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A New Excluded Volume Theory and Its Application to the Coexistence Curves of Aqueous Polymer Two-Phase Systems

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Received December 22, 1992; Revised Manuscript Received April 27, 1993

ABSTRACT: A theoretical description of the coexistence curves of binary and some ternary systems has been developed using methods of statistical geometry. It is based on the concept that macroscopically any molecular species in a solution is distributed at random and that every system composition on the binodal is a geometrically saturated solution of each solute in the presence of the other. The link between the model and thermodynamics is also established. The model has been applied to aqueous polydisperse polymer two-phase systems, and it is shown that when the molecular weights of the two phase-forming components are rather disparate, only one parameter, the effective excluded volume, is needed to reproduce experimental coexistence curves for polyethylene glycol-Dextran-water systems. The current model has been compared with some other approaches (lattice solution theories, treatments based on the Setschenow equation) and the deficiencies of the Setschenow equation and the current model have been commented on.

I. Introduction

Aqueous two-phase extraction systems, in which at least one of the components is a water-soluble polymer, are increasingly used in the isolation and purification of commercially important biomolecules¹⁻³ and can also be used to characterize the properties of many biologically active particulates including whole living cells.⁴⁻⁶ For these reasons there has been considerable activity in the search for suitable models that represent the partitioning behavior of particulates.

Several theoretical models for the partitioning of proteins have appeared and these have recently been reviewed.⁷⁻⁹ The construction of a protein partition model needs to be based on some reasonable theory of solutions. Some typical representations of polymer solutions, and those which can be modified to represent polymer solutions, have served as the starting points for modeling protein partitioning. These include the Flory-Huggins theory,¹⁰ the Scheutjens-Fleer lattice theory,¹¹ the Mc-Millan-Mayer solution theory,¹² Hill's alternative approach,¹³ and the similar approach of Edmond and Ogston,¹⁴ adopted respectively by Diamond and Hsu,¹⁵ Baskir *et al.*,¹⁶ Forciniti and Hall,¹⁷ Cabezas *et al.*,¹⁸ and King *et al.*¹⁹ Combination of these approaches with some other theories such as the renormalization group theory and scaling concepts is also useful in simplifying calculations.^{20,21} It has been indicated^{22,23} that the Flory-Huggins theory is not suitable for one of the two common systems used for two-phase extractions, *viz.* polyethylene glycol (PEG)-Dextran (Dex)-water. When one considers systems containing electrolytes, the lack of suitable theories for concentrated nonelectrolyte + electrolyte aqueous solutions has hampered the creation of protein partition models in polymer-inorganic salt aqueous two-phase systems.²⁴

Phase separation in aqueous two-phase systems must depend on the structure (*i.e.* the nature of the intermolecular forces) of the liquid phase, but the exact nature of this dependence is a contentious issue (see, *e.g.*, ref 25). Statistical mechanical-based theories of liquids and so-

lutions, almost invariably, have built into them simplifying assumptions, which make their mathematical formulation more tractable but do not in many cases necessarily reflect physical reality. In the current study we extend the approach suggested by Bernal²⁶ and treat molecules in a binary homogeneous system as soft objects that are randomly distributed and coherently assembled. The molecular features of a binary solution, in the present treatment, are described by one parameter, which we term the "effective excluded volume". It represents the acceptability of one component by a network constructed of the other component. Since the molecular packing in a solution is compact, in an ensemble average it is impossible for holes of any significant size to exist, which will admit additional molecules, unless the solution adjusts its structure. The description that we have adopted is mainly concerned with what can be termed geometrical aspects of solutions but, for the particular case of the aqueous two-phase systems that are in current use, leads to a rather simple mathematical expression (which we term the "binodal model") for coexistence curves (binodals).

It should be pointed out that the theory developed in the present paper is a branch of "statistical geometry", a term first suggested, some years ago, by Bernal.²⁶ An excellent and readable short review on work in this area has recently been presented by Reiss.²⁷

In all of the approaches mentioned above for aqueous two-phase systems, phase-forming polymers are treated as monodisperse and usually the number-average molecular weight is used to represent a polydisperse species. However, some studies^{28,29} have indicated that the molecular weight distribution of phase-forming polymers can significantly change phase diagrams of aqueous two-phase systems. As an extension, we have explored the problem of which molecular weight should be used in the present model to represent a polydisperse system.

II. Model Formulation

Any point on the binodal of an *i-j* binary system can be regarded as a saturated solution of species *i* in solvent *j* or of species *j* in solvent *i*. For an *i-j-0* binary-solute aqueous system (where 0 denotes water), we consider saturated solutions of one solute component in the aqueous solution containing the other solute. The term "saturated",

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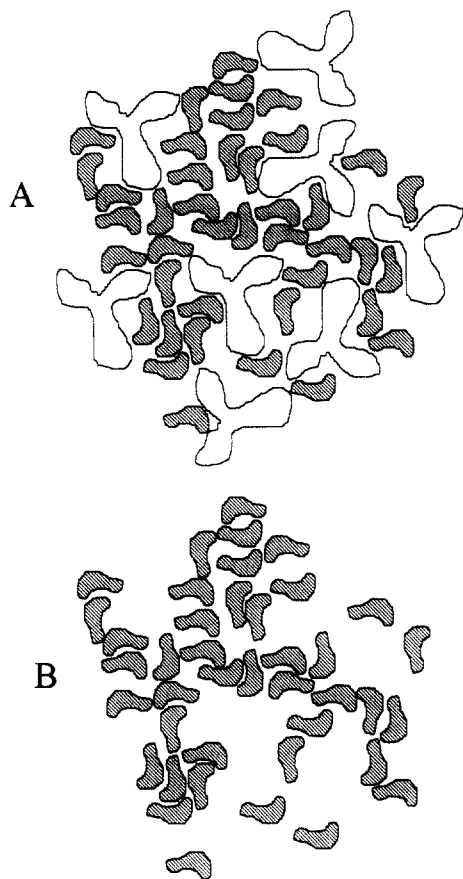


Figure 1. Schematic representations of geometrically saturated binary homogeneous systems (the compositions lie on the coexistence curve) with (A) an actual binary system and a binary system (B) where the larger sized species has been removed for an instant. The open objects represent the bigger *j* molecules and the shaded objects the smaller *i* ones.

as used here, refers to the space occupation and indicates that the volume available is constrained to the geometric limit. However, one of the solutes can still change its relative concentration and, within the two-phase region, the addition of more solute increases the lengths of the tie lines, but both of the two equilibrated phases remain saturated.

