from the lattice model of Baskir et al. Calculations were in good agreement with the experiments over the range of conditions investigated. Estimated phase polymer concentrations near the proteins were generally lower than those in the bulk, indicating that partitioning is primarily governed by steric repulsion of the protein from the phase polymers.

Introduction

Liquid–liquid extraction offers an attractive alternative to traditional methods for the separation of biomaterials since it may be operated continuously (rather than in batch mode) and may be scaled up relatively easily. One promising extraction method is the two-phase aqueous polymer separation technique, first introduced by Albertsson in the late 1950s.[1] In this technique, aqueous solutions of water-soluble polymers, such as poly(ethylene oxide) (PEO) and dextran, are mixed together to produce a thermodynamically stable, two-phase system. The concentration of each "phase-forming" polymer can be as low as 5% w/w or less; in such systems both phases are primarily aqueous, with the remainder of each phase being composed primarily of one of the phase-forming polymers.[1]

Since both phases of the two-phase aqueous polymer system are primarily aqueous, either phase provides a mild, protective environment for biomaterial particles. In addition, the interfacial tension between the phases is usually small, typically 0.0001–0.1 dyn/cm^2,[2] so that minimal stress is imposed on particles as they pass through the interfacial region during separation. Consequently, one can achieve purification of enzymes, for example, with very little loss of the enzyme activity.[1,3]

Although the efficacy of the two-phase aqueous extraction technique has been demonstrated for industrial-scale bioseparations,[3,4] the method cannot yet be widely applied to complex mixtures of biomaterials because the factors influencing biomaterial partitioning are not fully understood.[4] A number of recent studies address this problem.[5-8] Most of them attempt to correlate

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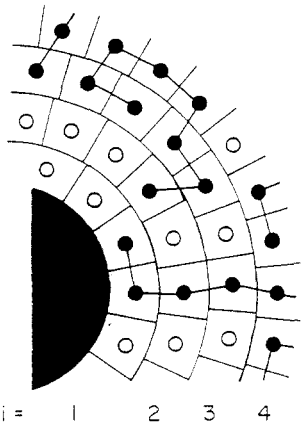

Figure 1. Lattice model for a spherical particle in an aqueous polymer solution. The lattice is shown here in two dimensions; however, the actual lattice would be three-dimensional. Only a portion of the lattice is shown.

the partitioning of biomaterials with physical data and other quantities measured from independent experiments and may ultimately provide a body of data that will help guide the design of two-phase aqueous polymer systems for specific separations; they do not, however, elucidate the physical interactions that govern partitioning behavior.

In a previous paper[9] we described a simple lattice model for examining the "molecular level" interactions of the phase-forming polymers with biomaterial particles in two-phase aqueous polymer systems. In that paper we indicated that the phase polymers affect the partitioning of biomaterials through two types of interactions: the energy interaction of the polymer chain segments with the biomaterial surface and the steric exclusion of the polymer chains from near the surface of the biomaterial. However, the data available to date are not sufficiently complete to permit systematic testing of models.

In this paper we more thoroughly investigate the effects of the phase-forming polymers on biomaterial partitioning by examining protein partitioning in water/PEO/poly(vinyl methyl ether) (PVME) two-phase systems. In particular, we examine the influence of the phase polymer molecular weight and the phase polymer concentration on the partitioning of proteins of various sizes.

Charge-related effects are not explicitly included in our model, but to a very good first approximation they can be assumed to be independent of polymer effects. They can be incorporated by the simple addition of a distribution potential term to our expression for the partitioning coefficient.[1,5]

The Lattice Model[9]

The lattice model is a Flory–Huggins model near a curved surface and employs an idealized picture for the particle in an aqueous polymer solution. (See Figure 1.) The particle, which, for simplicity, has been assumed to be (but is not required to be) a sphere, is at the center of the lattice. The lattice is made up of a series of concentric shells of lattice sites surrounding the particle. The shapes of the lattice shells are determined by the shape of the particle. The layers are indexed, beginning with the first layer ($i = 1$) at the particle surface.

Sites in the lattice contain either a single solvent molecule or else a "segment" of a polymer chain. The length scale of the lattice corresponds to about 4 Å per site. Polymer chains are represented as a series of segments placed contiguously in the lattice. All polymer chains of a given species are assumed to have the same number of segments, r. For PEO and PVME, a site accommodates roughly one monomer unit of the chain, so that the number of segments in the chain is taken to be equal to the polymer degree of polymerization. Due to polymer/protein interactions, the volume fractions of polymer chain segments in each lattice layer ($\phi_{2,i}$) are generally not the same as the one far from the particle in the bulk solution ($\phi_{2,*}$).

Energy interactions in the lattice occur between neighboring sites containing different components. There are two types of energy interactions: the solvent/polymer interaction energy (χ) and the polymer segment/protein surface interaction energy (χ_s). χ is the Flory–Huggins interaction parameter. χ_s is measured relative to the solvent interaction energy with the protein surface, so that for positive χ_s the interaction energy of the polymer segment with the protein surface is more favorable than that of the solvent with the protein surface.

The Need for a Single-Polymer System To Determine χ_s

In order to calculate partition coefficients for proteins from the lattice model, values for the system variables in each phase must be supplied. The polymer chain lengths (r) for the two-phase polymers are generally known; the size of the protein (R) can be estimated from hydrodynamic data (the hydrodynamic radius of the protein can be calculated from data for the diffusion coefficient using Stokes' law[10]); the concentration of polymer in each of the phases ($\phi_{2,*}$) can be found from the phase diagram for the particular system; values of χ for many polymer/solvent pairs are available in the literature, and reliable techniques have also been developed to measure χ.[11]

However, the polymer/protein interaction energies (χ_s) for various protein/polymer pairs are not known and cannot, in general, be measured directly. (It is possible to determine χ_s by differential adsorption,[12,13] but this technique is probably not applicable to proteins.) It is, therefore, necessary to devise a method to estimate χ_s. In particular, it is desirable to have a system in which partitioning depends on the interactions of only a single protein/polymer pair. Two-phase aqueous partitioning experiments are unsuitable for this, since partitioning in these systems depends on the interactions of two different polymers with the protein. (To fit the data, two adjustable χ_s values must be supplied to the model, making it difficult to determine accurate values for either one.)

One alternative for determining χ_s would be to fit model results to data for the precipitation of proteins in aqueous polymer solutions. By fitting the model to precipitation data for a given protein over a range of polymer molecular weights and concentrations, it would be possible to estimate χ_s for the given protein/polymer pair. This has been demonstrated previously for human serum albumin and PEO.[9] However, the lattice model is not truly appropriate for precipitation phenomenon since it presupposes the dilute limit of the protein in solution. (The model does not account for protein/protein interactions.) In addition, protein precipitation in aqueous polymer solutions generally requires the presence of a buffering salt at significant concentrations to ensure that protein precipitation occurs at relatively low polymer concentrations.[14] (At high polymer concentrations the high viscosity of the solution can make the precipitation of the protein difficult.)

A preferable alternative would be to examine the partitioning of the protein between an aqueous solution of the polymer and a pure aqueous phase. The problem is keeping the polymer in the polymer solution from mixing with the aqueous phase. In principle, this could be done with a semipermeable membrane. However, no such membranes exist at present that would be

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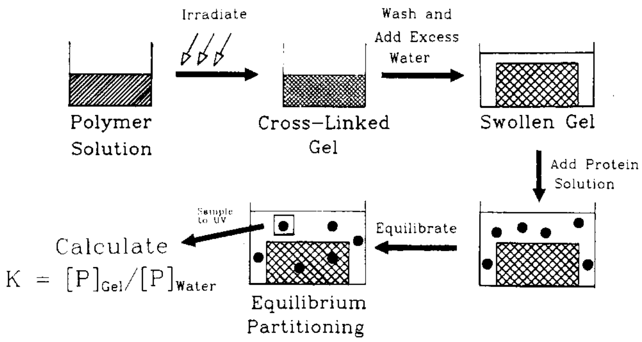

Figure 2. Schematic diagram of the hydrogel experiment.

appropriate for the systems of interest.

Consequently, to keep the aqueous polymer phase separate from the pure aqueous phase, we choose to limit the mobility of the polymer chains in the system by cross-linking the polymer to form a polymer hydrogel. In these experiments one phase is composed of a polymer network saturated with water. The presence of cross-links between the polymer chains limited the degree to which the gel could swell. Consequently, when an excess of water is added, a two-phase system consisting of the swollen hydrogel and a pure aqueous supernatant is formed. At their equilibrium swelling volume in water, the gels prepared here were typically composed of 2–20% w/w polymer. (See Figure 2 for a schematic diagram of the steps in the hydrogel experiment.)

