References and Notes

(1) Patel, G. N.; Chance, R. R.; Witt, J. D. *J. Chem. Phys.* **1979**, *70*, 4387. Patel, G. N.; Chance, R. R.; Witt, J. D. *J. Polym. Sci., Polym. Lett. Ed. 1978*, *16*, 607.
(2) Chance, R. R.; Patel, G. N.; Witt, J. D. *J. Chem. Phys.* **1979**, *71*, 206.
(3) Walters, G.; Painter, P.; Ika, P.; Frisch, H. *Macromolecules* **1986**, *19*, 888.
(4) Chance, R. R.; Baughman, R. H.; Muller, H.; Eckhardt, C. J. *J. Chem. Phys.* **1977**, *67*, 3616.
(5) Eckhardt, H.; Eckhardt, C. J.; Yee, K. C. *J. Chem. Phys.* **1979**, *70*, 5496.
(6) Rubner, M. F. *Macromolecules* **1986**, *19*, 2114. Rubner, M. F. *Macromolecules* **1986**, *19*, 2129.
(7) Sandman, D. J.; Samuelson, L. A.; Velazquez, C. S. *Polymer Commun.* **1986**, *27*, 242.
(8) Skrovanek, D. J.; Howe, S. E.; Painter, P. C.; Coleman, M. M. *Macromolecules* **1985**, *18*, 1676. Skrovanek, D. J.; Painter, P. C.; Coleman, M. M. *Macromolecules* **1986**, *19*, 699.
(9) Seymour, R. W.; Cooper, S. L. *Macromolecules* **1973**, *6*, 48. Schroeder, L. R.; Cooper, S. L. *J. Appl. Phys.* **1976**, *47*, 4310.
(10) Hannon, M. J.; Koenig, J. L. *J. Polym. Sci., Part A-2* **1969**, *7*, 1085.
(11) See, for example: Cameron, D. G.; Casal, H. L.; Mantsch, H. H. *Biochemistry* **1980**, *19*, 3665. Naselli, C.; Rabe, J. P.; Rabolt, J. F.; Swalen, J. D. *Thin Solid Films* **1985**, *134*, 173 and references therein.
(12) (a) Chance, R. R.; Eckhardt, H.; Swerdloff, M.; Federici, R. R.; Szobota, J. S.; Turi, E. A.; Boudreaux, D. S. In *Solid State Polymerization: The Structure and Properties of Lattice Controlled Processes*; Sandman, D. J., Ed.; American *Chemical Society*: Washingtron, D.C., *ACS Symp. Ser.* in press. (b) Eckhardt, H.; Boudreaux, D. S.; Chance, R. R. *J. Chem. Phys.* **1986**, *85*, 4116.
(13) Rubner, M. F., unpublished results.
(14) Downey, M. J.; Hamill, G. P.; Rubner, M. F.; Sandman, D. J.; Velazquez, C., to be published.
(15) Chance, R. R. *Macromolecules* **1980**, *13*, 396.
(16) Orchard, B. J.; Tripathy, S. K. *Macromolecules* **1986**, *19*, 1844.

Thermodynamics of the Separation of Biomaterials in Two-Phase Aqueous Polymer Systems: Effect of the Phase-Forming Polymers

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ABSTRACT: The liquid lattice theory of Scheutjens and Fleer for the adsorption of polymers from dilute solution to infinite flat surfaces was modified to be applicable to solid particles of arbitrary size and shape with homogeneous surface properties. The model was applied to globular proteins, assumed to be representable as hard spheres, and the Gibbs energy per particle was calculated as a function of the particle size, the polymer chain length, the polymer concentration, the Flory–Huggins interaction parameter, and the segment–surface interaction parameter. Applications of the model to the distribution of proteins between the phases of aqueous two-phase polymer systems and to protein precipitation from solution by the addition of poly(ethylene glycol) yielded results in good agreement with experimental data.

Introduction

Recent advances in the fields of medical technology and biochemistry have focused interest on understanding the nature of interactions between biological macromolecules and other natural and synthetic polymers. Such an understanding is critical to applications ranging from the development of biocompatible materials for artificial organs to the use of polymers as flocculating or stabilizing agents in biopolymer solutions. One particular area of interest to the biochemicals industry is the use of water-soluble polymers to separate colloidal bioparticle mixtures by using so-called "two-phase aqueous polymer systems".

It has long been known[1-4] that, in general, solutions containing two different polymers are immiscible and resist formation of a single phase even when the system is diluted with considerable amounts of a solvent that is completely miscible with the pure polymers. This behavior has been explained in the framework of polymer solution theory,[5-7] and many examples of three-component systems (polymer X/polymer Y/solvent) containing large two-phase regions in the phase diagram are presently known.[1-4]

In the late 1950s, Albertsson[1] discovered in his work with aqueous two-phase polymer systems that low concentrations of a fourth component, i.e., a third solute, added to such systems distributed unevenly between the two phases, so long as this new component was relatively "large" (solutes with dimensions roughly in the range 30 Å [proteins] to 10 μm [whole cells] were investigated). Albertsson exploited the preference of particles for one of the two phases successfully in designing a countercurrent extraction process for the separation and purification of biological macromolecules and colloidal systems.[1,8-10] Since then, extensive experimental research on these liquid–liquid extraction systems has been conducted in many laboratories (see ref 1, 11, and 12 for a literature review) and large-scale separation processes have been developed.[1,13,14] The technique has many practical advantages: the process may be operated continuously in a countercurrent configuration and is easily scaled up.[13] Perhaps most significantly, however, the technique provides a gentle, protective environment for the biological material since both phases in the biphasic system are comprised primarily of water. (Enzymes, for instance, typically retain a large fraction of their activity during such separations.[1,13])

Despite the success of aqueous two-phase polymer systems for biomaterial separation, the factors and mechanisms that bring about the uneven distribution of particles between the two phases are not understood. Some heuristic rules exist,[1,12] but no comprehensive theory has been developed to help guide the design of systems for the separation of specific mixtures. As a result, application of the technique requires tedious hit-or-miss experimentation.[13]

Here, we develop a model of the basic interactions and mechanisms acting in the distribution of particulates between two polymer solution phases. The model provides a detailed description of the particle in aqueous polymer solution but does not include the effects of salts and pH.

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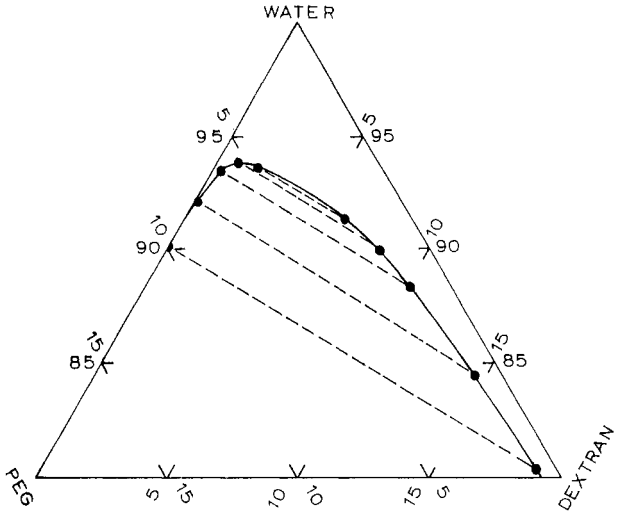

Figure 1. Phase diagram for the Dextran 68/PEG 6000 system at 20 °C from ref 1.

While it is, of necessity, a simplification of the true physical system, the model does permit an analysis of such systems. In addition, the model gives some insights into the precipitation of proteins from solution by the addition of water-soluble polymers,[15] a phenomenon which has, thus far, resisted successful interpretation.

System Considered

In general, the combination of aqueous solutions of two polymers forms a biphasic system. Often the polymer concentrations required for phase separation are low (<5% of the total system concentration by weight), so that each phase is composed mainly of water, with the remainder being primarily one of the two phase-forming polymers (for an example, see Figure 1). It is also common in biomaterial separations to add a buffer, e.g., sodium phosphate buffer, in order to make the solution isotonic; salt concentrations generally are on the order of 0.1 mol/L or less.

The partition coefficient, K_P, for particles P between the two phases A and B of such a biphasic system is defined as

$$K_P \equiv C_P^A / C_P^B$$

where C_P^A and C_P^B are the concentrations of the particles in phases A and B, respectively.

The considerations that follow are largely independent of the choice of particle and hold for silica or gold colloids, globular proteins, polystyrene lattices, etc. The main thrust of this work, however, is toward an understanding of the interactions of globular proteins with other polymers in aqueous solution, and the data used to assess the model are drawn exclusively from experiments with proteins. In this paper, therefore, we focus on globular proteins and often use the terms "particle" and "protein" interchangeably. It should be remembered, however, that the term "protein" is used to indicate a single molecule or stable associate; multimerization and aggregation phenomena are not considered here.

The partition coefficient for proteins has been found empirically to depend on a number of different factors, which are listed in Table I. To a first approximation, the particle interactions with each species in the phase may be examined independently,[10] so that

$$\ln K_P = \ln K_P^{polymer} + \ln K_P^{other} \quad (1)$$

where $K_P^{polymer}$ and K_P^{other} are the contributions to the particle distribution coefficient of the phase polymers and other factors (such as buffering salts, pH, etc.), respectively.

Table I
Factors Found Empirically To Govern Partitioning of Proteins

polymer-dependent factors	protein-dependent factors	ion-dependent factors
structure of polymers	protein charge	types of ions
M_w of polymers	size of protein	concn of ions
concn of polymers	shape of protein	solution pH

Here, we will examine only those factors that govern $K_P^{polymer}$. For the remainder of this paper, $K_P^{polymer}$ will be denoted simply by K_P.

Assuming the protein concentration to be sufficiently low to allow the application of ideal solution limits, the distribution coefficient may be written in terms of the Gibbs energy per particle as

$$K_P = \exp[(g_B - g_A)/kT] \quad (2)$$

where g_A and g_B are the particle Gibbs energies in phases A and B, respectively. (Strictly, from the model described here, changes in the Helmholtz energy are obtained; in the lattice model, however, it is assumed that there is no volume change of mixing. Since the system modeled is at constant pressure, changes in the Helmholtz energy are identical with changes in the Gibbs energy.)