At the outset we consider an *i*-*j* binary solution with number densities v_i and v_j , respectively, and assume that the molecules in the solution are distributed at random; see Figure 1A. As a consequence, the molecular interstitial space about either type of molecule will also have a random distribution. This is shown pictorially in Figure 1B, where the position of the molecular centers of species *i* is unchanged whereas the *j* molecules are imagined to have been removed from their former positions. The interstitial space distribution among species *i* is still represented by a random distribution. The probability $[P(V \geq V_{ji})]$ of there being no species *j* in an arbitrarily located volume V_{ji} is given by applying the Poisson distribution:

$$P(V \geq V_{ji}) = \frac{(v_i V_{ji})^0}{0!} e^{-v_i V_{ji}} = e^{-v_i V_{ji}} \quad (1)$$

An alternative derivation of eq 1 is given in Appendix A.

The volume V_{ji} in eq 1 is important in the present approach. We call this volume, V_{ji} , the "effective excluded volume (EEV)" of molecule *j* in a real *i*-*j* binary system, and it reflects the spacing of the nearest molecular centers of component *i*. From the derivation given in Appendix A, the EEV is clearly shown to represent the smallest

spacing of species *i* which will accept an individual *j* molecule. The relationship between the EEV and the van der Waals volume is such that the EEV is greater than the van der Waals volume because of molecular thermal motion. If the influence of the molecular size is the controlling factor, the EEV will be determined largely by the intrinsic size and shape of both species *i* and *j*. In the particular case when species *j* is much bigger than species *i*, the EEV then depends almost exclusively on the size and shape of molecule *j*. Molecular interactions, both attractive and repulsive, will also contribute to the EEV, and the EEV will, for example, be reduced if attractive forces predominate. For any given system the EEV is determined by the sizes and shapes of the constituent molecules and their molecular interactions, but at the moment we shall not consider these factors separately.

When viewed from the species *j* network, eq 1 becomes

$$P(V \geq V_{ji}) = e^{-v_j V_{ji}} \quad (2)$$

where V_{ji} is the EEV of component *i* in this *i*-*j* binary solution. V_{ji} and V_{ij} are generally different because of the different sizes of the two types of molecules.

The volume of the solution associated with species *j* can be divided into two parts:

$$V = V(V \geq V_{ji}) + V(V < V_{ji}) \quad (3)$$

where V is the solution volume. We call the first term $V(V \geq V_{ji})$ in eq 3 the effective available volume of species *j* (this is similar to the "available space" concept used in statistical geometry²⁷) and the second term $V(V < V_{ji})$ the effective unavailable volume of species *j* in this binary solution, respectively.

The spacing of the nearest *i* molecular centers arises predominantly through the existence of a great number of volume zones, each of which is at least as great as the EEV. Irregular molecular shapes are expected to reduce the EEV. For linear or highly unfolded molecules such as Dextran, which depart markedly from spherical shape, the EEV could be closer to the van der Waals volume of the corresponding species.

Obviously, $P(V \geq V_{ji})$ is equal to the volume fraction of the effective available volume (ϕ).^{30,31}

$$P(V \geq V_{ji}) = \phi = \frac{V(V \geq V_{ji})}{V} \quad (4)$$

For a saturated solution the effective available volume of species *j* is filled by component *j* using the EEV as its apparent molecular size. However, an assembly of molecules cannot generally pack completely to fill a space.³² Thus when eq 4 is combined with eq 1 the following expression applies:

$$e^{-v_i V_{ji}} = V_{ji} v_j + f_{ji} \quad (5)$$

where f_{ji} is the volume fraction of unfilled effective available volume after tight packing of *j* molecules into the network of species *i*.

Obtaining a general analytical expression for f_{ji} is very difficult as it depends on the relative geometrical shapes, sizes, and interactions of unlike molecules. However, since the *i* and *j* molecules are markedly different in size in the examples considered, f_{ji} will be very small and consequently will be neglected.³³ Thus we obtain from eq 5

$$e^{-v_i V_{ji}} = V_{ji} v_j \quad (6)$$

Given this, the effective available volume is completely filled by *j* molecules.

Table I. Some Polymer Molecular Weights Used in the Model Calculation

trade name	manufacturer	no.-av mol wt	wt.-av mol wt	root-mean-square-av mol wt	ref
PEG 4000	Union Carbide	3000-3700	<i>a</i>	~3774 ^b	4
PEG 6000	Union Carbide	6000-7500	<i>a</i>	~8000 ^b	4
PEG 3400	Aldrich	3400	<i>a</i>	3400 ^c	36
PEG 8000	Aldrich	8000	<i>a</i>	8000 ^c	36
PEG 4000	Fluka	3810	4097	3951	37
PEG 6000	Fluka	5318	5638	5476	37
PEG 3350	Union Carbide	3690	3860	3774	19
PEG 8000	Union Carbide	8920	11800	10259	19
Dex T500 or D48	Pharmacia	180000	460000	287750 ^b	4
Dex T500 (Lot 01852)	Pharmacia	234200	507000	344586	36
Dex T70 (Lot 02377)	Pharmacia	38400	72200	52654	36
Dex DX37	Fluka	52066	86173	66983	37
Dex DX48	Pfeifer & Langen	88252	214719	137657	37
Dex T500 (Lot 38624)	Pharmacia	167000	509000	291553	19
Dex T 70 (Lot 00356)	Pharmacia	37000	74540	52516	19

^a Data unavailable. ^b Average value from refs 4 and 38. ^c Number- and weight-average molecular weights are assumed to be the same.