The ability to cross-link vinyl polymers in solution by irradiation with both electrons[15–18] and γ rays[19–23] has been known for several decades. The gels employed here were made by bombarding an aqueous solution of the polymer with high-energy electrons, which caused the polymer to cross-link.

Proteins added to these two-phase systems distribute between the aqueous phase and the gel phase. At equilibrium, the distribution of protein can be determined by measuring the protein concentration in the aqueous phase by a simple UV measurement and then back-calculating the concentration of the protein in the gel.

Data from this simple experiment could be fit by the lattice model using a single adjustable χ_s parameter, except for those cases where the protein did not partition significantly into the gel. The χ_s parameters calculated from these experiments were then used to model protein partitioning in two-phase aqueous polymer systems that used the same proteins and polymers. Therefore, for those proteins that partitioned in the polymer hydrogel experiments, no adjustable parameters were employed for the modeling of their partitioning in the two-phase aqueous polymer systems.

It is noteworthy to realize that partitioning of the proteins into the gels could be modeled with values of χ_s independent of cross-link density and, hence, mesh size. This clearly indicates that the "steric exclusion effect" in a solution of linear chains is identical with the one in the loosely cross-linked gels employed here!

Systems Chosen

Although two-phase aqueous polymer systems for biomaterial extraction have traditionally been made from PEO and dextran, many different combinations of phase polymers may be used.[1] For this study, the phase polymers were chosen to best conform to the assumptions of the lattice model.[9] Specifically, this meant the choise of linear (unbranched) homopolymers with reasonably narrow molecular weight distributions and relatively small repeat units. Flexible polymers that behave like random coils in solution were also desirable. The phase polymers were chosen also to have similar chemical structures so that the effects of specific protein/polymer or solvent/polymer interactions would be small and would not overpower other effects.

TABLE I: Phase Polymer Structures

phase polymer	structure
poly(ethylene oxide)	$-(CH_2-CH_2-O)_n-$
poly(vinyl methyl ether)	$-(CH_2-CH(OCH_3))_n-$

PEO and PVME were chosen as the phase-forming polymers. (See Table I for structures.) PEO, a water-soluble, linear polyether, is available commercially in a wide range of narrow molecular weight fractions. PVME was chosen for this study because PEO and PVME have similar chemical structures, and, like PEO, PVME is a linear polymer.[24]

The partitioning behaviors of five proteins were examined in this work: bovine α-chymotrypsinogen A, hen egg lysozyme, ovalbumin, human serum albumin (HSA), and bovine serum albumin (BSA). Physical properties of the five proteins are summarized in Table II. Lysozyme is a small, prolate-ellipsoid-shaped protein with a molecular weight of 14 100 and a hydrodynamic radius of about 19 Å.[28] Chymotrypsinogen is a slightly ellipsoidal protein with a molecular weight of 23 200 and a hydrodynamic radius of about 23 Å.[28] Ovalbumin has a molecular weight of 45 000 and a hydrodynamic radius of 29 Å.[28] Human serum albumin (HSA) and bovine serum albumin (BSA) are "cigar-shaped" proteins with axial ratios of approximately 3.5–4.0.[29] The molecular weights of HSA and BSA are about 67 000–69 000, and their hydrodynamic radii are roughly 35 Å. The amino acid sequences of BSA and HSA are approximately 80% identical.[29]

Experimental Section

Materials. PEOs of three different molecular weights, labeled PEO4000, PEO7500, and PEO35000, were used in this study. Molecular weights were determined by size exclusion chromatography using a Hewlett-Packard HP-1090 liquid chromatograph equipped with two Toya Soda TSK 3000PW and one TSK 5000PW columns. The carrier solvent was pure water, flowing at a rate of 1.0 mL/min. The lowest molecular weight polymer, PEO4000, which was obtained from Polysciences (Warrington, PA; Lot No. 45971), had a number-average molecular weight (M_n) of 3520 and a weight-average molecular weight (M_w) of 3720, giving a polydispersity (M_w/M_n) of 1.06. PEO7500 was also purchased from Polysciences (Lot No. 62891), with M_n = 10 100 and M_w = 10 800 (M_w/M_n = 1.06). PEO35000, the highest molecular weight PEO used, was purchased from Fluka AG (M_w/M_n = 1.17, M_n = 29 400, M_w = 34 300).

PVME was obtained from Scientific Polymer Products (Ontario, NY) as a 50% solution in water. The polymer, designated as Lot No. 8, was a viscous, opaque solution with a nominal molecular weight of 100 000. The PVME was broadly distributed, with M_w/M_n = 2.1, M_n = 51 000, and M_w = 110 000.

Chymotrypsinogen, which was obtained from Sigma (Catalog No. C-4879, Lot No. 73F-8120), was type II from bovine pancreas, 6 times crystallized and lyophilized, and was essentially salt free.

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TABLE II: Physical Properties of Proteins[a]

property	BSA	HSA	ovalbumin	lysozyme	chymotrypsinogen
molecular weight	66700	69000	45000	14100	23200
intrinsic viscosity (η), cm^3/g	3.9 ± 0.2	4.2	4.3	2.7	2.5
coefficient of self-diffusion ($D_{20,w}$) × 10^7, cm^2/s	6.1 ± 0.2	6.1	7.4 ± 0.4	11.1 ± 0.5	9.5
$(f/f_0)_D$	1.31	1.29	1.21	1.22	1.20
$(f/f_0)_s$	1.33	1.34	1.14	1.22	1.19
partial specific volume ($\bar{V}$), mL/g	0.734	0.733	0.748	0.703	0.721
molecular dimensions, Å	41.6 × 140.9[b]	38 × 150[b]		45 × 30 × 30	50 × 40 × 40[c]
hydrodynamic radius,[d] Å	35.2	35.2	29.0	19.3	22.6
water uptake at 90% relative humidity and 25 °C, g of H_2O/g of protein	0.29		0.27	0.22	0.26
isoelectric pH	4.8[a]	4.9[e]	4.6–4.83[f]	11.0[e]	9.5[e]

[a] Information from ref 28, except where indicated. [b] From ref 29. [c] From ref 41. [d] Calculated from Stokes' law using diffusion coefficients. [e] From ref 30. [f] From ref 25.

Lysozyme, which was obtained from Sigma (Catalog No. L-6876, Lot No. 65F-8171), was grade I from chicken egg white, 3 times crystallized, dialyzed, and lyophilized, and contained approximately 90% protein, with the remainder primarily buffer salts such as sodium acetate and sodium chloride. Chicken egg albumin (ovalbumin) was obtained from Sigma (Catalog No. A-7641, Lot No. 43F-8050). Ovalbumin was grade VII, crystallized and lyophilized, and was essentially salt free. Human serum albumin was obtained from Sigma (Catalog No. A-1887, Lot No. 124F-9440) and was essentially fatty acid free. Bovine serum albumin was obtained from Sigma (Catalog No. A-7030, Lot No. 45F-0004) and was 98–99% albumin, with the remainder mostly globulins. By use of a refractive index detector and an ultraviolet light detector at 282 nm, all proteins investigated gave a single peak on an SEC trace. This is not necessarily an indication of extreme purity but might show a lack of resolving power of our analytical setup. It was felt, however, that for the purpose at hand the samples could be employed without further attempts at purification.

Water for all experiments was Omnisolv glass distilled water designated as suitable for liquid chromatography.

Preparation of Stock Solutions. Stock solutions of the PEO were made by mixing the appropriate weight of dry polymer with water. Concentrations were checked by drying a sample of the solution in a vacuum oven at 50 °C until the sample weight was constant. The stock solution concentrations for the PEO samples were 30% w/w for PEO4000 and 20% w/w for PEO7500 and PEO35000.

The PVME stock solution was made as follows. The polymer sample was diluted to a concentration of approximately 20%, and the polymer was then precipitated by heating the solution to about 60 °C. This produced a gummy white precipitate. The supernatant was discarded, and the precipitate was then redissolved in water to produce a stock solution with a concentration of 10% or 20% w/w. The concentration of the stock solution was checked by drying a sample of solution in a vacuum oven at 50 °C until the weight was constant (about 2 days).

Protein solutions were made to a 1.0% w/w solution. Protein solutions were not allowed to sit for more than 1 day before use, since solutions stored for longer periods showed signs of instability.

Preparation of Polymer Hydrogels. To make PEO hydrogels, 10 mL of a 20% PEO35000 solution was poured into a 9-cm-diameter Pyrex Petri dish. The Petri dish was then covered with a piece of saran wrap [poly(vinyl chloride-*co*-vinylidene chloride)], sealed with a rubber band, and refrigerated at 5 °C until irradiation. Samples were exposed to high-energy (2.5-MeV) electrons from a van de Graaf generator at a dose rate of either 1.25 or 2.5 Mrad per pass (approximately 0.125 or 0.25 Mrad/s). The number of passes was determined by the desired dose rate. Uniform dose through the depth of the sample was maintained by keeping sample thicknesses low (<5 mm) and by covering the samples with a thin polyethylene strip during irradiation. PEO samples were not cooled during irradiation.