To calculate the Gibbs energies, we proceed to develop a lattice-based model for the particle in a polymer solution phase. By use of statistical mechanics, an expression for the particle Gibbs energy (and, for the two phases, also the particle distribution coefficient) can be derived in terms of system parameters, thereby permitting an evaluation of the effects of the phase-forming polymers on the particle behavior.

Lattice Model

In order to simplify the problem of modeling a protein in an aqueous polymer solution, we make several assumptions.

Assumption 1. The phases contain protein, water, and only one of the two phase polymers. Most biological separations are performed in systems far from the critical point where the concentration of the minority polymer is small enough to be neglected.

Assumption 2. The phase compositions are known and do not change during the separation. Usually, the biomaterials are present at low enough concentrations that their partitioning does not change the polymer/water compositions of the phases.

Assumption 3. The phase polymer is linear (unbranched), homogeneous, and uniform with respect to molecular weight and constitution.

Assumption 4. The (biological) solute particle is a rigid solid of known size and shape.

Assumption 5. The (biological) solute particle has homogeneous surface properties.

Assumptions 4 and 5 represent a significant modification of true physical systems. Biological particles, clearly, do not have homogeneous surface features, nor have their sizes and shapes been well characterized. However, it is generally true that the phase-forming polymers do not bind specifically to sites on the biomaterials. (This is not true for polymers which have been modified to include affinity ligands for the biomaterial. We do not consider this case here.) As a simplification, we assume that the surface features of the biomaterial may be averaged over the enitre surface of the particle.

Under these assumptions, our problem reduces to one of modeling a rigid body of colloidal dimensions in an aqueous polymer solution. To do this, we have chosen to

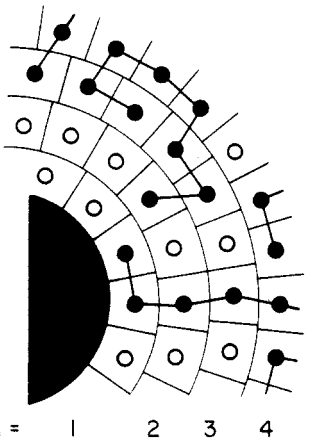

Figure 2. Lattice model for a spherical particle in an aqueous polymer solution. The lattice is shown here in two dimensions; however, the actual lattice would be three dimensional. Only part of the lattice is shown.

modify the liquid lattice model developed by Scheutjens and Fleer[16,17] for the adsorption of polymer chains to a flat plate. The solute (protein) is pictured as a solid body in the center of a curved lattice whose shape depends on the shape of the protein (see Figure 2). The lattice contains M shells of near-cubic lattice sites, the layer number i being indexed beginning with $i = 1$ at the surface of the particle. A lattice site must have dimensions such that a chain may be accommodated by the cell regardless of orientation; this corresponds, roughly, to 3.6 methylene units for alkyl chains.[18] The number of lattice sites in layer i (L_i) is a function of the shape of the particle and the layer number. For the simplest case, a spherical particle of radius R (R is measured in units of shell thickness), the dependence is given by

$$L_i = (4\pi/3)[3(R + i - 1)(R + i) + 1] \quad (3)$$

[The layer i of the lattice has a volume equal to $4\pi a^3/3[(R + i)^3 - (R + i - 1)^3]$, where a is the length of a side of a lattice site. A single lattice site has a volume of a^3, so we may write $L_i = 4\pi/3[(R + i)^3 - (R + i - 1)^3]$. Multiplying out the cubes and collecting terms gives eq 3.] For all calculations in this paper, we confine ourselves to spherical particles only; for the sake of generality, however, we will continue to use L_i in all formulas.

A lattice site in layer i is bordered, on the average, by Z sites. The "coordination number" Z for a curved hexagonal lattice (i.e., one which, very far from the particle at $i \rightarrow \infty$, approaches a plane hexagonal lattice) is

$$Z = 12 \quad (4)$$

The fraction of nearest-neighbor sites in layer j to a site in layer i is denoted as $\lambda_i(j-i)$, where

$$\begin{aligned} \lambda_i(j-i) = \lambda_i(0) &= 1 - 3(L_{i+1} + L_{i-1})/(ZL_i), \quad j = i \\ &= \lambda_i(1) = 3(L_{i+1}/L_i)/Z, \quad j = i + 1 \\ &= \lambda_i(-1) = 3(L_{i-1}/L_i)/Z, \quad j = i - 1 \\ &= 0, \quad j \notin \{i-1, i, i+1\} \end{aligned} \quad (5)$$

From these definitions, $\sum_{\nu=-1}^{1}\lambda_i(\nu) = 1$ except for layer 1, where $\sum_{\nu=-1}^{1}\lambda_i(\nu) = 1 - \lambda_1(-1)$. Each site in the lattice is occupied by either solvent or a polymer segment.

We follow Scheutjens and Fleer[16,17] by representing polymer chains in the lattice as a connected series of segments, each chain r segments long. We imagine the chain segments to be serially numbered. The position of a chain in the lattice may then be designated by writing a set of ordered pairs such that the first number in each pair specifies the index of the segment in the polymer chain and the second number the layer number in which the segment is located. For example, the position of a polymer chain with its first segment in layer i, its second in layer j, its third in layer k, and so forth, would be denoted as $(1,i)(2,j)(3,k)$.... This grouping of ordered pairs is called a *chain ordering*. Note that a chain ordering does not describe a single chain conformation but, rather, a set of n_c different chain conformations (for an example, see Figure 3). The values of the elements in the set, $\{n_c\}$, can be estimated by a procedure developed by Scheutjens and Fleer.[16,17]

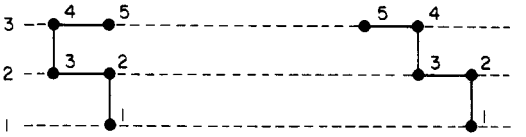

Figure 3. Two different polymer chain conformations for the chain ordering (1,1)(2,2)(3,2)(4,3)(5,3). Other conformations would also be possible. The way of enumerating the number of chain conformations for a given chain ordering is discussed in Appendix I.

Due to the presence of the particle (protein) in the center of the lattice, the concentration of polymer chain segments in the lattice will be anisotropic; the volume fractions in layer i of polymer chain segments, $\phi_{2,i}$, and solvent molecules, $\phi_{1,i}$, will be functions of the layer number i. The volume fractions are

$$\phi_{1,i} = n_{1,i}/L_i \qquad \phi_{2,i} = n_{2,i}/L_i \quad (6)$$

where $n_{1,i}$ is the number of solvent molecules and $n_{2,i}$ is the number of polymer chain *segments* in layer i. Since all sites in the lattice are occupied by either a solvent molecule or a chain segment, we have

$$\phi_{1,i} = 1 - \phi_{2,i} \quad (7)$$

Overall in the lattice, there are a total of $n_1 = \sum_i L_i\phi_{1,i}$ solvent molecules and $n_2 = \sum_i L_i\phi_{2,i}/r$ polymer chains.

Thermodynamic Quantities

The Gibbs energy may be expressed as

$$g = -kT \ln \Xi \quad (8)$$

where Ξ is the grand canonical partition function for the system consisting of one particle in the lattice. The grand canonical partition function is defined[19] by

$$\Xi = \sum_{\{n_c\}} Q_{\{n_c\}} \exp(\sum_i L_i\phi_{1,i}\mu_1/kT) \exp(\sum_i L_i\phi_{2,i}\mu_2/rkT) \quad (9)$$

where Q is the canonical partition function for the system and μ_1 and μ_2 are the chemical potentials of the solvent and the polymer. The canonical partition function for the system may be written[16,17] as

$$Q = (\Omega/\Omega^+) \exp(-\Delta U/kT) \quad (10)$$

where Ω is a combinatorial factor which describes the number of different ways of arranging the polymer chains and solvent molecules in the lattice, Ω^+ is the combinatorial factor for the same polymer chains in the pure bulk reference state, and ΔU, the internal energy of mixing, is the sum of the interaction energies for the given state of the solution. The reference state for the calculation of Q is pure solvent and pure disordered bulk polymer. We evaluate Q as follows.

The internal energy of mixing for a given state, ΔU is the sum of the interaction energies of unlike nearest neighbors in the lattice. There are three types of unlike nearest-neighbor contacts which may occur: (1) segment–surface, (2) solvent–surface, and (3) segment–solvent.