For those ternary systems in which strong, homotactic chemical binding occurs between the solvent molecules and there are strong heterotactic interactions between the solvent and each of the solutes, the expressions obtained above for the binary system are still valid since the spatial hindrance of solvent molecules to solutes is negligible compared with that between solute molecules. The systems that we wish to address (PEG-Dex-H₂O) are such that both of the polymers are very hydrophilic and consequently will be strongly hydrated (*i.e.* there are intense, attractive molecular interactions between the polymers and water), and the solvent water molecules are associated through hydrogen bonds. In ternary systems such as these, water molecules necessarily occupy a certain proportion of the solution volume, and so as a correction, we consider the influence of the water by denoting the EEV of *j* molecules in an aqueous solution of species *i* as V_{jio} . This latter term incorporates the effect arising from the presence of the volume and interactions of the third component, water, and so we rewrite eqs 1 and 6 as

$$P(V \geq V_{jio}) = e^{-V_{jio}v_i} \quad (7)$$

$$e^{-V_{jio}v_i} = V_{jio}v_j \quad (8)$$

It is clear from what has been stated that EEVs, in the *i*-*j* binary and *i*-*j*=0 ternary solutions, respectively, and the van der Waals volume of species *j* are related by

$$V_{jio} > V_{ji} > V_j \quad (9)$$

where V_j is the van der Waals volume of component *j*.

The forms of eqs 8 and 6 are the same and so, in effect, a PEG-Dex-water ternary system can be treated as one or two pseudobinary system(s).

Using the transformation relationship between molecular number density and weight concentration of solute components:

$$v_s = \frac{\rho N_a w_s}{M_s} \quad s = i, j \quad (10)$$

where w_s is the weight percent concentration of component *s*, ρ is the solution density, N_a is Avogadro's number, and M_s is the molecular weight of component *s*.

The solution density is almost unchanged in PEG-Dex aqueous two-phase systems⁴ and will be treated as a constant. We define the scaled EEV, V_{jio}^* , by

$$V_{jio}^* = \rho N_a V_{jio} \quad (11)$$

By using eqs 10 and 11 in eq 8, we get

$$\ln \left(V_{jio}^* \frac{w_i}{M_i} \right) + V_{jio}^* \frac{w_i}{M_i} = 0 \quad (12)$$

Equation 12 is important since it allows the coexistence curve of a binary-solute aqueous system to be described using only one parameter, V_{jio}^* .

However, in practical situations phase-forming polymers are polydisperse, *i.e.* have a range of molecular weights. It is found (see Table I) that PEG has relatively small polydispersity, whereas Dextran is usually significantly polydisperse. Since the weight-average molecular weight is the mean of the monomer number fraction of the same molecular weight in the total monomers of the system, the average molecular dimension is therefore known to be related to the weight-average molecular weight of the constituent polymer.³⁴ We would expect that the EEV should relate to the average size of both PEG and Dextran molecules and is thus related to the weight-average molecular weight for each polymer. The influence of polydispersity on the mean molecular weight, $\langle M \rangle$, in the term $V_{jio}^* (\sum_k w_k) / \langle M \rangle$ of eq 12 for a polydisperse system may thus be expressed by:

$$\langle V^* \rangle \frac{\sum_k w_k}{\langle M \rangle} = \sum_k \left(V_k^* \frac{w_k}{M_k} \right) \quad (13)$$

where w_k is the weight percent of the *k*-mer and $\langle V^* \rangle$ is the average scaled EEV of a polydisperse species.

If the molecular weight distribution for each polymer is available, eq 13 can be applied directly. However, this is rarely the case and, much more frequently, only the number- and weight-average molecular weights are known. In Appendix B, a derivation is given to show that generally³⁵

$$\langle M_n \rangle < \langle M \rangle < \langle M_w \rangle \quad (14)$$

where $\langle M_n \rangle$ and $\langle M_w \rangle$ are number- and weight-average molecular weights, respectively.

As an approximation, we take the geometrical average of the number- and weight-average molecular weights, which coincidentally is the root-mean-square-average molecular weight, $\langle M_{rms} \rangle$:

$$\langle M \rangle = \langle M_{rms} \rangle = \sqrt{\langle M_w \rangle / \langle M_n \rangle} \quad (15)$$

We use this in eq 12 for PEG-Dex-water systems and so

$$\ln \left(\langle V^* \rangle_{210} \frac{w_2}{\langle M_{rms} \rangle_2} \right) + \langle V^* \rangle_{210} \frac{w_1}{\langle M_{rms} \rangle_1} = 0 \quad (16)$$

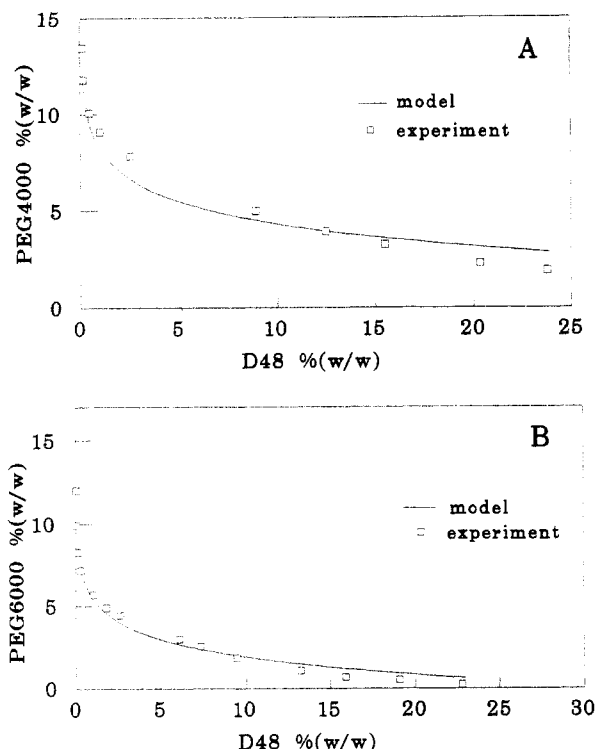


Figure 2. Comparison of experimental⁴ and predicted PEG-Dex-water coexistence curves at 293 K for (A) the PEG4000-Dex D48 system and (B) the PEG6000-Dex D48 system.