PVME gels were made from a stock solution of 10% PVME by a procedure similar to that for PEO gels. Since PVME has a cloud point at roughly 32 °C,[24] the temperature of the PVME samples was controlled during irradiation by packing the Petri dishes in ice. The ice was replaced after every 2.5-Mrad dose, so that the sample temperature was maintained below ambient (25 °C).

Following irradiation, samples were placed in glass vials with approximately 200 mL of pure water and allowed to swell for 2 days at 20 °C. Samples of the swollen gel weighing approximately 1–5 g were then cut from the gel and used either for determining the polymer concentration in the gel or for protein partitioning experiments. Polymer concentration in the gels was determined by drying the gels in a vacuum oven at 50 °C.

Protein Partitioning between Polymer Hydrogels and Water. In partitioning experiments, a 3.5-cm-diameter disk was cut from the hydrogel, weighed, and placed in a small, wide-mouth glass vial. Approximately 5 mL of 0.1% w/w protein solution was then added to the vial. The system was allowed to sit for 2 days at 20 °C to reach equilibrium. The solution surrounding the gel was then drained and filtered with a 0.22-μm Millex GV filter from Millipore. The concentration of protein in the solution was determined by UV absorbance measurements at either 278 or 290 nm. The concentration of the protein in the gel was then calculated from the protein concentrations in the initial and final solution samples, assuming that the gel weight and volume remained constant during the experiment. Partitioning was carried out at neutral pH.

Protein Partitioning in Two-Phase Aqueous Polymer Systems. Two-phase systems were made by weighing the appropriate amounts of the two polymer stock solutions and pure water into clear disposable polystyrene centrifuge tubes (15-mL capacity) to make 10-g total system weight. For protein partitioning experiments, systems were made so that the system concentration of protein was 0.2% w/w. All weights were measured to 0.0001 of a gram. Protein partitioning was performed at neutral pH. The samples were shaken to thoroughly mix the system, after which the samples were centrifuged for 90–120 min at 3300 rpm in a Beckman Model J2-21 centrifuge. The centrifuge was thermostated at 20 ± 3 °C. Systems that did not separate completely were centrifuged for an additional 90 min and were subsequently stored overnight in a 20 °C room.

When complete phase separation was achieved, a sample was pipetted from the top phase, and then the bottom of the centrifuge tube was broken off to allow a sample to be taken directly from the bottom phase. Phase samples were then diluted by a factor of 10 (or 20, for very concentrated samples) to reduce the concentration of all components in the sample to below 2% w/w. Samples were filtered with 0.2-μm Millex GV disposable filters from Millipore.

After preparation, samples were analyzed by using a Hewlett-Packard HP-1090 liquid chromatograph operating in isocratic (size exclusion chromatography) mode. For analysis of samples for phase diagram measurements, the carrier solvent was pure water. For analysis of samples for protein partitioning, the carrier solvent was water and 0.1 M sodium phosphate buffer (pH 7). Two Toya Soda TSK 3000PW and one TSK 5000PW column purchased from Varian Instruments along with the recommended column guard were installed for separating component peaks. The

system was operated at a flow rate of 1.0 mL/min and thermostated at a temperature of 26 or 34 °C. Temperature was chosen to optimize separation since PVME elution time is very sensitive to column temperature in this range. A Hewlett-Packard 1037A refractive index (RI) detector and a Hewlett-Packard filter photometric (UV) detector at a wavelength of 282 nm were used.

Data from the HPLC detectors were collected on an IBM XT personal computer using a Data Translation DT2801 series analog-to-digital converter and the ASYST data acquisition software package from McMillan publishers. Component concentrations were calculated from the peak areas by using appropriate calibration standards.

In general, only the protein peaks were seen on the UV detector trace, while both polymers and the protein peaks were visible on the refractive index detector trace. Protein concentrations were, therefore, determined from the UV detector trace, while the polymer concentrations were determined from the RI trace after suitably accounting for the protein peaks.

Results and Discussion

A. The Polymer Hydrogel Experiment. *(1) Polymer Concentration in the Polymer Hydrogel.* In general, the concentration of the polymer in the swollen gel depends on a combination of the initial polymer concentration in solution, the polymer molecular weight, and the radiation dose.[15,17-22] This may be understood most easily in terms of the molecular weight of the polymer chain between cross-links formed under various conditions (also called the "interjunction molecular weight"). The greater the interjunction molecular weight (i.e., the less tightly the gel is cross-linked), the more the gel can swell in solution.[26] This relationship can be written[17,18]

$$\frac{1}{M_c} = \frac{2}{M_n} - \frac{(v/V_1)[\ln(1 - v_{2s}) + v_{2s} + \chi v_{2s}^2]}{v_{2r}\left[\left(\frac{v_{2s}}{v_{2r}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2s}}{v_{2r}}\right)\right]} \quad (1)$$

where M_c is the average interjunction molecular weight, M_n is the number-average molecular weight of the polymer used to make the gel, v is the specific volume of the polymer, V_1 is the molar volume of the solvent, v_{2s} is the polymer volume fraction in the swollen network, v_{2r} is the polymer volume fraction in the cross-linked network before swelling, and χ has its usual meaning. v_{2r} is generally equal to the volume fraction of the polymer in the polymer solution before cross-linking.

One would expect the number of cross-links formed to be a function of the energy put into the system, which is proportional to the radiation dose. For a given number of cross-links (i.e., for a given radiation dose) the interjunction molecular weight should increase with increasing initial polymer molecular weight (see eq 1). The interjunction molecular weight at a given radiation dose would also be expected to increase as the initial polymer concentration in solution increases. Increasing the number of chains in solution will decrease the number of cross-links per chain and, therefore, will increase the interjunction molecular weight.

For the gels produced in this study, the polymer molecular weight and concentration in solution were kept constant, so that the polymer concentration in the swollen gel was controlled by varying the radiation dose applied to each gel. The relationship between the dose and the final polymer concentration in the swollen gel for PEO35000 and PVME is shown in Figure 3. Here the polymer concentrations in the gels were measured after the gels, which were in contact with pure water, had reached their equilibrium swollen volumes.

Table III shows the molecular weight of polymer between cross-links and the number of monomer units between cross-links calculated from eq 1. The values for the equation variables were determined as follows. The number-average molecular weight of PEO35000 was 29 400 (see Materials and Methods), and that of PVME was 51 000. χ for PEO in water is 0.44.[27] The χ value for PVME in water is not known; however, since experiments were carried out at 20 °C, which is close to PVME's cloud point of 32 °C,[24] the PVME under these conditions should be close to a

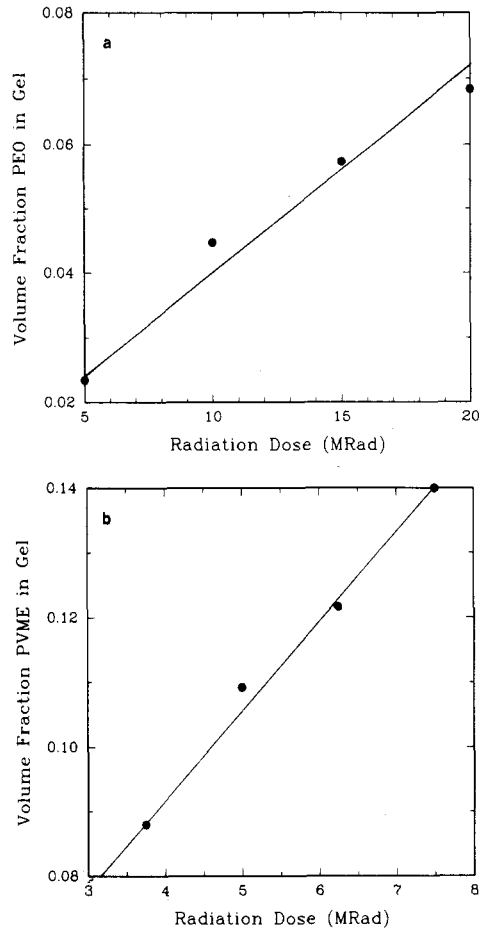

Figure 3. Concentration of polymer in the polymer hydrogel (weight percent) as a function of the radiation dose for gels made from (a) PEO, 20% w/w solution, and (b) PVME, 10% w/w solution.