The first two interactions are adsorption energies for the polymer and the solvent, whereas the third interaction is the energy of mixing for the polymer and the solvent. The contribution to the internal energy from the adsorption of polymer and solvent to the surface (1 and 2 above) is given by

$$n_{2,1}u_{2|s} + n_{1,1}u_{1|s}$$

where $n_{2,1}$ and $n_{1,1}$ are, respectively, the number of polymer chain segments and solvent molecules in the first layer of the lattice (see above), and $u_{2|s}$ and $u_{1|s}$ are the corresponding adsorption energies.[16]

The total segment–solvent energy depends on the number of segment–solvent contacts in the lattice. On the average, a molecule in layer i will have $Z\lambda_i(-1)$ site contacts with cells in layer $i-1$. A fraction $\phi_{2,i-1}$ of these contacts will be with polymer segments and $\phi_{1,i-1}$ with solvent molecules. Using the Bragg–Williams approximation *within a layer*,[16] we may express the number of polymer contacts of a solvent molecule in layer i as $Z[\lambda_i(-1)\phi_{2,i-1} + \lambda_i(0)\phi_{2,i} + \lambda_i(1)\phi_{2,i+1}]$. Summing these contacts over all of the $n_{1,i}$ solvent molecules in layer i gives the total number of polymer–solvent contacts as

$$\sum_i n_{1,i}Z[\lambda_i(-1)\phi_{2,i-1} + \lambda_i(0)\phi_{2,i} + \lambda_i(1)\phi_{2,i+1}] \quad (11)$$

Defining the average site volume fraction for polymer segments, $\langle\phi_{2,i}\rangle$, and for solvent molecules, $\langle\phi_{1,i}\rangle$, as

$$\langle\phi_{2,i}\rangle = [\lambda_i(-1)\phi_{2,i-1} + \lambda_i(0)\phi_{2,i} + \lambda_i(1)\phi_{2,i+1}] \quad (12)$$

$$\langle\phi_{1,i}\rangle = [\lambda_i(-1)\phi_{1,i-1} + \lambda_i(0)\phi_{1,i} + \lambda_i(1)\phi_{1,i+1}] \quad (13)$$

the total number of segment–solvent contacts becomes

$$Z\sum_i n_{1,i}\langle\phi_{2,i}\rangle = Z\sum_i n_{2,i}\langle\phi_{1,i}\rangle \quad (14)$$

From eq 14 we may now write the expression for the energy term in eq 9 as

$$\Delta U/kT = (n_{2,1}u_{2|s} + n_{1,1}u_{1|s})/kT + \chi\sum_i n_{1,i}\langle\phi_{2,i}\rangle$$

$$\Delta U/kT = L_1(u_{1|s}/kT - \chi_s\phi_{2,1}) + \chi\sum_i n_{1,i}\langle\phi_{2,i}\rangle \quad (15)$$

where χ is the Flory–Huggins interaction parameter (defined as $\chi = Zu_{1|2}/kT$), and χ_s is the relative adsorption energy of a polymer chain segment with respect to the solvent and is defined by[16,19]

$$\chi_s \equiv (u_{1|s} - u_{2|s})/kT \quad (16)$$

χ_s is positive when the segments are attracted to the surface, negative when they are repelled from the surface, and zero when chain segments and solvent molecules interact equally with the surface.

The combinatorial factor Ω/Ω^+, required in eq 10, is calculated by determining the number of ways of placing a set of polymer chains with specified orderings in the lattice. The derivation, following closely the work of Scheutjens and Fleer,[16,17] is rather lengthy; our development differs from theirs in that L_i cannot be assumed to be constant here. Details may be found in Appendix I. Briefly, an expression is developed for the number of ways of placing a single chain with a given ordering in the lattice. This expression is summed over all the chains with the given ordering and then summed over all the chain orderings in the set to give the combinatorial factor for the system. The final expression for the combinatorial factor (see Appendix I) is

$$\ln(\Omega/\Omega^+) = -\sum_i n_{1,i}\ln(\phi_{1,i}) + \sum_i (n_{2,i}/r)\ln L_i - \sum_c n_c \ln(rn_c/\omega_c) \quad (17)$$

where n_c is the number of chains with the given chain ordering c and ω_c is the product of λ factors for the chain ordering c.

To determine the equilibrium concentration profile of chain segments, the partition function is maximized with respect to the set $\{n_c\}$ of polymer chain orderings to determine the equilibrium set of orderings. This set is expressed in terms of a quantity which Scheutjens and Fleer[16] call the "free segment probability". The free segment probability is a statistical weighting factor which expresses the preference for a free polymer chain segment to be in a given layer of the lattice instead of in the bulk.[16] The free segment probability is defined by[16,17] (see Appendix II)

$$\ln p_i = \chi_s\delta_{1,i} + \ln\phi_{1,i} + \chi(\langle\phi_{2,i}\rangle - \langle\phi_{1,i}\rangle) - [\ln\phi_{1,*} + \chi(\phi_{2,*} - \phi_{1,*})] \quad (18)$$

where the subscript * indicates the bulk value of the given parameter and $\delta_{1,i}$ is the Kronecker delta.

A closed form system of equations for the volume fractions is found by expressing the probability of individual chain conformations in terms of the free segment probabilities. (A complete derivation may be found in ref 16. The main details are given in Appendix IV for the convenience of the reader.) The concentration profile is calculated by iterative solution of the system of equations by using a modified Newton–Raphson technique.[17]

Once the concentration profile is known, the system partition function may be calculated from[16] (eq 17, 18, and II-8 were substituted into eq 10)

$$-\ln Q = n_2\ln\phi_{2,*} + \sum_i n_{1,i}\ln\phi_{1,i} + \sum_i n_{2,i}\ln p_i + \Delta U/kT \quad (19)$$

From the partition function, the Gibbs energy per particle is then found to be (see Appendix III)

$$g/kT = L_1(u_{1|s}/kT - \chi_s\phi_{2,1}) + \sum_i L_i[\phi_{1,i}\ln(\phi_{1,i}/\phi_{1,*}) + \phi_{2,i}\ln p_i - (\phi_{1,i} - \phi_{1,*}) - (\phi_{2,i} - \phi_{2,*})/r] + \chi\sum_i L_i[\phi_{1,i}(\langle\phi_{2,i}\rangle - \phi_{2,*}) - \phi_{1,*}(\phi_{2,i} - \phi_{2,*})] \quad (20)$$

where $u_{1|s}$ is the adsorption energy for the solvent, $\phi_{2,*}$ and $\phi_{1,*}$ are the bulk volume fractions of the polymer chain segments and solvent molecules, r is the length of a polymer chain in lattice units, and p_i is the free segment probability for layer i. The first term accounts for the adsorption energy of the polymer chains and solvent molecules to the surface. The middle term (first summation) gives the combinatorial entropy of the system. The last term (second summation) is the enthalpy of mixing for the polymer chains and the solvent molecules in the lattice with the given concentration profile.

Therefore, the Gibbs energy particle may be calculated, given the following: r, the length of the polymer chain in lattice units; χ, the Flory–Huggins interaction parameter; χ_s, the segment–surface interaction parameter; $\phi_{2,*}$, the bulk volume fraction of polymer chain segments; and $\{L_i\}$, the shape and size of the solute. Substituting the expression for the Gibbs energy per particle into eq 2 gives the distribution coefficient for a particle as a function of system variables.

Computational Procedure

The Gibbs energy per particle in the system was computed following the procedure described above with a suitably modified version of a computer program by Scheutjens and Fleer.[16,17] The principal changes are due to the fact that L_i is not a constant in the curved lattice.

The $\lambda_i(j)$ ($j \in \{-1, 0, 1\}$) are, therefore, dependent on the local curvature of the lattice. All computations were conducted on spherical-shell lattices, with L_i and $\lambda_i(j)$ given by the equations displayed above. The results shown in the following section were calculated using the guidelines listed below for determining the system parameters.

Polymer Chain Length. Polymer chains are measured in lattice length units, which were chosen somewhat arbitrarily as 1 unit = 4 Å. Since a lattice site contains roughly 3.6 methylene units,[18] when comparison with experimental data is sought, r is taken to be equal to the degree of polymerization of the polymer for calculations involving Dextran and PEO.

Polymer–Solvent Interaction Parameter. The Flory–Huggins interaction parameter for a given polymer–solvent combination may be found either from tabulated values in the literature or from independent experiments, such as osmometry. For water-soluble polymers, χ is generally within the range $0.3 < \chi < 0.5$,[6,21,22] but values for PEO and Dextran are clustered about $\chi \approx 0.45$. For PEO we have used $\chi = 0.44$,[6] and for Dextran, we have used $\chi = 0.46$.[21]

Polymer–Solute Interaction Parameter. Tabulated values for χ_s are not available in the literature, and to our knowledge, independent experimental techniques have not been developed to determine it. Consequently, χ_s is an "adjustable" parameter. As will be demonstrated, however, it is possible in practice to determine a range for χ_s by using the model to reproduce the form of the experimental data. Once χ_s for a given polymer–protein pair has been determined, the value should not vary from one experiment to the next. Note that higher values of χ_s indicate a more favorable interaction between the polymer and the protein. It should, therefore, also be possible to determine relative values of χ_s for a series of proteins of a similar size with the same polymer by passing the proteins through a column packed with the polymer. Those proteins which elute at a later time would be expected to have a higher value of χ_s.

Bulk Polymer Concentration. The bulk concentration of the polymer was chosen to be the same as that indicated from the phase diagram of the two-phase system.

Solute Size and Shape. For all calculations it has been assumed that the protein particle is a rigid sphere whose radius is given by the experimental hydrodynamic radius. The radius, R, is expressed in lattice units (1 lattice unit = 4 Å).

Results and Discussion

Effect of Polymer–Solvent Interaction Parameter. The Flory–Huggins interaction parameter measures the quality of the solvent for the polymer. [Recall that $\chi < 0$ indicates that water is a good solvent for the polymer, $\chi = 0$ means that water is an athermal solvent for the polymer, and $\chi = 0.5$ means that a linear polymer of infinite molecular weight is just insoluble in water (i.e., Θ conditions).] Calculations indicate that over the range $0.3 < \chi < 0.5$, the Gibbs energy of a particle decreases slightly, changing very little (for a representative sample, see Figure 4). This result is in agreement with Monte Carlo simulations of chain adsorption to surfaces.[29] Consequently, for the rest of our calculations we have chosen $\chi = 0.44$, corresponding to the value of χ for PEO in water.

Effect of the Protein–Polymer Interaction Parameter. Two principal molecular mechanisms dominate the Gibbs energy per particle in an aqueous polymer phase—the relative energy of interaction of the nearest neighbor contacts of the polymer chain segments with the protein surface, and the entropy loss incurred by the polymer

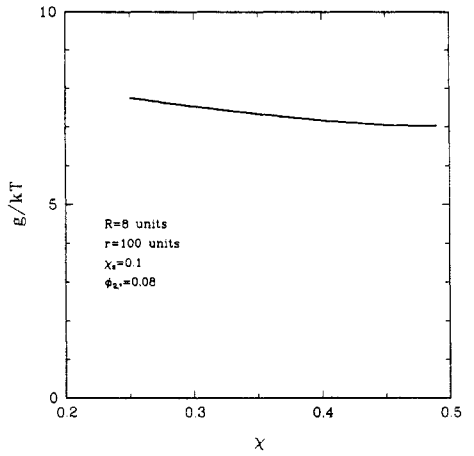

Figure 4. Gibbs energy per particle as a function of the polymer–solvent interaction parameter χ.