where subscripts 1 and 2 refer to PEG and Dextran, respectively. Equation 16 is the expression that will be used in comparison with experimental results.

III. Comparison with Experimental Results

The binodal model, eq 16, was tested against experimental data for PEG-Dex aqueous two-phase systems. It is apparent from eq 16 that w_2 can be expressed as an explicit function of w_1 . The EEV can easily be computed using an appropriate least-squares algorithm. In the current article we focus on the experimentally determined systems with the PEG-Dex pairs, PEG8000-Dex T500 and PEG3350-Dex T70, reported by Albertsson,⁴ Diamond and Hsu,³⁶ Forciniti *et al.*,³⁷ and King *et al.*¹⁹ For the data from the first source, a system that is close to that of PEG4000-Dex T70 was chosen. These polymers cover the types of PEG and Dextran produced by the principal manufacturers (Table I). The method used by Diamond and Hsu to determine PEG is different from that used by the other workers. We have used the molecular weights determined or reported by the different authors, or when no molecular weight is reported,⁴ the average given by the manufacturer is assumed.^{4,38}

In Figures 2–5 the data points are from the sources cited above and the curves are the binodals obtained by correlation with the present model. In all of the systems studied, the binodal model correlates with the reported data within the limits of the accuracy of the experiments.

Table II shows the EEV (V_{210}^*), obtained by curve-fitting experimentally measured PEG-Dex-H₂O binodals, and the corresponding correlation coefficients. The trend is that the larger the molecular weight of either phase-forming polymer, the higher is the EEV. For PEG-Dex aqueous two-phase systems, the EEV (V_{210}^*) appears to be in the range $(1-8) \times 10^5$ kg kmol⁻¹ as the Dextran average molecular weights vary from 0.5×10^5 to 3.4×10^5 .

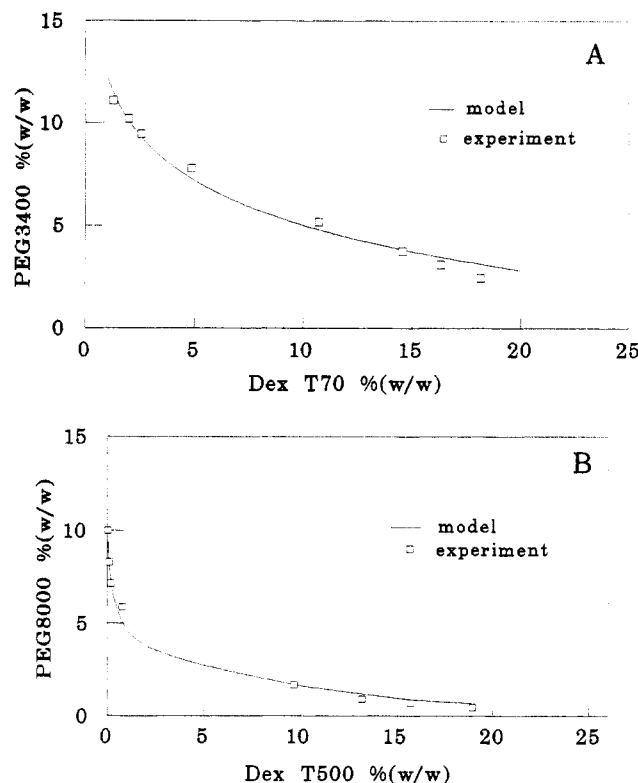


Figure 3. Comparison of experimental³⁶ and predicted PEG-Dex-water coexistence curves at 295 K for (A) the PEG3400-Dex T70 system and (B) the PEG8000-Dex T500 system.

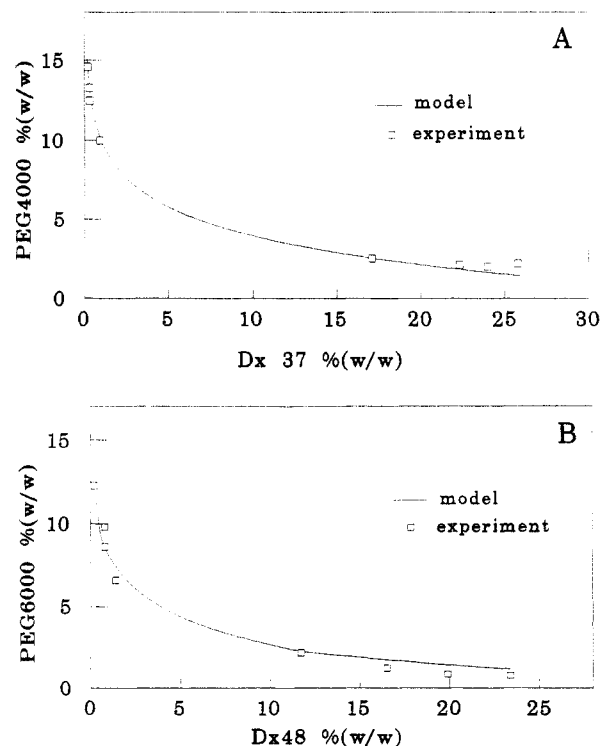


Figure 4. Comparison of experimental³⁷ and predicted PEG-Dex-water coexistence curves at 298 K for (A) the PEG4000-Dx37 system and (B) the PEG6000-Dx48 system.