TABLE III: Physical Characteristics of Polymer Hydrogels

radiation dose, Mrad	vol % polymer in swollen gel[a]	M_c[b]	N[c]	moles of cross-links per gram of solution ($\times 10^5$)[d]	$\langle r^2 \rangle^{1/2}$,[e] Å
		PVME			
3.75	8.8	2350	40	4.25	44
5	10.9	1630	28	6.13	36
6.25	12.2	1350	23	7.43	33
7.5	14.0	1040	18	9.61	29
		PEO			
5	2.3	11200	255	1.78	83
10	4.5	7000	159	2.86	66
15	5.7	4980	113	4.01	56
20	6.8	3790	86	5.27	49

[a] Measurements were obtained from gels that had been allowed to research their equilibrium swollen volume when in contact with a supernatant of pure water. [b] Average molecular weight between cross-links, calculated from eq 1 assuming χ = 0.49 for PVME and 0.44 for PEO. [c] Average number of monomer units between cross-links. [d] Based on the polymer concentration in solution before cross-linking. [e] Estimated distance between cross-links (see text).

Θ state. Therefore, χ for PVME and water was estimated to be 0.49 for all calculations. Note, however, that the calculated values for the interjunction molecular weight depend very strongly on the value of χ and M_n, so that these estimates for the PVME gels may be in error by as much as a factor of 2.

In general, the PVME hydrogels contained a higher polymer volume fraction than the PEO hydrogels, even though the doses applied to the PVME gels were lower. Calculations with eq 1 show that the numbers of monomer units between cross-links were significantly greater for the PEO gels than for the PVME gels. This could be attributed, in part, to the higher polymer concentrations in the solutions used to make the PEO gels. However,

calculations indicate that, for a given dose, the number of cross-links formed in the PVME gels is roughly 3–4 times that of the PEO gels (Table III). This indicates that the energy required to form a cross-link is lower for PVME than for PEO. In the PEO hydrogels, cross-links are believed to form by a complex set of reactions that result in the bonding of carbon atoms on two different PEO chain segments and the abstraction of H_2.[18] For the PVME hydrogels the set of cross-linking reactions is not known, but it is possible that abstraction of the pendant methoxy groups or of methyl hydrogen is also involved.

(2) *Protein Partitioning between Polymer Hydrogels and Water.* Of the proteins studied, only two—lysozyme and chymotrypsinogen—partitioned substantially into both the PEO and the PVME gels. Ovalbumin significantly partitioned into the PEO gels, but not the PVME gels. Neither BSA nor HSA partitioned significantly into either gel, so that χ_s values for these proteins could not be determined from the gel experiments.

The apparent low tendency of the albumins to partition into the polymer hydrogels may be a reflection of a very one-sided distribution coefficient or due to kinetic reasons. If the protein/polymer interaction energy is not very favorable, the proteins will favor the aqueous phase. This tendency is amplified for larger proteins, such as the albumins, since larger biomaterials partition more unevenly than small particles in two-phase systems.[1] Strong partitioning into the aqueous phase would make experimental determination of the partition coefficient difficult, particularly for gels with high polymer concentrations. Calculations for $\chi_s = 0.15$ show that, for partitioning of BSA or HSA in PEO gels at a PEO volume fraction of 4% w/w, the estimated partition coefficient ($[P]_{gel}/[P]_{aqueous}$) is $K = 0.04$. This would be difficult to measure by the gel partition experiment.

Another possibility is that the polymer gels were too tightly cross-linked to permit the proteins to enter the gel, i.e., that the "mesh sizes" were so small as to physically prohibit the proteins from the gels. Average distances between cross-links were approximated roughly by the root-mean-squared end-to-end distance ($\langle r^2\rangle^{1/2}$) of unperturbed chains of molecular weight M_c; this should be a lower bound to the mesh sizes. $\langle r^2\rangle^{1/2}$ can be written[26]

$$\langle r^2\rangle^{1/2} = (CbM_c/M_0)^{1/2}l \qquad (2)$$

where C is the characteristic ratio for the polymer, b is the number of bonds in the backbone of the repeat unit, M_c is the interjunction molecular weight, M_0 is the molecular weight of the repeat unit, and l is the bond length (in angstroms). Values of $\langle r^2\rangle^{1/2}$ given in Table III were calculated for PEO gels with $C = 4.0$, $b = 3$, $M_0 = 44$, and $l = 1.51$ Å.[42] l was calculated as the root-mean-square average for the monomer unit consisting of one C–O bond ($l = 1.45$ Å) and two C–C bonds ($l = 1.54$ Å). Values of $\langle r^2\rangle^{1/2}$ were calculated for PVME gels with $C = 10.0$ (the characteristic ratio for PVME in benzene[42]), $b = 2$, $M_0 = 58$, and $l = 1.54$ Å. The distances between cross-links estimated from eq 2 for PEO range from 49 to 83 Å, indicating that PEO gels are not so tightly cross-linked as to prohibit the proteins from entering the gel. The estimated distances between cross-links for the PVME gels, on the other hand, are 29–44 Å, so that protein exclusion from the PVME gels may be possible.

The most likely reason for the difficulty in measuring partition coefficients for bovine and human serum albumin is the slow diffusion of the proteins into the gels. For large particles such as BSA and HSA the diffusion coefficients of the particle in the gel can be as low as 3×10^{-10} cm^2/s,[18] so that the length of the equilibrium partitioning experiment would have to be on the order of weeks. Experiments of this time scale were not possible since the protein solutions were not sufficiently stable.

For those proteins for which the gel partitioning worked well, calculations from the model were compared with the partitioning data, using the measured polymer concentrations in the gels. The values of χ were taken to be the same as those for the calculation of the interjunction molecular weight; the value used for χ of PVME in water may not be accurate, but this is of little consequence since the model calculations were very insensitive to the value of χ.[9] For all calculations the proteins were assumed to be spheres with radii equal to the hydrodynamic radii as determined from diffusion coefficients found in the literature.[28] The polymer molecular weight was assumed to be infinite for the calculations.

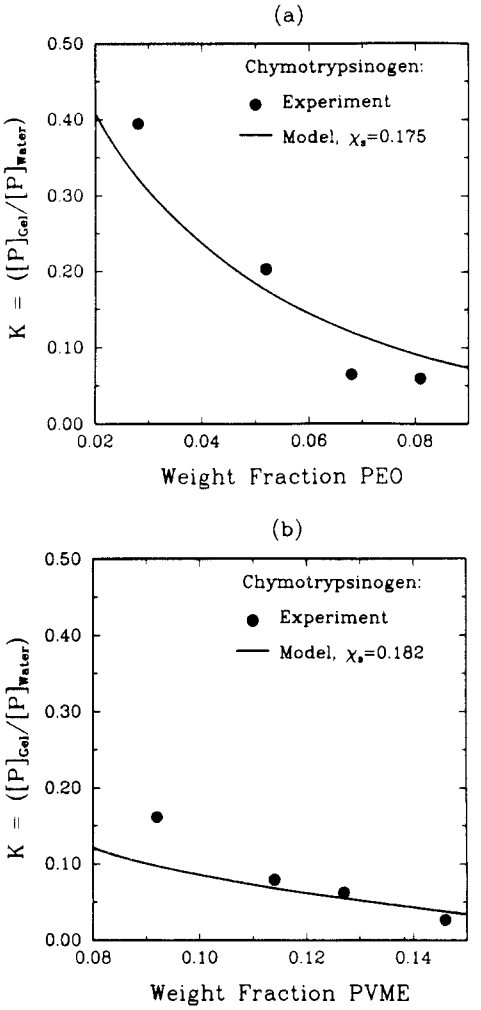

Figure 4. Chymotrypsinogen partition between polymer hydrogels and water at 20 °C. (a) PEO gel, (b) PVME gel.

Results for the partitioning of chymotrypsinogen into the PEO and PVME gels as a function of the polymer concentrations in the gels are shown in Figure 4. The fact that protein partition coefficients were less than one and decrease with phase polymer concentration indicates that in each case the protein favored pure water over the hydrogel. This demonstrates that the overall polymer/protein interaction was repulsive. However, in each case, the value for χ_s was positive, indicating that the interaction energy of the polymer chain segments with the protein surface was (slightly) attractive. The protein/polymer interaction energy appears, therefore, to ameliorate the repulsive interaction of steric exclusion.

Ovalbumin partitioning in PEO gels showed the same behavior as chymotrypsinogen. Model fit to the data shows excellent agreement over the range of polymer concentrations examined. (See Figure 5.)

Lysozyme partitioned differently than chymotrypsinogen and ovalbumin. (See Figure 6.) With both the PVME and the PEO gels, the lysozyme was found to favor the gel phase over the aqueous phase, and the preference for the gel phase increased with increasing polymer concentration in the gel. This behavior cannot be explained by steric interactions alone, since steric exclusion is solely repulsive. It appears that the polymer/protein energy of attraction is strong enough to overcome the repulsive effects of steric exclusion. This is reflected in the high χ_s values that gave the best model fit to the data.