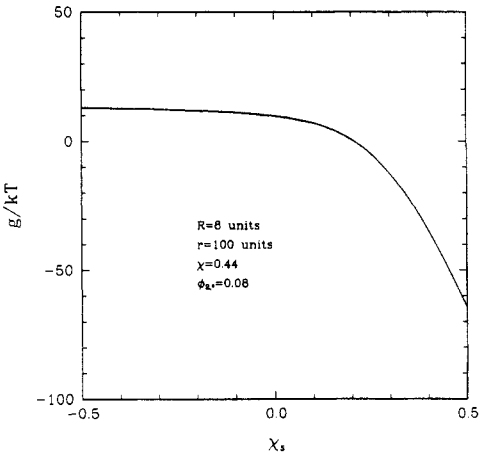

Figure 5. Gibbs energy per particle as a function of the polymer–particle interaction parameter χ_s.

chains when they are moved from the bulk to the region near the protein surface.[30] Figure 5 contains a plot of g/kT vs. χ_s; it contains two regions. For $\chi_s < 0$, the polymer chain segments are energetically repelled from the surface of the protein. Since there is little or no polymer in the layer next to the protein surface, the change in the particle Gibbs energy with χ_s is very small.

When $\chi_s > 0$, the concentration profile changes from surface depletion to surface excess of polymer as χ_s increases. Here the particle Gibbs energy depends on both the protein–polymer interaction energy and the steric repulsion of the polymer chains from the protein's domain. The attraction energy of polymer chains to the surface of the particle tends to lower the Gibbs energy, but this is offset by steric exclusion of the polymer chains from the surface region, which raises the Gibbs energy. At values of χ_s of about 0.2, the attractive forces almost exactly compensate the steric repulsion of the polymer chains from the protein surface so that the Gibbs energy of the particle is zero. The Gibbs energy is very sensitive to the choice of χ_s in this region and decreases rapidly with increasing χ_s.

For large values of χ_s ($\chi_s \gg 0$), the polymer chains are tightly adsorbed to the protein surface. The polymer chains cover almost the entire particle surface, so that the concentration profile of segments does not change much with χ_s. Consequently, the Gibbs energy per particle changes linearly with χ_s.

Effect of Bulk Polymer Concentration. As the concentration of polymer in the bulk is increased, the Gibbs energy per particle may increase or decrease, de-

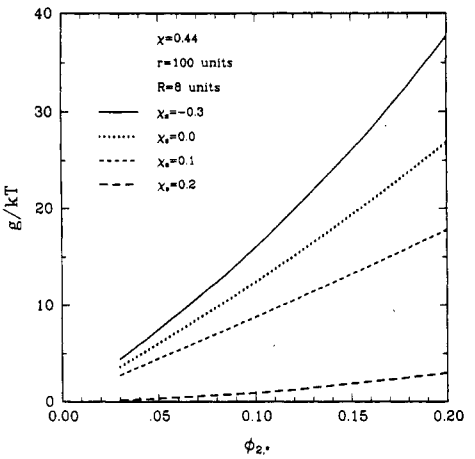

Figure 6. Gibbs energy per particle as a function of bulk polymer concentration for different values of χ_s.

pending on the polymer–particle interaction energy. If polymer chains are strongly attracted to the particle surface ($\chi_s \gg 0$), increasing the bulk polymer concentration will decrease the Gibbs energy because it will lower the concentration gradient of polymer chains near the surface. If the polymer chains are repelled from the surface, or only weakly attracted to it, increasing the bulk polymer concentration will increase the Gibbs energy since the concentration gradient of polymer chains near the surface will be steeper.

Figure 6 shows plots of the Gibbs energy per particle as a function of the polymer concentration for different values of χ_s. In general, the slope of the curve increases with decreasing χ_s. This happens because increasing the polymer concentration, when the polymer–particle interaction is less favorable, forces the polymer into the surface region, thereby increasing the concentration gradient near the surface and the Gibbs energy.

In general, a plot of Gibbs energy vs. bulk polymer concentration will be roughly linear when χ_s is less than 0.2. The slope of the curves will change with the polymer chain length for shorter polymer chains (see Figure 7). The slopes of the curves increase with increasing molecular weight when χ_s is less than zero (Figure 7a) and decrease when χ_s is greater than zero (Figure 7c,d). This can be seen more clearly in Figure 8 where the slopes of a–d in Figure 7 are plotted as a function of the polymer chain length. This behavior is due to a trade-off between the entropy of mixing and the conformational entropy (see the following section on the effect of polymer chain length for more detail).

These calculations may be compared with the data of Atha and Ingham[15] for the precipitation of human serum albumin (HSA) from aqueous PEG solutions. Although the mechanism of protein precipitation is not fully understood, the solubility of a protein in solution must be determined by its Gibbs energy. A comparison of the slope of the solubility curves with the slope of the curves of g/kT vs. $\phi_{2,*}$ is shown in Figure 8. Curves calculated from the model using a range of values for χ_s are shown to demonstrate the trends which may be reproduced. The data of Atha and Ingham show that the slope of the precipitation curve for HSA increases with increasing PEG molecular weight. The calculated trends are in agreement with the data when $\chi_s > 0$, indicating an attractive polymer–protein interaction. However, this interaction cannot be too favorable ($\chi_s > 0.2$—see Figure 7e) or else the slope of the protein free-energy curve would actually be negative (indicating that the polymer helps solubilize the protein). This was not observed.[15] A value of $\chi_s = 0.15$, however, gives excellent agreement with the data.

The experimental results are in contradistinction to calculations from the excluded volume model used by Atha and Ingham,[15] which predicted that the slopes of the solubility curves should increase with *decreasing* polymer

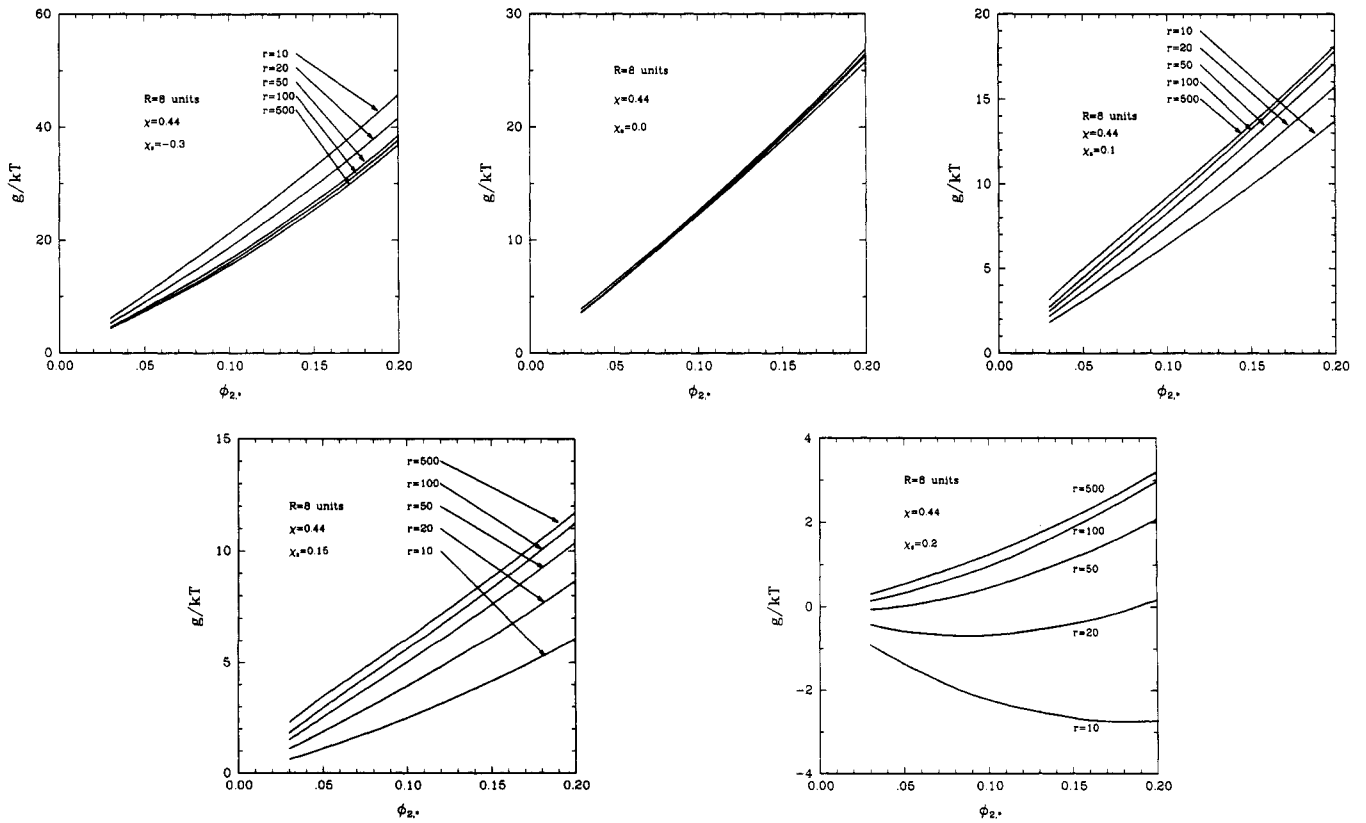

Figure 7. Gibbs energy per particle as a function of the bulk polymer concentration for different polymer chain lengths. Different plots show different values of χ_s: (a, top left) −0.3, (b, top middle) 0.0, (c, top right) 0.1, (d, bottom left) 0.15, (e, bottom right) 0.2.