IV. Discussion

The Flory-Huggins polymer lattice solution theory¹⁰ is applicable only for solutions containing chain polymers at intermediate concentrations. This arises partly from the fact that there is a distribution of voids in liquid phase structures. In our representation, the voids in a liquid are, on average, too small to hold another solvent or solute

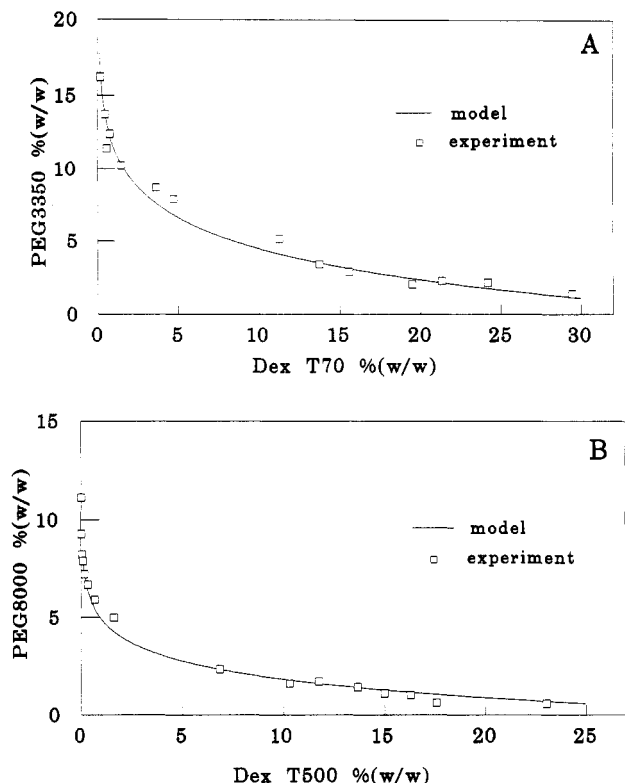


Figure 5. Comparison of experimental¹⁹ and predicted PEG-Dex-water coexistence curves at 298 K for (A) the PEG3350-Dex T70 system and (B) the PEG8000-Dex T500 system.

Table II. Some Effective Excluded Volumes of Dextran in PEG-Dex-Water Systems

PEG-Dex aqueous systems	calculation of the EEV	
	$10^5 V_{210}^*$ (kg kmol ⁻¹)	corr coeff
PEG3350-Dex T70 at 298 K ^a	1.23	0.990
PEG3400-Dex T70 at 295 K ^b	1.08	0.993
PEG4000-Dx37 at 298 K ^c	1.50	0.997
PEG4000-D48 at 293 K ^d	1.96	0.969
PEG6000-D48 at 298 K ^c	2.62	0.970
PEG6000-D48 at 293 K ^d	4.97	0.987
PEG8000-Dex T70 at 295 K ^b	6.13	0.978
PEG8000-Dex T500 at 298 K ^a	7.59	0.996

^a System from ref 19. ^b System from ref 36. ^c System from ref 37. ^d System from ref 4.

molecule (or one of its segments). Recently, Bawendi and Freed³⁹ systematically corrected the Flory-Huggins model by adopting a model for polymer solutions in which the lattice structure is occupied by solvent molecules, polymer segments, or voids. This treatment certainly brings the theory closer to molecular reality, but in our view the importance of voids is overemphasized. Our treatment based on random distribution and closer packing of the molecules produces a simple relationship that represents PEG-Dex aqueous two-phase systems rather well. This binodal model recognizes in part the complexity of the many-body nature of molecular interactions in a real solution. In a binary or pseudobinary solution, the effective available volume is accessible to each *j* molecule with a characteristic size (the EEV). The structure of simple pure liquids can be approximated by the random packing of equal sized spheres; similarly, the structure of a binary solution in the present treatment can be represented by the random packing of two different sized objects. Monte Carlo studies of the packing of different sized spheres have shown that, as the diameter ratio of the spheres is increased, the packing fraction also increases.³³ For the molecules studied here, even closer packing is likely

to occur because of the substantial molecular weight difference and the unfolded shapes of the two types of phase-forming polymers.

If we take the binodal model one step further, the behavior of a pseudobinary (and certainly binary) system may be expressed by defining a criterion function for phase separation, *F*, from eqs 5, 10, and 11 as

$$F = V_{ji0} v_j - e^{-v_i V_{ji0}} + f_{ji0} \\ = \frac{V_{ji0}^* w_j}{M_j} - e^{-V_{ji0}^* w_j / M_j} + f_{ji0} \quad (17)$$

where *f*_{ji0} is similar to *f*_{ji} in eq 5 but includes the influence of the size of the water molecules.

If the concentration of species *j* exceeds that for close filling, the system will become unstable and, as a consequence, will separate into two saturated solutions. Conversely, if the concentration of the *j* component is lower than that required for close filling, one phase will be produced. These can be expressed as

$$\left. \begin{array}{ll} \text{one phase region:} & F < 0 \\ \text{binodal:} & F = 0 \\ \text{heterogeneous phase (including two-phase):} & F > 0 \end{array} \right\} \quad (18)$$

Note that *F* is related exponentially to the EEV (*V*_{ji0}^{*}) which concurs with the experimental fact that phase formation is very sensitive to polymer molecular weight.⁴

Although the above description is geometric in nature, a relation with thermodynamics may be established. We will comment on this, but it is not our intention to develop this exhaustively at the moment.