In contrast to the experiments with chymotrypsinogen and ovalbumin, the model's fit to the lysozyme partitioning data was poor for both PEO and PVME gels. The correct trend was

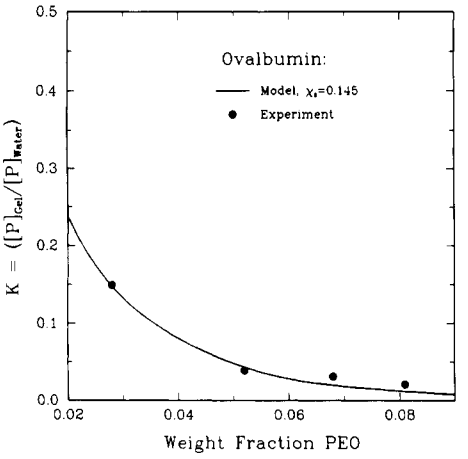

Figure 5. Ovalbumin partition between PEO hydrogel and water at 20 °C.

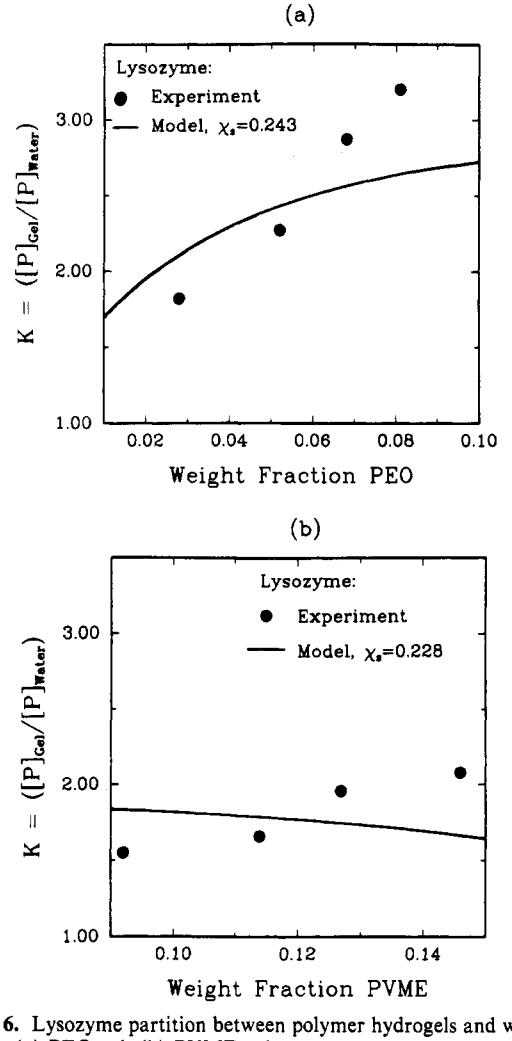

Figure 6. Lysozyme partition between polymer hydrogels and water at 20 °C. (a) PEO gel, (b) PVME gel.

demonstrated for the PEO gel, but for the PVME gel the partition coefficients calculated from the model decreased with the polymer concentration in the gel—the opposite of what was actually observed. No single value of χ_s can account for both the partition coefficient and its concentration dependence; χ_s values chosen to reproduce the trend shown by the data cause the predicted partition coefficients to be much greater than the measured values.

***B*. Protein Partitioning in Two-Phase Aqueous Polymer Systems.** Partitioning of the five proteins was examined in the PEO7500/PVME two-phase system for different phase polymer concentrations. The partitioning of chymotrypsinogen was further studied as a function of the PEO molecular weight.

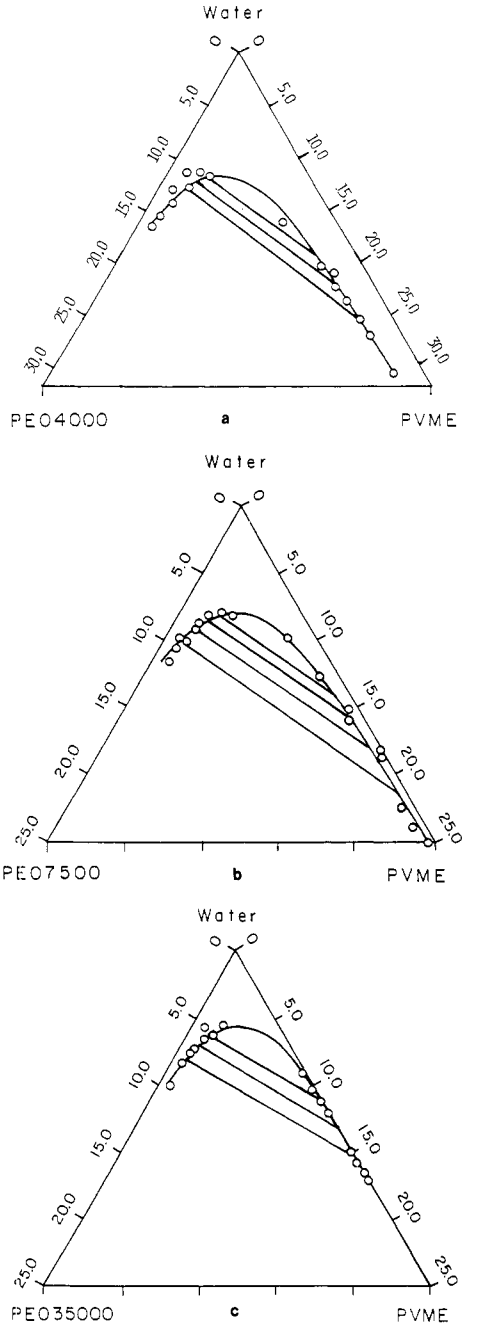

Figure 7. Phase diagram for the PEO/PVME two-phase systems. Concentrations of all components are given in weight percents. (a) PEO4000/PVME, (b) PEO7500/PVME, (c) PEO35000/PVME.

(*1*) *PVME/PEO Phase Diagrams.* The phase diagrams for the three PEO/PVME two-phase systems are shown in Figure 7a–c. The data points indicate experimental equilibrium values, and tie lines through the composition points for the systems in which protein partitioning was studied are also shown. Concentrations of the two phases for the systems in which protein partitioning was investigated are shown in Table IV. In general, the PVME phase in these two-phase systems contains a higher overall polymer concentration than the PEO phase because the PVME molecular weight is higher than that of the PEO.[26] (The number of polymer chains corresponding to a given weight fraction of polymer in solution is greater for PEO than for PVME. Therefore, the mixing entropy of a system consisting of a PEO-rich and a PVME-rich phase, each of equal weight fraction of polymer, is increased if the solvent moves from the PVME-rich phase to the PEO-rich phase.)

(*2*) *Protein Partitioning as a Function of the Phase Compositions.* For all the proteins examined, the protein partitioning became more one-sided as the phase polymer concentrations were

TABLE IV: Representative Phase Compositions[a] for PEO/PVME Two-Phase Systems

overall composition			PEO-rich phase			PVME-righ phase		
PEO	PVME	water	PEO	PVME	water	PEO	PVME	water
			PEO4000/PVME					
6.0	9.0	85.0	7.8	3.6	88.6	3.4	16.8	79.8
7.0	9.0	84.0	9.4	2.8	87.8	3.0	19.4	77.6
8.0	9.0	83.0	10.6	2.2	87.2	2.8	22.8	74.4
			PEO7500/PVME					
4.0	6.0	90.0	5.2	2.8	92.0	1.2	13.2	85.6
3.0	10.0	87.0	6.2	2.2	91.6	1.0	15.4	83.6
3.0	12.0	85.0	7.3	1.7	91.0	0.6	18.4	81.0
6.0	8.0	86.0	8.7	1.3	90.0	0.2	22.6	77.2
			PEO35000/PVME					
2.0	7.0	91.0	4.9	1.2	93.9	0.2	10.8	89.0
2.0	9.0	89.0	6.0	1.0	93.0	0.1	13.0	86.9
2.0	11.0	87.0	7.2	0.7	92.1	0.1	14.9	85.0

[a] All compositions given as percent of total system or phase weight.