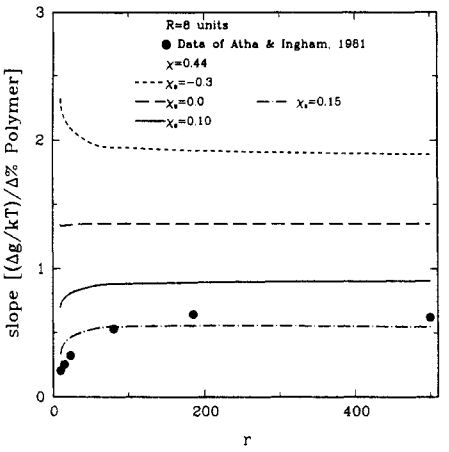

Figure 8. Slope of the curves of Gibbs energy vs. bulk polymer concentration. Model calculations are from the slopes of the curves shown in parts a–d in Figure 7. Data are for human serum albumin.[15]

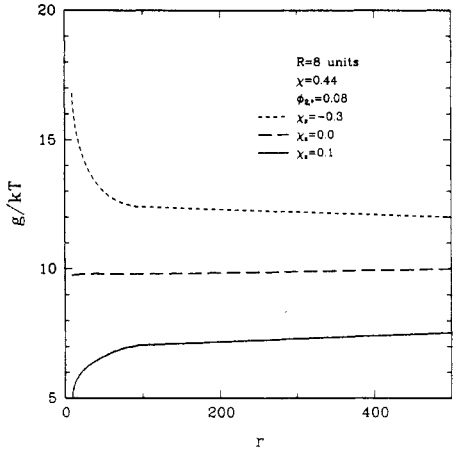

Figure 9. Gibbs energy of the particle as a function of the polymer chain length r for three cases of polymer–particle interaction: repulsive ($\chi_s = -0.3$), athermal ($\chi_s = 0$), and attractive ($\chi_s = 0.10$).

molecular weight. This happens because the excluded volume model contains an inherent assumption that there exists a repulsive interaction between the polymer chains and the solute particle—an assumption which, in this case, does not appear to be valid.

When χ_s is equal to 0.2, the Gibbs energy per particle may decrease initially before increasing (see Figure 7e). This occurs because the energy of attraction of the polymer to the surface is just beginning to overcome the steric repulsion of the chains from the surface. At low bulk polymer concentrations, increasing the polymer concentration allows more polymer chains near the surface without substantially decreasing the entropy of mixing. Increasing the bulk polymer concentration, however, steepens the concentration gradient of chain segments near the surface, causing the Gibbs energy to increase.

Effect of Polymer Chain Length. Increasing the chain length of the phase polymer may increase or decrease the particle Gibbs energy, depending on the value of χ_s. (Figure 9 shows the results of representative computations.) This is because the entropic losses for $\chi_s < 0$ are larger for shorter chains, whereas for $\chi_s > 0$ they are greater for longer chains. When $\chi_s < 0$, there is little polymer near the surface, and the decrease in the entropy of mixing incurred by keeping the many small chains from the surface is larger than that for keeping the few longer chains away (fewer translational degrees of freedom are reduced), so that the Gibbs energy of the particle will be greater for

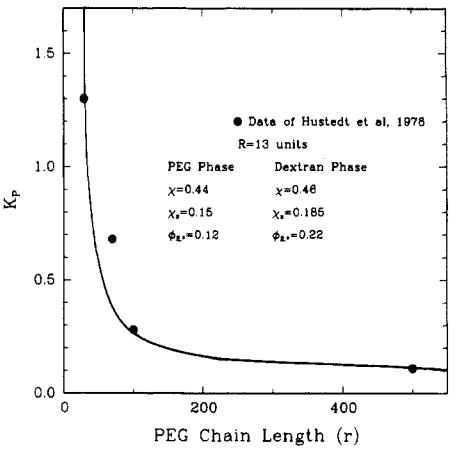

Figure 10. Distribution coefficient for pullulanase in a 12% PEG 6000/1% Dextran T500 biphasic system. Model calculations (solid line) are compared to the data of Hustedt et al.[23]

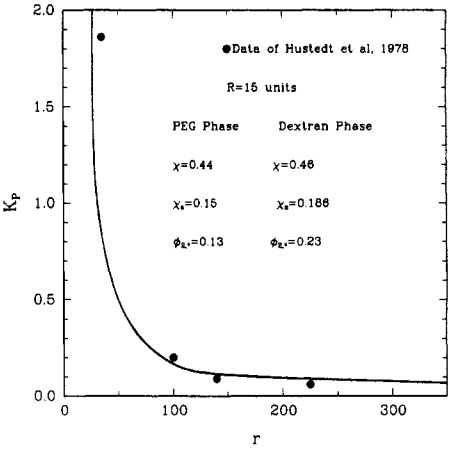

Figure 11. Distribution coefficient for phosphorylase in 9.3% PEG 6000/7% Dextran T500 biphasic system. Model calculations are compared to the data of Hustedt et al.[23]

lower molecular weight polymer at any given bulk polymer concentration. When $\chi_s > 0$, the interaction energy favors polymer near the protein, but steric effects are antagonistic to this trend and "exclude" chains from the surface region, since chains which are near the surface suffer a loss of conformational entropy relative to those in the bulk. This conformational entropy loss will be greater for longer chains. In all cases investigated, however, the Gibbs energy per particle reaches an asymptotic limit as the chain length increases, so that the particle Gibbs energy changes little for polymer chains greater than 100 units.

We compared the results of this model to the experimental results of Hustedt et al.[23] for the partitioning of pullulanase and phosphorylase in PEG/Dextran systems. In order to make this comparison, we assumed reasonable values for each χ_s, i.e., $\chi_s = 0.15$ in the PEO phase and $\chi_s = 0.185$ and 0.186 in the Dextran phase. Phase polymer concentrations were found from phase diagrams given in ref 1. Other parameters were chosen as stated earlier in this paper. The hydrodynamic radii of pullulanase and phosphorylase were calculated from diffusion coefficient data;[24,25] they are 51 Å for pullulanase and 66 Å for phosphorylase. Values thus calculated are shown in Figure 10 and 11 together with the experimental data. The curves calculated from the model are in good agreement with the experiments of Hustedt et al.[23] They suggest that the polymer chains are slightly attracted to the protein surface, although not so strongly as to be adsorbed. In fact, despite the slight attraction, there is still a surface depletion of

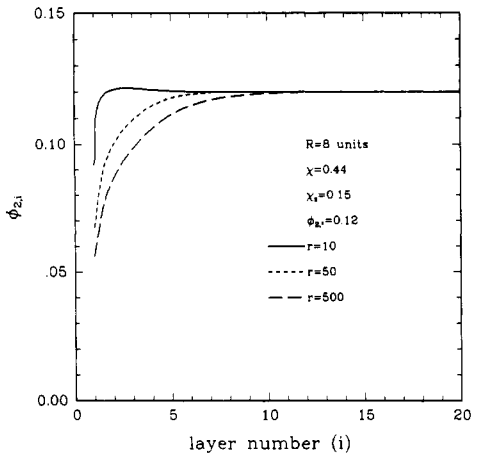

Figure 12. Concentration profile of polymer chain segments near the particle surface for different polymer chain lengths.

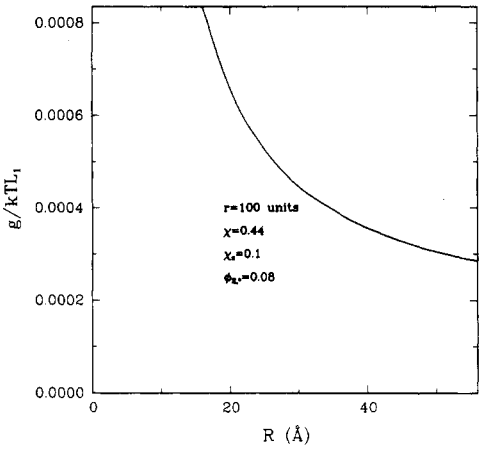

Figure 13. Gibbs energy per particle surface site as a function of particule radius.

the polymer. Typical density profiles for the PEG phase are displayed in Figure 12. They show that, as the chain length of the polymer is increased, the concentration of polymer chains near the particle surface decreases. This depletion of chain segments near the surface is caused by the decrease of the conformational "freedom" which a polymer chain near the surface suffers, relative to a chain in the bulk. The entropy loss is smaller for a shorter chain. The effect reaches an asymptotic limit with increasing chain length since the loss of conformational entropy for chains near the surface is counterbalanced by the entropy of mixing.

Effect of the Particle Radius. In general, if small particles "perturbed" the solution in exactly the same way as large particles, the Gibbs energy per particle could be expected to be proportional to the surface area of the particle, since the interfacial energy per particle is a function of its surface area. The model indicates, however, that this is not the case: calculations show that the Gibbs energy per surface site decreases to a limiting value as the particle size increases (for an example, see Figure 13). This is because smaller particles (with higher surface curvature) "perturb" a larger solution volume per surface area than do large particles (with lower surface curvature). The concentration profile of polymer chain segments near the surface of small particles is, therefore, slightly different from that of large particles, which approach, in the infinite limit, the profile for a flat plate. In this region the particle Gibbs energy is directly proportional to its surface area.

These predictions of the model can be compared with the experimental findings of Atha and Ingham,[15] who

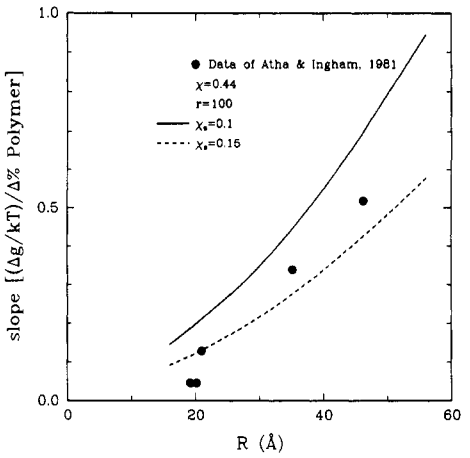

Figure 14. Slope of the curves of particle Gibbs energy vs. bulk polymer concentration as a function of the particle radius. (This plot is similar to Figure 8, except that the abscissa is the particle radius instead of the polymer chain length.) Data are from the solubility data of Atha and Ingham.[15] The proteins, in order of increasing radius, are lysozyme, α-lactalbumin, chymotrypsin, human serum albumin, and aldolase.

found that the slopes of the solubility curves for different size proteins were an almost linear function of the particle radius. Figure 14 shows the experimental results for several proteins along with the theoretical values computed using some of the same values for the parameters as for Figure 8 (no further fitting was conducted). Although agreement is not quantitative, the predicted trend agrees with the experimental observations.