Using Einstein's equation⁴⁰ for fluctuations of thermodynamic equilibrium gives for the current case

$$P(V \geq V_{ji0}) \cong e^{-\Delta A_{ji0} / kT} \quad (19)$$

where ΔA_{ji0} is the difference between the Helmholtz free energy of the system to create an empty region with volume *V*_{ji0} and that of the mean value in this *i-j=0* pseudobinary solution. *k* is the Boltzmann constant, and *T* is the absolute temperature.

Combination of eqs 7, 11, and 19 yields

$$V_{ji0}^* = \frac{\rho N_a}{v_i} \frac{\Delta A_{ji0}}{kT} \quad (20)$$

Assuming there is no change in the total volume of the system with temperature (*i.e.* the system has zero isobaric thermal expansibility), then the following relationship applies:

$$\left(\frac{\partial V_{ji0}^*}{\partial T} \right)_V = - \frac{\rho N_a}{k v_i} \frac{\Delta U_{ji0}}{T^2} \quad (21)$$

where ΔU_{ji0} is the internal energy change for the same process as that associated with ΔA_{ji0} . Since ΔU_{ji0} must be greater than zero for the systems under consideration (*i.e.* external work is needed), then $(\partial V_{ji0}^* / \partial T)_V$ must always be negative at any temperature suitable for the binodal model. It is therefore readily concluded that the EEV must decrease when the temperature increases. Figure 6 gives some results obtained from Albertsson's data⁴ to illustrate how temperature influences the EEV. For a given polymer molecular weight, it is found that the EEV is higher at 273 K than at 293 K. In association with eqs 17 and 18, this trend is also in agreement with the experimental observation that polymer two-phase systems change from two phase to one phase as the temperature is raised.^{41,42} Figure

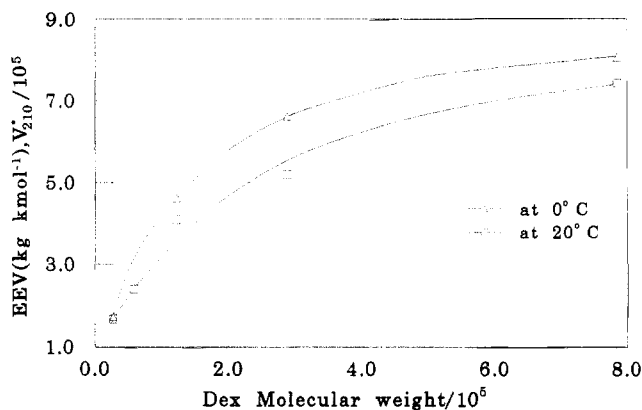


Figure 6. Influence of Dextran molecular weight and temperature on the effective excluded volume (EEV) of Dex in PEG aqueous solutions. Experimental data are taken from ref 4. Some PEG and Dex molecular weights used in this correlation are not listed in Table I.

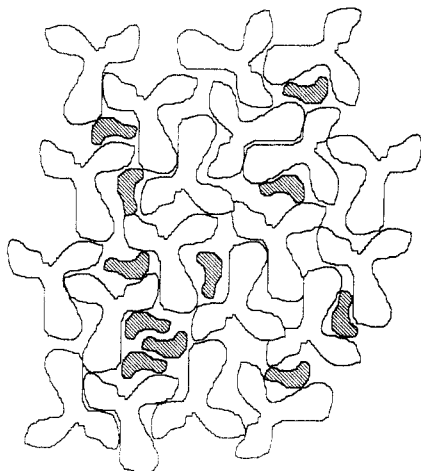


Figure 7. Schematic illustration of the limitation of the binodal model. The larger closed objects represent Dex, and the smaller, PEG.

6 further indicates that the change of the EEV with Dextran molecular weight is marked at lower molecular weights, but less marked at higher molecular weights.

The model developed does have its limitations, which can be illustrated by reference to Figures 1 and 7. Given that Dextran molecules are much bigger than PEG molecules, two divergent situations for the compositions on the binodal of an aqueous two-phase system might exist. Unless the concentration of PEG is extremely low, the Dextran molecules are separated randomly by PEG molecules (Figure 1A). When the Dextran concentration becomes so high that the Dextran molecules touch each other, as shown in Figure 7, some PEG molecules can occupy the space between Dextran molecules without interfering with the number density of Dextran. Under these conditions, the model used will become less precise since the distribution of Dextran molecules is governed primarily by other Dextran molecules rather than by PEG molecules. Generally, however, the concentrations of phase-forming polymers used in protein partitioning studies will not fall into this composition region. In such studies the concentrations of the polymer species tend to be similar. This accounts for the asymmetrical features of PEG–Dex aqueous two-phase diagrams. In the bottom, Dex-rich phase, there is little change in the concentration of PEG as the Dextran concentration is increased, and in the PEG-rich top phase, conversely, there is usually very little Dextran present.

In some theories such as those of Scheutjens and Fleer¹¹ and Flory and Huggins,¹⁰ the macromolecular species is defined as being a linear homopolymer but variations in segmental number are allowed. In contrast, the concepts used in the present model should apply when the phase-forming polymers are highly branched, which is the case for Dextran molecules.^{43,44} The results in Figure 6 indicate that it might be possible to establish a relationship between the EEV and the molecular weight of a phase-forming polymer, and we intend to investigate this problem.

Vainerman *et al.*⁴⁵ extended and applied the Setschenow empirical equation⁴⁶ to aqueous two-phase systems and observed that the experimental phase diagrams for some PEG–Dex systems satisfy the following relationship:

$$\ln \frac{w_1^T}{w_1^B} = k_S (w_2^B - w_2^T) \quad (22)$$

where k_S is the Setschenow coefficient. Superscripts T and B refer to top and bottom phases, respectively.