TABLE V: Protein/Polymer Interaction Parameters (χ_s)

protein	polymer	χ_s
bovine serum albumin	PEO	0.150[a]
bovine serum albumin	PVME	0.176[b]
human serum albumin	PEO	0.150[a]
human serum albumin	PVME	0.170[b]
ovalbumin	PEO	0.145[c]
ovalbumin	PVME	0.162[b]
chymotrypsinogen	PEO	0.175[c]
chymotrypsinogen	PVME	0.182[c]
lysozyme	PEO	0.243[c]
lysozyme	PVME	0.228[c]

[a] Estimated from model comparison to protein precipitation data in aqueous PEO solutions. [b] From best model fit to the two-phase partitioning data. [c] Measured by model fit to data on protein partitioning between polymer hydrogels and water.

increased. (See Figure 8.) This observation agrees with data in the literature.[1] In comparing the model to the experimental data, where possible, we used polymer/protein interaction parameters determined by the polymer hydrogel experiments. For chymotrypsinogen and lysozyme, χ_s values for both polymer/protein pairs were obtained from the hydrogel experiments, so that the model calculations *contained no adjustable parameters.* χ_s for PEO/ovalbumin was also obtained from the gel experiment. For PEO/HSA and PEO/BSA it was assumed that $\chi_s = 0.15$ since this value has been shown to give accurate predictions for the precipitation of HSA in aqueous PEO solutions.[9] All values of χ_s for PVME with the albumins were chosen so as to give a good fit to the data. The selected values for χ_s are displayed in Table V.

A comparison of experimental data and calculated protein partition coefficients from the model is displayed in Figure 8a–e. Partition coefficients, calculated with no adjustable parameters, for chymotrypsinogen and lysozyme exhibit the same trends as shown by the data and give values of the correct order of magnitude. As expected from the poor model fit for lysozyme partitioning in the hydrogel experiment, the data for the calculated partition coefficient showed substantial deviation from experimental values. The agreement of the predicted chymotrypsinogen partitioning with the data was good. For both proteins, the measured dependence of the protein partition coefficient on the phase polymer concentration was somewhat greater than that anticipated by the model.

Calculations fit the data well for the albumins, which is not surprising, given that the values of χ_s for PVME with the proteins were adjusted for this fit. Interestingly, BSA partitioned significantly more evenly than HSA, even though both proteins are structurally very similar.[29] The model calculations show that the differences in partitioning of BSA and HSA could have been caused by a very small difference in the χ_s value for the two proteins with PVME ($0.006kT$). The large surface area of these proteins means that there are many contacts of polymer chain segments with the protein surface, so that even a small change in the energy per contact can have a significant effect on protein partitioning behavior.

All values of χ_s fell within the narrow range of 0.14–0.25. Since both polymers have similar chemical structures, χ_s for a given protein should not be radically different for the two polymers. However, it is interesting that, for the range of proteins examined, the values of χ_s fall within such a narrow span. The serum albumins are relatively large and are negatively charged at neutral pH,[29] whereas chymotrypsinogen and lysozyme are small and positively charged at neutral pH.[30] Yet all proteins partitioned more favorably into the PEO phase, indicating that charge-related factors were probably not dominant in these experiments.

In addition to calculating partition coefficients, the lattice model can be used to calculate the polymer concentration profiles near the protein surface. The nature of the concentration profiles provides some insight into the relative strength of the various protein/polymer interactions. For all the proteins studied except lysozyme, the calculated concentration of polymer near the protein surface was less than that in the bulk phase. This indicates that the steric exclusion of the polymer chains from the solution near the proteins was the dominant polymer/protein interaction for these proteins.

Steric exclusion is an entropic effect, caused by the constraints that the presence of a solid surface places on the conformational freedom of the polymer chains near the surface. Polymer chains in the bulk solution can take on a greater number of conformations than those near the surface and, consequently, have a higher entropy and a lower Gibbs energy. Consequently, the steric constraints that the presence of a protein in solution places on neighboring polymer chains lead to a reduction in the polymer concentration near the protein surface relative to that in the bulk phase. This can be seen in Figure 9, where the difference between the polymer volume fraction in the first layer of the lattice (i.e., at the protein surface) and in the bulk phase ($\phi_{2,1} - \phi_{2,*}$) is plotted as a function of the polymer volume fraction in the bulk phase. This difference, called depletion, increases with the bulk polymer volume fraction.

For the range of proteins examined, the best model fit to the data was obtained for small positive values of χ_s, meaning that the polymer chain segments are slightly attracted to the protein surface. The protein/polymer interaction energy, therefore, serves to dampen the purely repulsive effect of steric exclusion. For all the proteins except lysozyme, however, the repulsive effect of steric exclusion is stronger than the attractive energy interactions, so that the net protein/polymer interaction is repulsive.

Except for lysozyme, all proteins would favor a pure aqueous solution over both PVME-rich and PEO-rich phases. Since the PVME-rich phases contained higher concentrations of polymer than the PEO-rich phases (see phase diagrams, Figure 7), the proteins were more strongly repulsed by the PVME-rich phases.

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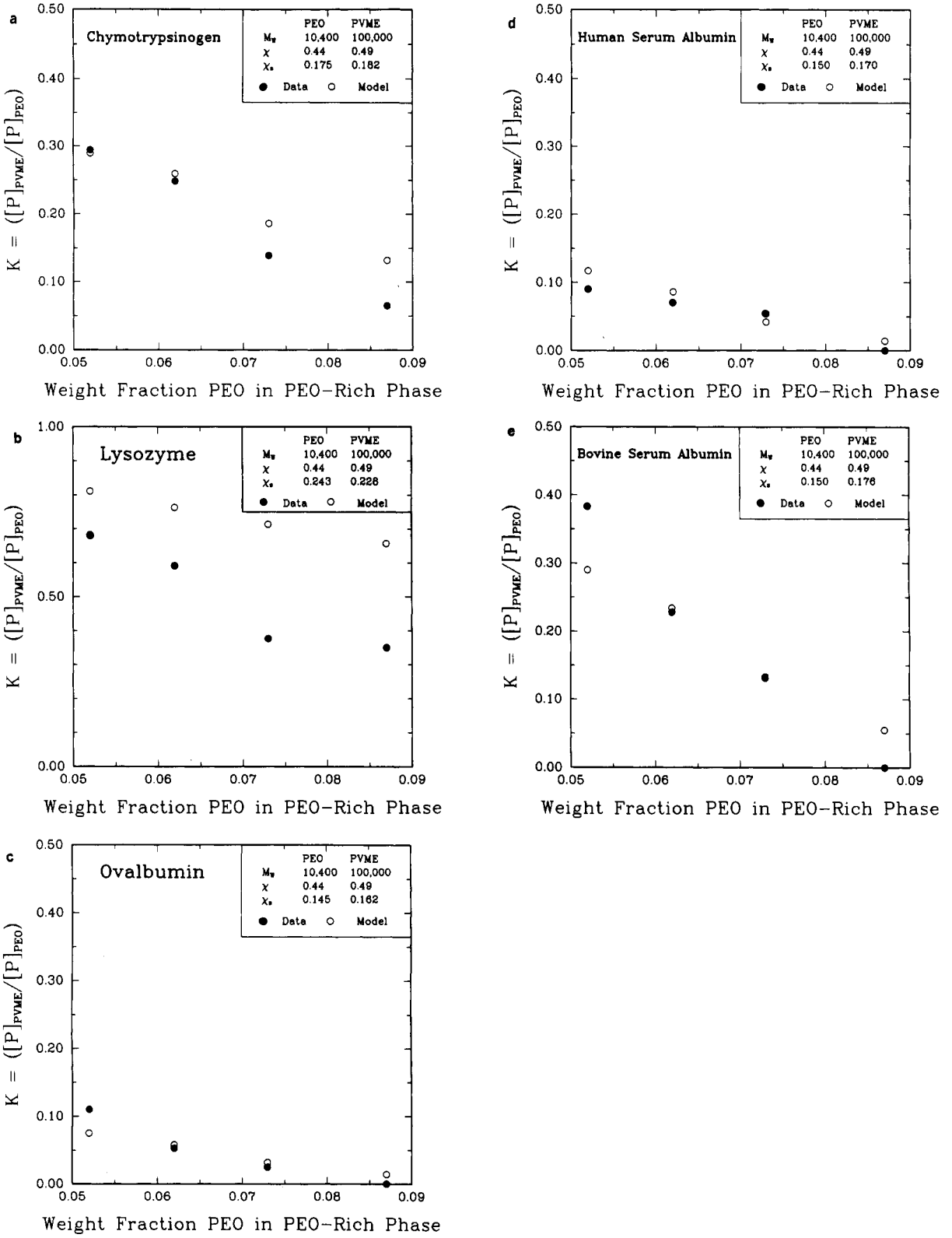

Figure 8. Protein partitioning in PEO7500/PVME two-phase systems. System compositions can be found in Table IV. Model calculations were made with χ_s values from hydrogel experiments where possible (see text for complete discussion). (a) Chymotrypsinogen, (b) lysozyme, (c) ovalbumin, (d) human serum albumin, (e) bovine serum albumin.