Conclusions

The model presented here is an extension of traditional lattice theory to account for the presence of a surface of specifiable curvature rather than assuming the flat plate limit.[16,19] The model, therefore, takes into account the biomaterial size and shape as a factor determining its interaction with the surrounding polymer solution.

It is possible to make a priori calculations of the partition coefficient of colloidal particles, e.g., biomaterials, in biphasic aqueous polymer systems, given the bulk polymer concentration in the phase $\phi_{2,*}$, the polymer chain length r, the particle's size and shape, the Flory–Huggins interaction parameter χ, and the polymer–protein interaction parameter χ_s. The model may also be used to estimate the particle solubility as a function of the polymer concentration and chain length.

Application of the model to globular proteins (assumed to be spherical in shape) substantiates the view expressed by other authors[29,30] that the contributions of the phase polymers to the Gibbs energy of the particle are primarily the result of the energy of interaction of the polymer with the surface and the steric exclusion of the polymer chains from the region near the surface. These two factors cause the concentration of polymer chains near the particle surface to differ from that in the bulk. As expected, model computations demonstrate that steric exclusion increases with increasing polymer chain length and concentration. The model also shows, however, that the partitioning can be extremely sensitive to the polymer–protein interaction energy. This agrees with the observations of Albertsson,[9] Zaslavsky,[26–28] and others that protein partitioning is very sensitive to the relative hydrophobicities of the two phases.

The sensitivity of the model to χ_s indicates that the surface structure of the biomaterial plays an important role in determining its partitioning behavior. In order to gain a complete understanding of biomaterial partitioning, the assumption of biomaterial surface homogeneity (assump-

tion 5) should be relaxed. It is interesting to note, however, that the data for many different proteins were fit by the model using a very narrow range of values for χ_s ($0.1 < \chi_s < 0.2$). This seems to show that the surface inhomogeneities of the proteins do not dramatically affect the magnitude of protein–polymer interactions.

At present, we do not know of any method for experimentally determining values of χ_s for different polymer–protein combinations. Observations of biomaterial behavior in aqueous polymer solutions can, however, narrow the range within which χ_s lies. Since the value of χ_s for a given protein–polymer combination is constant, it may be possible to experimentally determine values for χ_s by fitting the model to protein precipitation data for different chain lengths and concentrations of the polymer.

The lattice model provides a starting point for the development of a quantitative understanding of the thermodynamics of colloidal particles, including biomaterials, in biphasic systems. Future work is needed to accurately describe the interactions of the polymer with a nonhomogeneous surface, to determine the influence of charge related factors—particularly added salts—on partitioning, and to determine the importance of polymer/salt interactions on particle distribution.

Acknowledgment. This work was supported by the National Science Foundation through the Biotechnology Process Engineering Center at MIT. Partial support for U.W.S. through the Texaco–Mangelsdorf professorship at MIT is also gratefully acknowledged. We thank Dr. J. M. H. M. Scheutjens for the enlightening explanation of his work.

Appendix I. Evaluating Ω/Ω^+

Given a set of polymer chain orderings $\{n_c\}$, we need to calculate the number of ways of placing the chains in the lattice. To do this, we follow Scheutjens and Fleer[16,17] and begin by calculating the number of ways of placing a single chain in a specified ordering c in the lattice. Suppose we have a dimer with the ordering $(1,i)(2,j)$. If we define ν_i as the number of previously occupied sites in layer i, then, using the Bragg–Williams approximation in each layer of the lattice, the probability that a site in layer i is unoccupied is $(1 - \nu_i/L_i)$. Therefore, the first segment may be placed in any one of the L_i $(1 - \nu_i/L_i)$ unoccupied sites in layer i. The second segment may be placed in one of the sites in layer j adjoining layer i. For a rectilinear lattice, this is given simply by $\lambda(j-i)Z$, where Z is the coordination number of the lattice and $\lambda(j-i)$ is equal to $\lambda(1)$, $\lambda(-1)$, or $\lambda(0)$ depending on whether $j - i = 1$, 0, or -1.[16,17] For a curved lattice, however, the curvature of the lattice introduces an artificial directionality in placing the chains. Suppose, for instance, that the dimer was placed with one segment in layer i and the second segment in layer $i + 1$. From the definition of λ used in the main body of this paper, if we place the segment in layer i first, there are $3(L_{i+1}/L_i)$ connections between the layers; however, if we place the segment in layer $i + 1$ first, there are $3(L_i/L_{i+1})$ connections. Clearly, the order in which the segments are placed in the lattice should not change the number of possible conformations.

To correct this problem, we introduce the convention that the number of connections between a site in layer i and a site in layer j is equal to the geometric mean $Z(\lambda_i(j-i)\lambda_j(i-j))^{1/2}$. Consequently, the number of ways of placing the second segment is $Z(\lambda_i(j-i)\lambda_j(i-j))^{1/2}(1 - \nu_j/L_j)$, where the second term is the probability that a site in layer j is unoccupied. Therefore, the overall number of ways of placing the chain is given by

$$L_i(1 - \nu_i/L_i)Z(\lambda_i(j-i)\lambda_j(i-j))^{1/2}(1 - \nu_j/L_j)$$

Extending this argument to an r segment chain, we find that the number of ways of placing the chain in the lattice is given by

$$L_{k(1,c)}\prod_{s=1}^{r-1} Z(\lambda_{k(s,s+1,c)}\lambda_{k(s+1,s,c)})^{1/2}\prod_{s=1}^{r}(1 - \nu_{k(s,c)}/L_{k(s,c)})$$

where the subscript $k(s,c)$ is used to denote the layer k in which segment s of a polymer chain of ordering c is located, and $\lambda_{k(s,s+1,c)}$ is defined as

$$\begin{aligned}\lambda_{k(s,s+1,c)} &= \lambda_k(0), \quad k(s+1,c) = k(s,c)\\ &= \lambda_k(1), \quad k(s+1,c) > k(s,c)\\ &= \lambda_k(-1), \quad k(s+1,c) < k(s,c)\end{aligned}$$

For the spherically symmetric hexagonal curved lattice defined earlier in the main body of this paper, $Z(\lambda_{k(s,s+1,c)}\lambda_{k(s+1,s,c)})^{1/2}$ is equal to $Z - 3(L_{i+1} + L_{i-1})/L_i$ if $k(s,c) = k(s+1,c)$ or is equal to 3 if $k(s,c) \neq k(s+1,c)$. For $R > 5$ lattice units, $Z - 3(L_{i+1} + L_{i-1})/L_i$ is approximately equal to 6 (deviation less than 6%). Therefore,

$$\prod_{s=1}^{r-1} Z(\lambda_{k(s,s+1,c)}\lambda_{k(s+1,s,c)})^{1/2} \approx \lambda(1)^a\lambda(0)^b Z^{r-1} \qquad \text{(I-1)}$$

where the values of $\lambda(1) = {}^1/_4$, $\lambda(0) = {}^1/_2$, and $Z = 12$ are the same as for a flat hexagonal lattice. The exponents a and b correspond to the number of bonds between chain segments in different layers and chain segments in the same layer, respectively.

In addition, since any chain segment may be arbitrarily chosen as the first segment to be placed, we must use the geometric mean

$$\prod_{s=1}^{r} L_{k(s,c)}{}^{1/r}$$

as the number of sites available for the first chain segment placed.

The number of ways of placing a chain in ordering c is therefore given by

$$\omega = \omega_c Z^{r-1}\prod_{s=1}^{r} L_{k(s,c)}{}^{1/r-1}[L_{k(s,c)} - \nu_{k(s,c)}] \qquad \text{(I-2)}$$

where ω_c is defined as in eq 17 in the main body of this paper. Rewriting this in terms of layer numbers, we get

$$\omega = \omega_c Z^{r-1}\prod_{i=1}^{M}\prod_{\nu_i=0}^{r_{i,c}-1} L_i{}^{1/r-1}(L_i - \nu_i)$$

$$\omega = \omega_c Z^{r-1}\prod_{i=1}^{M} L_i{}^{(1/r-1)r_{i,c}}\prod_{\nu_i=0}^{r_{i,c}-1}(L_i - \nu_i) \qquad \text{(I-3)}$$

where $r_{i,c}$ denotes the number of segments that a chain in conformation c has in layer i. The number of ways of placing all n_c chains in the lattice is

$$\omega(n_c) = \omega_c{}^{n_c} Z^{(r-1)n_c}\prod_{i=1}^{M} L_i{}^{(1/r-1)r_{i,c}n_c}\prod_{\nu_i=0}^{(r_{i,c}-1)n_c}(L_i - \nu_i) \qquad \text{(I-4)}$$

For the entire set of chain conformations $\{n_c\}$, we have

$$\omega(\{n_c\}) = Z^{(r-1)n_2}\prod_c(\omega_c{}^{n_c}/n_c!)\prod_{i=1}^{M} L_i{}^{(1/r-1)n_{2,i}}\prod_{\nu_i}^{n_{2,i}-1}(L_i - \nu_i) \qquad \text{(I-5)}$$

where the factor $1/(n_c!)$ accounts for the indistinguishability of the conformations in a given ordering c. Arranging all of the solvent molecules over the remaining $L_i - n_{2,i}$ sites gives an additional factor of

$$\prod_{i=1}^{M} 1/n_{1,i}!\prod_{\nu_i=n_{2,i}}^{L_i-1}(L_i - \nu_i) \qquad \text{(I-6)}$$

Therefore, the combinatorial factor Ω may be written as

$$\Omega = Z^{(r-1)n_2}\prod_c(\omega_c^{n_c})/n_c!\prod_{i=1}^{M}L_i^{(1/r-1)n_{2,i}}(L_i!/n_{1,i}!) \quad \text{(I-7)}$$

The reference state combinatorial factor Ω^+ has been derived by Flory[5] and may be written[16] as

$$\Omega^+ = [(rn_2)!/(n_2!)](Z/rn_2)^{(r-1)n_2} \quad \text{(I-8)}$$

for pure disordered polymer in the bulk state. Combining these two equations gives eq 17 in the main body of this paper.