Another expression is readily obtained from eq 2 by following similar procedures to those used in establishing eq 16:

$$\ln w_1 = \ln \left(\frac{\langle V^* \rangle_{120}}{\langle M \rangle_1} \right) - \frac{\langle V^* \rangle_{120}}{\langle M \rangle_2} w_2 \quad (23)$$

where $\langle M \rangle$ is the average molecular weight of a polydisperse species and $\langle V^* \rangle_{120}$ is the average scaled EEV of PEG in the PEG–Dex–water pseudobinary solution. Applying eq 23 to the top and bottom phases at equilibrium leads to

$$\ln \frac{w_1^T}{w_1^B} = \frac{\langle V^* \rangle_{120}}{\langle M \rangle_2} (w_2^B - w_2^T) \quad (24)$$

Consequently, in the present formulation, k_S is equivalent to $\langle V^* \rangle_{120} / \langle M \rangle_2$, which is a constant for a given system.

However, given that both the solubilities of PEG and Dextran in water are very high, there is a contradiction between the approach given by Vainerman *et al.*⁴⁵ and the well-established fact that the Setschenow equation is only applicable when the concentration of the solutes is very low. If we express the Setschenow equation⁴⁷ as

$$\ln w_1 = \ln w_1^* - k_S w_2 \quad (25)$$

where w_1^* is the solubility of PEG in water. Then comparison of eq 23 with eq 25 leads to $w_1^* = \langle V^* \rangle_{120} / \langle M \rangle_1$. This cannot usually be true since the EEV is a parameter of the ternary (PEG–Dextran–water) system while the PEG solubility in water, w_1^* , is only determined by the binary (PEG–water) system. Our conclusion therefore is that the binodal model provides algebraic similarities with the Setschenow equation and this explains the apparent success of approaches based on the Setschenow expression. However, the shortcomings in the Setschenow approach are apparent.

V. Concluding Remarks

In the past, thermodynamic theories have been applied, with some success, to studies of aqueous two-phase extraction systems. In the present work, we have developed and applied an alternative approach, which is geometrical in nature. The fundamental assumptions made in the model are (a) the spatial distribution of any one species follows an exponential law and (b) solute molecules of one species, characterized by what we have

termed the effective excluded volume, almost fill the corresponding effective available volume.

Application of this model to some PEG-Dex aqueous two-phase systems satisfactorily reproduces experimental results. Combination of the present concepts with fluctuation theory allows a connection to be made to thermodynamic variables.

The problem of polydispersity of polymer molecules has been treated using semitheoretical considerations. Comparisons of the current theory with lattice polymer solution theories and treatments based on the Setschenow equation have been made and serve to highlight some deficiencies in the earlier treatments as well as show their links to the present approach.

It is perhaps appropriate to mention here that we are currently extending this treatment to the important and outstanding problems of the partitioning of soluble particulates and the precipitation of macromolecules by polymers or salts.

Acknowledgment. We gratefully acknowledge financial support by the Science and Engineering Research Council under Grant No. GR/G19824. We also acknowledge the help of Dr. D. R. Grey in checking some of the mathematical derivations.

Appendix A: Derivation of Eq 1⁴⁸

For a randomly distributed point set in a three-dimensional space with number density v_i , from an arbitrary point E_0 , the probability that a point will be in a small volume shell ($E_0 + V, E_0 + V + dV$), proportional, apparently, to dV , will be independent of whether or not there is a point at E_0 . Given no point at E_0 , let the probability that the first point appears in ($E_0 + V, E_0 + V + dV$) be $\varphi(V)dV$. $\varphi(V)$ is the probability density function of finding "holes" with a volume of at least V . We may then write the spacing distribution by

$$\begin{aligned}\varphi(V)dV &= P\{N(dV) = 1, N(V) = 0\} \\ &= P\{N(dV) = 1|N(V) = 0\}P\{N(V) = 0\}\end{aligned}\quad (\text{A1})$$

where N is the number of points and $N(V)$ is the number of points in volume V . $P\{N(V) = 0\}$ is the probability that the internal volume V contains no point, and $P\{N(dV) = 1|N(V) = 0\}$ is the conditional probability that the volume shell dV contains one point when there is no point in the volume V .

The second factor on the right hand side of eq A1 can be expressed as

$$P\{N(V) = 0\} = \int_V^\infty \varphi(x) dx \quad (\text{A2})$$

and is the probability that the spacing is larger than V . The first factor can be expressed as

$$P\{N(dV) = 1|N(V) = 0\} = \lambda_{01}(V)dV \quad (\text{A3})$$

where $\lambda_{01}(V)$ is a function of hole size V . It is easy to prove that $\lambda_{01}(V)$ has the following characteristic:

$$\int_0^\infty \lambda_{01}(V) dV = \infty \quad (\text{A4})$$

We therefore have

$$\varphi(V) = \lambda_{01}(V) \int_V^\infty \varphi(x) dx \quad (\text{A5})$$

Solving eq A5 gives

$$\varphi(V) = C_1 \lambda_{01}(V) e^{-\int_0^V \lambda_{01}(x) dx} \quad (\text{A6})$$

where $C_1 = \varphi(0)/\lambda_{01}(0)$ and is a constant.

To determine the value of C_1 , we use the following restriction:

$$\int_0^\infty \varphi(V) dV = 1 \quad (\text{A7})$$

Substituting eq A6 into eq A7 and using eq A4, one finds $C_1 = 1$.