The protein partitioning for chymotrypsinogen and the albumins is, therefore, the result of an exclusionary mechanism, in which the protein is "pushed" more strongly out of the PVME phase than out of the PEO phase.

The decrease in the protein partition coefficient with increasing phase polymer concentration is also due to steric exclusion. For systems made at compositions farther from the critical point of the phase diagram, the difference between the polymer concentration in the PVME phase and the PEO phase is greater. As the phase polymer concentration in the system is increased, both phases become more concentrated, but the polymer concentration rises faster in the PVME phase than the PEO phase, so that the degree of "exclusion" of the protein from the PVME phase also increases more rapidly than that in the PEO phase. This leads to stronger protein partitioning into the PEO phase.

The increase in exclusion between proteins and polymer with increasing polymer concentration has also been seen in protein precipitation experiments with PEO.[14,31-35] In these experiments,

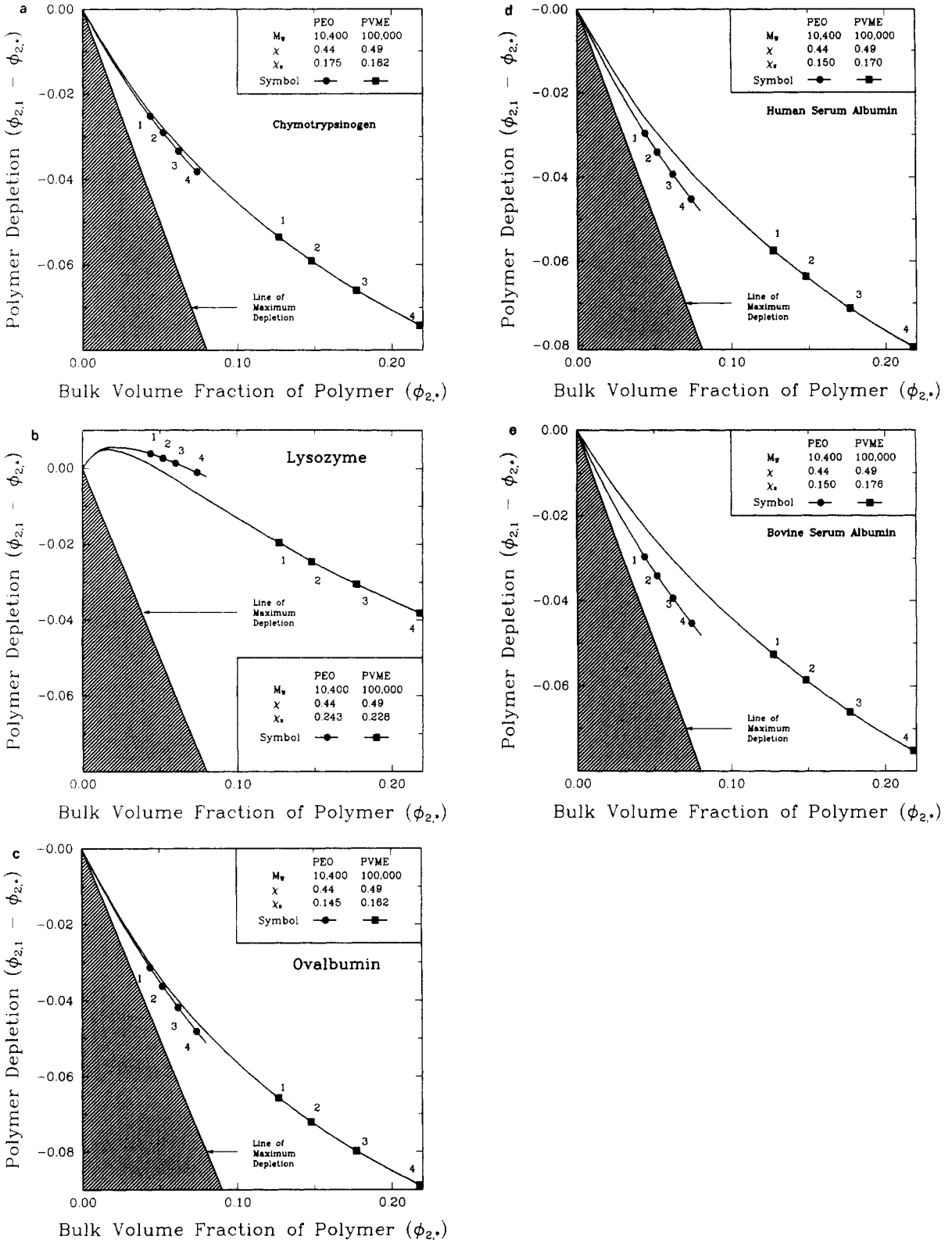

Figure 9. Surface depletion of polymer at the protein surface calculated from the lattice model for the protein partition experiments shown in Figure 8. Symbols on the curves correspond to the actual phase compositions for the systems studied. (a) Chymotrypsinogen, (b) lysozyme, (c) ovalbumin, (d) human serum albumin, (e) bovine serum albumin. ●, PEO curve; ■, PVME curve.

protein solubility in aqueous PEO solutions has been shown to decrease with increasing polymer concentration for a number of different proteins.[14] Lee and Lee,[35] using density measurements and analysis by multicomponent thermodynamic theory, have demonstrated that this effect is due, in part, to the fact that the polymer concentration near the protein surface is less than that in the bulk and that the disparity between the surface and bulk concentrations increases with increasing bulk polymer concentration. Comparison of lattice model calculations with the protein precipitation data of Atha and Ingham gave similar results.[9]

The exclusion mechanism which appeared to govern the partitioning of the other proteins is not operative for lysozyme. In

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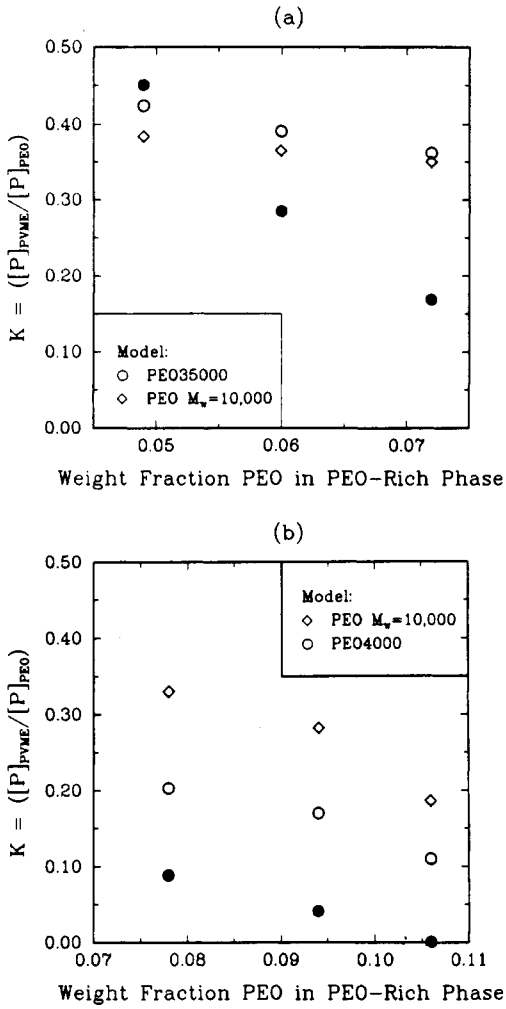

Figure 10. Partition of chymotrypsinogen as a function of the PEO molecular weight. See table IV for system compositions. Systems: (a) PEO35000/PVME, (b) PEO4000/PVME. Model calculations were made using the same parameters as those shown in Figure 8a except for the PEO molecular weights. Calculations are for actual PEO molecular weights (○) and for PEO molecular weight of 10000 (◇).

this case, the gel partitioning experiments showed that the protein/polymer interaction energy is favorable enough to *enhance* the solubility of the protein in the aqueous polymer phases (relative to pure water). Hence, the partitioning of lysozyme in two-phase aqueous polymer systems is strongly influenced by net attractions of the protein to the phase polymers. This can be seen from the surface depletion plot (Figure 9a) which shows that the depletion of phase polymers from the surface of lysozyme is much smaller than for the other proteins studied. The relatively even partitioning of lysozyme can be attributed to the fact that lysozyme is much smaller than the other proteins investigated and has less surface area for energy contacts. This also agrees with the generally observed trend that small particles partition more evenly in two-phase systems than large ones.[1]