Appendix II. The "Free Segment Probability"

To calculate the equilibrium distribution of polymer chains, the grand canonical partition function must be maximized with respect to the set of polymer chain orderings $\{n_c\}$ to find the equilibrium set of chain orderings $\{n_e\}$. Taking the derivative of the combinatorial entropy term (eq 17) gives

$$(\partial \ln (\Omega/\Omega^+)/\partial n_e) = \sum_{i=1}^{M} r_{i,e} \ln \phi_{1,i} - \ln r + r - 1 + \ln (\omega_e/n_e) + \sum_{i=1}^{M}(r_{i,e}/r) \ln L_i \quad \text{(II-1)}$$

where $r_{i,e}$ = the number of segments that a polymer chain in its equilibrium ordering e has in layer i and ω_e = the product $\lambda(1)^a\lambda(0)^b$ for a chain in its equilibrium ordering e. (See Appendix I.) The derivative of the energy term (eq 15) is

$$(\partial(-\Delta U/kT)/\partial n_e) = \sum_{i=1}^{M} r_{i,e}\{\chi_s\delta_{1,i} + \chi(\langle\phi_{2,i}\rangle - \langle\phi_{1,i}\rangle)\} \quad \text{(II-2)}$$

where $\delta_{1,i}$ is the Kronecker delta function, so that, specifically, $\delta_{1,i} = 1$ when $i = 1$, and zero otherwise.

The grand canonical partition function for the system is given by

$$\Xi = \sum_{\{n_c\}} Q_{\{n_c\}} \exp(\sum_i L_i\phi_{1,i}\mu_1/kT) \exp(\sum_i L_i\phi_{2,i}\mu_2/rkT) \quad \text{(II-3)}$$

Maximizing the grand canonical partition function with respect to $\{n_c\}$ to obtain the equilibrium set of chain orderings, we find

$$\partial \ln \Xi/\partial n_e = kT(\partial \ln Q/\partial n_e) + \mu_2 - r\mu_1 = 0 \quad \text{(II-4)}$$

where we have used $(\partial\phi_{1,i}/\partial n_e) = -r$. Therefore, we have

$$-kT(\partial \ln Q/\partial n_e) = \mu_2 - r\mu_1 \quad \text{(II-5)}$$

Combining (II-1), (II-2), and (II-5) gives

$$\mu_2 - r\mu_1 = -kT(\sum_{i=1}^{M} r_{i,e} \ln \phi_{1,i} - \ln r + r - 1 + \ln (\omega_e/n_e) + \sum_{i=1}^{M} r_{i,e}[\chi_s\delta_{1,i} + \chi(\langle\phi_{2,i}\rangle - \langle\phi_{1,i}\rangle)] + \sum_{i=1}^{M}(r_{i,e}/r) \ln L_i) \quad \text{(II-6)}$$

which reduces to

$$\ln (n_e/(\prod_{i=1}^{M} L_i^{r_{i,e}/r}) = \ln ((1/r) \exp[(\mu_2 - r\mu_1)/kT]) + \ln (\omega_e) + (r - 1) + \sum_{i=1}^{M} r_{i,e}[\ln \phi_{1,i} + \chi_s\delta_{1,i} + \chi(\langle\phi_{2,i}\rangle - \langle\phi_{1,i}\rangle)] \quad \text{(II-7)}$$

Defining

$$C \equiv (1/r) \exp[(\mu_2 - r\mu_1)/kT] = \text{constant}$$

we get

$$(n_e/(\prod_{i=1}^{M} L_i^{r_{i,e}/r})) = (C\omega_e)\prod_{i=1}^{M} P_i^{r_{i,e}} \quad \text{(II-8)}$$

where P_i, the free segment probability, is defined[16] as

$$\ln P_i = \ln \phi_{1,i} + \chi_s\delta_{1,i} + \chi(\langle\phi_{2,i}\rangle - \langle\phi_{1,i}\rangle) \quad \text{(II-9)}$$

Substituting the bulk concentration values into eq II-9, we get the bulk free segment probability P_* as

$$\ln P_* = \ln \phi_{1,*} + \chi(\phi_{2,*} - \phi_{1,*}) \quad \text{(II-10)}$$

Consequently, the free segment probability, *referenced to the bulk*, is given by

$$\ln p_i = \ln (P_i/P_*)$$

$$\ln p_i = \chi_s\delta_{1,i} + \ln \phi_{1,i} + \chi(\langle\phi_{2,i}\rangle - \langle\phi_{1,i}\rangle) - \{\chi(\phi_{2,*} - \phi_{1,*}) + \ln \phi_{1,*}\} \quad \text{(II-11)}$$

Appendix III. The Gibbs Energy per Particle

From statistical thermodynamics, the Helmholtz energy per particle may be written in terms of the grand partition function for the particle in solution (Ξ) as

$$a = -kT \ln \Xi \quad \text{(III-1)}$$

In this lattice model, it is assumed that the volume of the system remains constant. Therefore, since the system pressure is also constant, the Gibbs energy is equal to the Helmholtz energy. For a single particle, the Gibbs energy may be written as

$$g = -kT \ln Q - (n_1^\sigma\mu_1 + n_2^\sigma\mu_2) + n_1^T\mu_1 + n_2^T\mu_2 \quad \text{(III-2)}$$

where Q = canonical partition function for the system, n_1^σ and n_2^σ = surface excess number of molecules of solvent and polymer, n_1^T and n_2^T = the total number of solvent and polymer molecules in the system, and μ_1 and μ_2 = chemical potential of the solvent and polymer molecules, respectively.

Using the phase without the particle as the reference state, we have

$$g^* = -kT \ln Q^* = n_1^T\mu_1 + n_2^T\mu_2 \quad \text{(III-3)}$$

where g^* is the reference-state Gibbs energy and Q^* is the canonical partition function for the bulk phase without the particle. Note that Q^* is simply the canonical partition function for a polymer solution at the given bulk concentration.

From these definitions, we have

$$g^{\text{excess}} = g - g^*$$

$$g^{\text{excess}} = -kT \ln Q - (n_1^\sigma\mu_1 + n_2^\sigma\mu_2) \quad \text{(III-4)}$$

where g^{excess} is the change in the Gibbs energy of the phase upon addition of the particle. For the rest of this derivation and in the body of the paper, g^{excess} is denoted by g.

The excess Gibbs energy per particle may be calculated in terms of the concentration profile by using eq 19 and the following equations:

$$n_1^\sigma = \sum_i L_i[\phi_{1,i} - \phi_{1,*}] \quad \text{(III-5)}$$

$$n_2^\sigma = \sum_i L_i[\phi_{2,i} - \phi_{2,*}]/r \quad \text{(III-6)}$$

$$\mu_2/kT = 1 - \phi_{2,*} - r\phi_{1,*} + \ln \phi_{2,*} + r\chi\phi_{1,*}(1 - \phi_{2,*}) \quad \text{(III-7)}$$

$$\mu_1/kT = 1 - \phi_{1,*} - \phi_{2,*}/r + \ln \phi_{1,*} + \chi\phi_{2,*}(1 - \phi_{1,*}) \quad \text{(III-8)}$$

Equations III-5 and III-6 are simply definitions of the surface excess,[16] and eq III-7 and III-8 have been derived by Flory.[5] The resulting equation for g is eq 20 in the main body of the text.

Appendix IV. Computational Procedures

The following development draws heavily on the original work of Scheutjens and Fleer.[16,17] The reader is directed to these papers for a more rigorous and detailed description.

In general, a randomly chosen segment s in a polymer chain of r segments will have some probability of being in layer i. The statistical weighting factor for this chain segment is designated as $P(s,i:r)$. The number of chain segments in a layer i will be proportional to the sum over all segments in the chain of these statistical weighting factors, so that

$$n_{2,i}/\sum_i n_{2,i} = \sum_{s=1}^{r} P(s,i:r)/\sum_i \sum_{s=1}^{r} P(s,i:r) \qquad \text{(IV-1)}$$

Therefore, if we can find the statistical weights $P(s,i:r)$, we may calculate the number of chain segments $n_{2,i}$ in each layer. This is done by a two-step procedure. First, $P(s,i:r)$ is expressed in terms of the end segment statistical weighting factors $P(i,s)$ and $P(i,r-s+1)$. A matrix procedure is then used to obtain the end segment statistical weights.

To find $P(s,i:r)$ it is helpful to conceptualize a polymer chain as a combination of smaller polymer chains. For example, a 10-segment polymer chain may also be thought of as two 5-segment polymer chains with a bond connecting one "end segment" of the first chain with one "end segment" of the second chain. Each individual chain may take on any conformation, so long as their connected end segments are positioned adjacent to each other.

In the lattice model we may calculate the statistical weighting factor $P(s,i:r)$ in terms of the end segment statistical weights of the smaller chains. Therefore, from the Bragg–Williams approximation, the statistical weighting factor for any individual chain in a given ordering c to have its sth segment in layer i, $P(s,i:r)_c$, may be expressed as the joint probability that a chain of s units and a chain of $(r - s + 1)$ units both have their end segment in layer i. The statistical weighting factor associated with the probability that a chain of t segments in a chain ordering c has its last segment in layer i is designated as $P(t,i)_c$, so that

$$P(s,i:r)_c = P(s,i)_c P(r-s+1,i)_c/P_i \qquad \text{(IV-2)}$$

The factor P_i corrects for the fact that we have double counted the segment in layer i since both of the "shorter" polymer chains are assumed to have their last segment in layer i. $P(s,i:r)$ is found by summing over all the possible chain orderings, so that

$$P(s,i:r) = \sum_c P(s,i:r)_c$$

$$P(s,i:r) = (1/P_i) \sum_{c(1,s)} P(s,i)_c \sum_{c(s,r)} P(r-s+1,i)_c$$

$$P(s,i:r) = P(s,i)P(r-s+1,i)/P_i \qquad \text{(IV-3)}$$

where $c(1,s)$ and $c(s,r)$ indicate the chain orderings for the first s segments and the last $(r - s + 1)$ segments of the chain, respectively.