The probability of finding interstitial spacings that are greater than the volume V_{ji} is given by

$$P(V \geq V_{ji}) = \int_{V_{ji}}^\infty \varphi(V) dV = e^{-\int_0^{V_{ji}} \lambda_{01}(x) dx} \quad (\text{A8})$$

The value of $\lambda_{01}(V)$ in eq A8 should be determined by another restriction:

$$\int_0^\infty V \varphi(V) dV = 1/v_i \quad (\text{A9})$$

where $1/v_i$ represents the mean volume occupied by each point. Using eqs A4 and A6 and the result $C_1 = 1$, eq A9 can be changed to

$$\int_0^\infty e^{-\int_0^V \lambda_{01}(x) dx} dV = \frac{1}{v_i} \quad (\text{A10})$$

$\lambda_{01}(V)$ can theoretically be any function satisfying eq A10, but the minimum hole volume or the EEV (see earlier) should be such that the centers of the two particles do not overlap. Under this condition, the locations of particle centers can be regarded as a completely random distribution (the random spheres model, proposed by Weissberg⁴⁸). The value of $\lambda_{01}(V)$ for this case should be a constant, e.g., C_2 :

$$\lambda_{01}(V) = C_2 \quad (\text{A11})$$

From eq A10 $C_2 = v_i$. Hence eq A8 becomes eq 1.

Appendix B: Analysis of the Average Molecular Weight

We may express the EEV for every subcomponent k of a polydisperse species as

$$V_k^* = aM_k^{1.5} + b \quad (\text{B1})$$

where a and b are two positive constants.

Note that the average molecular size of a polydisperse species is related to its weight-average molecular weight.³⁴ We therefore have the average scaled EEV:

$$\langle V^* \rangle = a \langle M_w \rangle^{1.5} + b \quad (\text{B2})$$

Represent eqs B1 and B2 as

$$V^*(x) = aM^x + b \quad (\text{B3})$$

where corresponding $V^*(x)$ is an expansion of the EEV and x is an independent variable.

Equations B1 and B2 now become special cases of eq B3 at $x = 1.5$. Substitution of eq B3 into eq 13 by regarding $V^*(x)$ as V^* yields after rearrangement

$$\langle M(x) \rangle = \frac{(a \langle M_w \rangle^x + b) \sum_k w_k}{\sum_k \left[(aM_k^x + b) \frac{w_k}{M_k} \right]} \quad (\text{B4})$$

where $\langle M(x) \rangle$ is expanded from $\langle M \rangle$.

Since the following relationship is generally true

$$\langle M_w \rangle > \langle M_n \rangle > 0 \quad (\text{B5})$$

it is readily established

$$\langle M_n \rangle < \langle M(x) \rangle < \langle M_w \rangle \quad x = 1, 2 \quad (\text{B6})$$

The derivative of $\langle M(x) \rangle$ is

$$\frac{d\langle M(x) \rangle}{dx} = \frac{a \sum_k w_k}{[\sum_k (aw_k M_k^{x-1} + bw_k/M_k)]^2} \times$$

$$\{a \langle M_w \rangle^x [(\ln \langle M_w \rangle) \sum_k (M_k^{x-1} w_k) - \sum_k (w_k M_k^{x-1} \ln M_k)] +$$

$$b [\langle M_w \rangle^x (\ln \langle M_w \rangle) \sum_k (w_k/M_k) - \sum_k w_k M_k^{x-1} \ln M_k] \}$$
(B7)

Now we only need to prove the following inequalities in the range $x \in [1, 2]$:

$$\ln \langle M_w \rangle > \frac{\sum_k [(w_k M_k^{x-1}) \ln M_k]}{\sum_k (w_k M_k^{x-1})}$$
(B8)

and

$$\langle M_w \rangle^x \ln \langle M_w \rangle > \frac{\sum_k (w_k M_k^{x-1} \ln M_k)}{\sum_k (w_k/M_k)}$$
(B9)

To give the evidence of eqs B8 and B9, we need to know the specific form of the molecular weight distribution function. As seen from Table I, the ratio of weight- and number-average molecular weights of Dextran are in the range 1.7–3.1. These data are close to a distribution investigated by Flory⁴⁹ for linear condensation polymers, from which the theoretical value of this ratio is close to 2. We follow the same law and therefore the number fraction of k -mer in the total number of molecules of all sizes is given by

$$N_k = p^{k-1}(1-p) \quad (B10)$$

where p is a characteristic constant. Apparently, the polymer molecular weight is nearly the product of monomer molecular weight, M_0 , and its polymerization number, k , i.e. $M_k = kM_0$.

The number- and weight-average molecular weights are therefore given by

$$\left. \begin{aligned} \langle M_w \rangle &= \frac{1+p}{1-p} M_0 \\ \langle M_n \rangle &= \frac{1}{1-p} M_0 \end{aligned} \right\} \quad (B11)$$

In terms of the following inequality

$$\frac{\sum_k (w_k M_k^{3/2})}{\sum_k w_k} > \frac{\sum_k (w_k M_k^{x-1} \ln M_k)}{\sum_k (w_k M_k^{x-1})}$$
(B12)

we obtain from eq B10

$$\frac{\sum_k (w_k M_k^{3/2})}{\sum_k w_k} = M_0^{3/2} \frac{\sum_k (k^{5/2} p^{k-1})}{\sum_k (k p^{k-1})}$$
(B13)

since

$$\frac{\sum_k (k^3 p^{k-1})}{\sum_k (k p^{k-1})} > \frac{\sum_k (k^{5/2} p^{k-1})}{\sum_k (k p^{k-1})} \quad (B14)$$

and

$$\left. \begin{aligned} \sum_1^\infty (k p^{k-1}) &= \frac{1}{(1-p)^2} \\ \sum_1^\infty (k^3 p^{k-1}) &= \frac{1+4p+p^2}{1-p} \end{aligned} \right\} \quad (B15)$$

Comparing eqs B8, B12, and B13 through eqs B14 and B15, we find that when the molecular weight of a species is very large, then $p \rightarrow 1$ and eq B8 results.

In a similar way by using, e.g., the following inequality

$$\frac{\sum_k (w_k M_k^2)}{\sum_k (w_k/M_k)} > \frac{\sum_k (w_k M_k^{x-1} \ln M_k)}{\sum_k (w_k/M_k)} \quad (B16)$$

eq B9 can also be proved.

Hence, at least for high molecular weight polymers, eq 14 is true.

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