(3) Partitioning as a Function of Phase Polymer Molecular Weight. To examine the effect of phase polymer molecular weight on protein partitioning, experiments with chymotrypsinogen in PEO/PVME two-phase systems were performed using three different molecular weights for PEO. In general, the chymotrypsinogen was found to partition increasingly into the PVME phase as the PEO molecular weight was increased from 3520 to 29400 (see Figure 10), in agreement with trends previously shown in the literature for protein partitioning in two-phase systems.[1,36]

This result is not solely the effect of the PEO molecular weight on the strength of the PEO/chymotrypsinogen interactions,

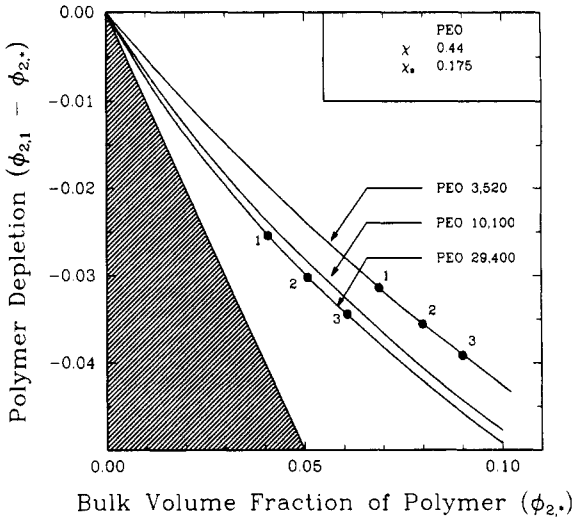

Figure 11. Surface depletion of PEO calculated from the lattice model for chymotrypsinogen. Filled circles indicate the actual PEO volume bulk fraction for the partitioning experiments in the PEO4000 and PEO35000 systems.

however, since changing the PEO molecular weight alters the compositions of the phases. (See phase diagrams, Figure 7.) To separate the two effects, model calculations were first performed for the PEO4000 and PEO35000 systems using the actual phase compositions but assuming a PEO molecular weight of 10000 (the approximate molecular weight of PEO7500). The same calculations were then made using the actual PEO molecular weights for each of the systems. No parameters were adjusted to improve the fit of the model to the data. The results of these calculations are shown in Figure 10. The model reproduces the trends shown by the data, but the fit is not quantitative.

In Figure 11, the calculated depletion of the phase polymer from the protein surface is plotted as a function of the bulk polymer concentration for each of the molecular weights of PEO investigated. According to the calculations, as the PEO molecular weight increases, so does the difference between the volume fraction of PEO at the protein surface and in the bulk phase. This finding agrees with the data of Lee and Lee, who found that the deficiency of PEO in the domain of calf brain tubulin in aqueous PEO solutions increased with increasing PEO molecular weight.[35]

The change in the difference between the volume fraction of PEO next to the protein and that in the bulk phase is seen to decrease with increasing PEO molecular weight, however, as an infinite molecular weight limit is reached. (The model calculations for PEO7500 and PEO35000 exhibit a smaller difference than those for PEO4000 and PEO7500.) This decrease in the effect of PEO molecular weight on PEO/protein exclusion is in agreement with previous protein partitioning data[36] and with data for the precipitation of proteins in aqueous PEO solutions.[14]

***C.* Interpretation of χ_s.** χ_s is a measure of the energy preference for a polymer chain segment, rather than a solvent molecule, to be next to the protein surface. When χ_s is positive, the polymer chain segment is more attracted to the surface than is the solvent molecule. Protein molecules are generally "hydrophilic".[37] (Dry proteins take up roughly 0.25 g of water per gram of protein at 90% relative humidity.[28,38]) Although both PEO and PVME contain polar groups,[37] it seems unlikely that the energy interactions of the polymer segments with charged or polar regions on the protein would be more favorable than that of water.

Aside from polar and charged groups, many proteins also have significant nonpolar surface regions with which water interacts unfavorably.[30,38] To minimize the interactions of polar water groups with the nonpolar surface groups, the water molecules

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TABLE VI: Strengths of Molecular Interactions at Ambient Temperatures[a]

type of interaction	interaction energy[b]
C–H covalent bond (in CH_4)	172
ion/dipole interaction (water/Na^+)	39
hydrogen bond	4–16
hydrophobic effect	4–5
van der Waals	1
typical χ_s value	0.18

[a] All values, except that for χ_s, are from ref 37. [b] In kT.

orient themselves so as to form an ordered surface.[39,40] The surface energy of these water/nonpolar surfaces can be quite high, due to the surface tension of water and the ordered state of the water molecules.[37] The interactions of water with nonpolar materials produce an "entropic" driving force which acts to minimize the hydrophobic surface area in aqueous solutions (the less hydrophobic surface area, the less solvent ordering in the system). This effect, known as the hydrophobic effect or hydrophobic bonding, makes the aggregation of hydrophobic surfaces in aqueous solution much stronger than it would be in the absence of water.[37,40]

The polymer molecules in solution probably interact more favorably with the hydrophobic regions of the protein than the water molecules, and this could lead to a net positive value of χ_s. Although χ_s is intended to be an enthalpy parameter only, it is advantageous to let it encompass a contribution from the change in entropy due to the displacement of highly ordered water molecules near the nonpolar region. (This entropy change is otherwise not accounted for in the lattice model, since only the conformational entropy of the polymer chains and the entropy of mixing contribute to the system entropy.)

Could the hydrophobic effect account for the interactions that have been seen in this modeling? To better understand the strength of these interactions, χ_s can be compared to some typical molecular interactions. From Table VI, it can be seen that χ_s is less than even the strength of van der Waals interactions. One must bear in mind, however, that χ_s is the polymer segment interaction energy with a surface patch on a protein with a *homogeneous* surface, so that χ_s is an average polymer segment/protein surface interaction energy. If polymer segment interaction energies with nonhydrophobic surface regions are not favorable, then since hydrophobic regions cover only a small fraction of the total protein surface, a strong polymer segment interaction energy with a hydrophobic surface patch could easily average out over the entire surface of the particle to a small χ_s value. Judging from the values in Table VI, this type of "average" energy interaction could easily account for the values of χ_s obtained.

It appears, therefore, that χ_s could reflect the favorable interaction of the phase polymers with hydrophobic regions on the proteins. Since χ_s is an averaged quantity, it is not possible to correlate it with specific physical quantities. However, the values obtained for χ_s are physically reasonable when compared with other intermolecular forces.

Summary and Conclusions

The partition coefficients of five proteins—BSA, HSA, ovalbumin, chymotrypsinogen, and lysozyme—in PEO/PVME two-phase aqueous polymer systems have been measured and the data have been compared to results from a lattice model developed recently.[9] Model calculations require the measurement of the protein/polymer interaction parameter χ_s for the protein with both phase-forming polymers. Values of χ_s for chymotrypsinogen and lysozyme and one value of ovalbumin were estimated by fitting the model to data obtained for the partitioning of the proteins between water and polymer hydrogels made from PEO and PVME.

Using these values of χ_s, it was possible to predict partition coefficients for the partitioning of the proteins in PEO/PVME two-phase systems. Model predictions were in good agreement with data for lysozyme and chymotrypsinogen partitioning in PEO7500/PVME two-phase systems. Predictions of chymotrypsinogen partitioning as a function of PEO molecular weight were not in quantitative agreement with data but demonstrated the correct trends.

The albumins did not partition significantly enough into either of the hydrogels to obtain χ_s values from measurements on hydrogels. Consequently, for these proteins the model was fit to partitioning data from two-phase aqueous polymer system. All values of χ_s fell within the range 0.14–0.25.

For all of the proteins except lysozyme, model calculations indicated that partitioning was due to repulsion of the protein from the two phases. The PVME phases exclude the proteins more strongly since they contain a higher concentration of polymer. Lysozyme, on the contrary, interacts with the polymers favorably enough to overcome the steric exclusion effects.

The partition of chymotrypsinogen is seen to increasingly favor the PVME phase as the molecular weight of the PEO is increased. This is due both to the greater steric exclusion between the higher molecular weight PEO and the protein and to the change in phase composition caused by the changing molecular weight. The steric exclusion effect approaches a limit with increasing molecular weight.

In summary, for the proteins examined in this work, the lattice model calculations are in agreement with the data obtained. Also, the lattice model provides physical insight through calculations of the polymer concentration profiles near the protein surface. The molecular level description provided by the model gives a degree of detail not available from other models currently available.

The value of the model is tempered somewhat by the unrealistically simplistic description of the protein as a sphere with homogeneous surface properties. Clearly, this picture must be improved if the model is to provide an accurate description of protein/polymer interactions. Also, the model must be modified to incorporate charge related effects.

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Registry No. PEO, 25322-68-3; PVME, 9003-09-2; water, 7732-18-5; chymotrypsinogen, 9035-75-0; lysozyme, 9001-63-2.

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