To find the end segment statistical weighting factors in terms of the free segment probabilities, we use the fact that a chain whose end segment is in layer i must have its penultimate segment in either layer i or one of the adjacent layers, $i - 1$ and $i + 1$. Consequently, the end segment statistical weight for a chain of some given length may be expressed in terms of the end segment statistical weights for chains one unit shorter. Therefore

$$P(i,s) = P_i[\lambda(-1)P(i-1,s-1) + \lambda(0)P(i,s-1) + \lambda(1)P(i+1,s-1)]$$

$$P(i,s) = P_i \sum_{j=i-1}^{i+1} \lambda(j-i)P(j,s-1) \qquad \text{(IV-4)}$$

The λ factors here are not taken as functions of the layer number for the reasons outlined in Appendix I. By recursively applying eq IV-4, we may eventually derive expressions for all the end segment statistical weights in terms of the free segment probabilities. The end segment statistical weights for all chains may be computed by using a matrix multiplication procedure.[16,17] Therefore, using (IV-3) in conjunction with (IV-4), we may find $P(s,i:r)$ in terms of the set of P_i. We may then use the $P(s,i:r)$ in eq IV-1 to find the set of $n_{2,i}$ in terms of the P_i. Combining eq IV-1 with the definition of the free segment probability (eq 17), we obtain a system of simultaneous equations for the $\{n_{2,i}\}$, which allow us to calculate the $\{\phi_{2,i}\}$.

Appendix V. List of the Symbols Used in This Paper

*	bulk value for the parameter
a	Helmholtz energy for the system
C_P^A, C_P^B	biomaterial concentration in phase A, phase B
c, e	specification of a chain ordering
g, g^{excess}	Gibbs energy of the system with respect to the reference state, excess Gibbs energy
i, j, etc.	layer number
K_P	distribution coefficient for a particle in a biphasic system
k	Boltzmann constant
$k(s,c)$	number of the layer in which the sth segment of a chain with the ordering c is located
L_i	number of lattice sites in layer i
M	number of layers of sites in the lattice
n_1, n_2	number of solvent molecules, polymer chains, in the system
n_c	number of polymer chains with the ordering c
$\{n_c\}$	set of polymer chain orderings
$n_{1,i}$, $n_{2,i}$	number of solvent molecules, polymer chain *segments*, in layer i
n_1^σ, n_2^σ	surface excess number of solvent molecules, polymer chains
P_i	"free segment probability" in layer i
$P(s,i:r)$	statistical weighting factor for the sth segment of an r segment chain to be in layer i
$P(s,i:r)_c$	statistical weighting factor for a chain with the ordering c which has its sth segment in layer i
$P(s,i)$	statistical weighting factor for a chain of s segments to have its last segment in layer i
$P(s,i)_c$	statistical weighting factor for a chain of s segments in the ordering c whose last segment is in layer i
p_i	"free segment probability" in layer i, referenced to the bulk (P_i/P_*)
$Q_{\{n_c\}}$	canonical partition function for a given set of chain orderings $\{n_c\}$
R	radius for a spherical biomaterial
r	number of segments in a polymer chain
$r_{i,c}$, $r_{i,e}$	number of segments that a chain in the ordering c or e has in layer i
s, t, etc.	segment indexing number
T	absolute temperature
U	internal energy of the system

$u_{1|s}$, $u_{2|s}$ adsorption energy for a solvent molecule, polymer chain segment

Z number of nearest neighbors for a lattice site (coordination number)

$\delta_{i,j}$ Kronecker delta, if $i = j$, $\delta_{i,j} = 1$; otherwise $\delta_{i,j} = 0$

$\lambda_i(j-i)$ fraction of nearest neighbors in layer j around a site in layer i

$\lambda_{(s,s+1,c)}$ fraction of sites, located in the layer where segment $s + 1$ is located, around a site in the layer where segment s is located; $\lambda_{(s,s+1,c)}$ equals $\lambda(0)$, $\lambda(-1)$, $\lambda(1)$ if segment s is in the same layer as, the layer above, or the layer below segment $s + 1$

μ_1, μ_2 chemical potential of a solvent molecule, polymer chain

ν_i number of previously occupied sites in layer i

Ξ grand canonical partition function for the system

$\phi_{1,i}$, $\phi_{2,i}$ volume fraction of solvent molecules, polymer chain segments in layer i

$\phi_{1,*}$, $\phi_{2,*}$ volume fraction of solvent molecules, polymer chain segments in the bulk

$\langle\phi_{1,i}\rangle$, $\langle\phi_{2,i}\rangle$ site average volume fraction of solvent molecules, chain segments in layer i (defined in eq 11, 12)

χ Flory–Huggins interaction parameter (polymer–solvent)

χ_s differential energy adsorption parameter (defined in eq 15)

Ω, Ω^+ combinatorial entropy for the system, for the reference state (amorphous bulk polymer)

ω, $\omega(n_c)$, $\omega(\{n_c\})$ number of ways of placing the first polymer chain in the lattice, number of ways of placing all n chains with the given ordering c in the lattice, number of ways of placing a set of chains $\{n_c\}$ with the orderings $\{c\}$ in the lattice

ω_c product of the λ factors for a chain ordering c

Registry No. PEG 6000, 25322-68-3; dextran T500, 9004-54-0.

References and Notes

(1) Albertsson, P. A. *Partition of Cell Particles and Macromolecules*; Wiley: New York, 1986.
(2) Beijerinck, M. W. *Kolloid-Z.* **1910**, *7*, 16.
(3) Dobry, A.; Boyer-Kawenoki, F. *J. Polym. Sci.* **1947**, *2*, 90–100.
(4) Kern, R. J.; Slocombe, R. J. *J. Polym. Sci.* **1955**, *15*, 183–192. Kern, R. J. *J. Polym. Sci.* **1956**, *21*, 19–25.
(5) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University: Ithaca, NY, 1953.
(6) Edmond, E.; Ogston, A. G. *Biochem. J.* **1968**, *109*, 569–576.
(7) Scott, R. L. *J. Chem. Phys.* **1949**, *17*, 279.
(8) Albertsson, P. A. *Adv. Protein Chem.* **1970**, *24*, 309–341.
(9) Albertsson, P. A. *Endeavor* **1977**, *1*, 69–74.
(10) Albertsson, P. A. *J. Chromatogr.* **1978**, *159*, 111–122.
(11) *Partitioning in Aqueous Two-Phase Systems*; Walter, H., Brooks, D. E., Fisher, D., Eds.; Academic: New York, 1985.
(12) Walter, H. *Methods Cell Sep.* **1977**, *1*, 307–354.
(13) Kula, M. R.; Kroner, K. H.; Hustedt, H. *Adv. Biochem. Eng.* **1982**, *24*, 73–118.
(14) Albertsson, P. A.; Andersson, B. *J. Chromatogr.* **1981**, *215*, 131–141.
(15) Atha, D. H.; Ingham, K. C. *J. Biol. Chem.* **1981**, *256*, 12108–12117.
(16) Scheutjens, J. M. H. M.; Fleer, G. J. *J. Phys. Chem.* **1979**, *83*, 1619–1635.
(17) Scheutjens, J. M. H. M.; Fleer, G. J. *J. Phys. Chem.* **1980**, *84*, 178–190.
(18) Dill, K. A.; Flory, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 676–680.
(19) Roe, R. J. *J. Chem. Phys.* **1974**, *60*, 4192–4206.
(20) Vink, H. *Eur. Polym. J.* **1971**, *7*, 1411–1419.
(21) Gekko, K. In *Solution Properties of Polysaccharides*; Brant, D., Ed.; ACS Symposium Series 150; American Chemical Society: Washington, DC, 1981; pp 416–438.
(22) Klenin, V. J.; Klenina, O. V.; Shvartsburd, B. I.; Frankel, S. Y. *J. Polym. Sci. Polym. Symp.* **1974**, *44*, 131.
(23) Hustedt, H.; Kroner, K. H.; Stach, W.; Kula, M.-R. *Biotechnol. Bioeng.* **1978**, *20*, 1989–2005.
(24) Eisele, B.; Rasched, I. R.; Wallenfels, K. *Eur. J. Biochem.* **1972**, *26*, 62–67.
(25) *Biology Data Book*, Altman, P. L.; Dittmer, D. S., Eds.; Federation of American Societies for Experimental Biology: Bethesda, MD, 1972; Vol. 1, p 374.
(26) Zaslavsky, B. Yu.; Miheeva, L. M.; Mestechkina, N. M.; Rogozhin, S. V. *J. Chromatogr.* **1982**, *253*, 139–148.
(27) Zaslavsky, B. Yu.; Miheeva, L. M.; Mestechkina, N. M.; Rogozhin, S. V. *J. Chromatogr.* **1982**, *240*, 21–28.
(28) Zaslavsky, B. Yu.; Miheeva, L. M.; Rogozhin, S. V. *Biochim. Biophys. Acta* **1979**, *588*, 89–101.
(29) Higuchi, A.; Rigby, D.; Stepto, R. F. T. In *Symposium on Adsorption from Solution*; Ottewill, R. H.; Rochester, C. H.; Smith, A. L., Eds.; Academic: New York, 1983; pp 273–286.
(30) Bellemans, A. *Colloid and Interface Science, Proceedings of the International Conference in San Juan* (1976); Academic: New York, 1977; pp 551–557.