Review

Theory of phase formation in aqueous two-phase systems

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Abstract

Currently there are a number of different mathematical models for phase equilibria in aqueous two-phase systems available. This diversity can create some confusion for model users, since most models seem to perform reasonably well. Choosing a model, thus, becomes rather a difficult task. In trying to address this problem, the principal models and the relevant theory available are reviewed. A discussion of osmotic viral expansions, lattice theory, group contribution, scaling ideas, excluded volume, electrostatics and other modeling approaches is presented. The strengths of the different approaches are critically evaluated and suggestions offered. Choosing a model, however, requires sophistication because each model is typically best at representing only a few particular aspects of system behavior, and the intended use of the model must be considered. Some suggestions for future work are also given.

Keywords: Reviews; Aqueous two-phase systems; Phase formation

Contents

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1. Introduction

The fact that two or more phases form in water with the addition of incompatible polymers or polymers and salts has been well known for several decades. Interested readers are referred to excellent reviews on the development of the subject by Albertsson [1,2] and by Walter et al. [3]. The experimental aspects of these phenomena have been carefully studied and have been well documented by Albertsson [1] and by Walter et al. [3]. The fact that almost any solute will show partitioning behavior between the liquid phases has also been well known, and the experimental aspects of this behavior have also been well studied and documented [1,3]. Walter et al. [4] have comprehensively reviewed recent progress in the field of aqueous two-phase systems. Very recently, Zaslavsky [5] has published a monograph covering most topics in the area of aqueous two-phase partitioning. Zaslavsky gives a compilation of experimental results together with his interpretations of these results.

One should note that an understanding of the known experimental results in terms of fundamental molecular properties, molecular parameters and interactions has started to emerge only recently, perhaps over the last decade. The objective of this article is then to present an overview of the most fundamental existing theories of phase formation and phase equilibria. Solute partitioning will be the subject of another article. However, this article is not meant to be an exhaustive compilation of every piece of work that has been published on the subject. Rather, the aim is to explain the principal ideas that

have emerged, and it is, thus, representative rather than encyclopedic. The topic of aqueous two phase systems formed with polyelectrolytes as phase forming polymers is not discussed. The reason is that I am not aware of any theoretical model for the phase behavior of these types of systems. The development of such a model, however, is entirely possible.

There are roughly four schools of thought in the area of modeling of phase formation: (1) models based on osmotic viral expansions descended from the original work of Edmond and Ogston, (2) models based on extensions of lattice theories such as the Flory–Huggins theory, (3) models incorporating integral equation theory as a major element and (4) models that do not fall into the above categories, such as group contribution schemes and excluded volume approximations. All of these models will often have added-on expressions for electrostatic forces to account for the effect of interactions involving charged species.

2. Polymer solution regimes

All of the aqueous two-phase systems known are solutions of polymers. It is, therefore, appropriate to initiate a discussion on the theory of aqueous two-phase systems by first briefly reviewing the behavior of polymer solutions. In particular this will focus on the differences between dilute and concentrated polymer solutions.

When the concentration of polymer molecules in a solution is approaching zero there are always many layers of solvent molecules separating the polymer
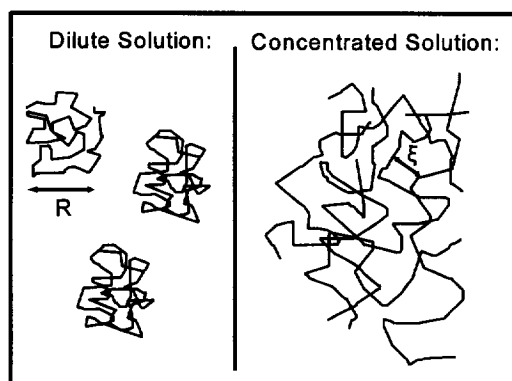
Fig. 1. In a dilute polymer solution the polymer coils are separated from each other and the relevant length scale is the end-to-end distance (R). In a concentrated solution the polymer coils intertwine and the relevant length scale is the correlation length or mesh size (ξ).

molecules from each other. This is the regime of a dilute polymer solution illustrated in Fig. 1. It should be noted, however, that if we use polymers of higher and higher molecular mass, the volume occupied by each polymer molecule becomes larger and larger, and the concentration range over which a dilute regime exists becomes very small although the number of polymer molecules per unit volume may still be very small. Whether the solution regime is dilute or concentrated, depends not only on the molar concentration of the polymer but also on its molecular mass. As the volumes occupied by individual polymer molecules start to overlap, because their concentration increases for a given polymer molecular mass, the polymer chains start to entangle and the solution smoothly evolves from a collection of individual polymer molecules to a mesh-like structure as illustrated in Fig. 1. It is possible to do a simple calculation for the polymer concentration c^* called the crossover concentration at which the polymer chains just start to overlap. One such calculation has been presented by Abbott et al. [6] for poly(ethylene glycol)s (PEGs) in water. Although, there are, in fact, several slightly different ways of performing the calculation [7] all of which give similar results. The calculation that we present here is based on the results from the monograph by Des Cloizeaux and Jannink [7] which we believe to be most accurate. The relevant expressions are

$$c^* = \frac{3^{3/2}}{R^3}, \qquad R \approx 2.5105 R_g \qquad (1)$$

where R is the end-to-end distance of the polymer chain and R_g is the radius of gyration of the polymer. The relation between R and R_g is an approximate result obtained for a very long linear polymer with excluded volume [7]. The radius of gyration R_g can be measured from static light scattering experiments [8]. For the case of polyethylene oxide p in water, the radii of gyration data of Cabane and Duplessix [9,10] can be represented as a function of polymer molecular mass by a fitted equation:

$$R_g = 0.13245 M_p^{0.62} \qquad (2)$$

where R_g is given in $\mathring{A}$ and M_p is the molecular mass of the polymer (g/mol). This data is used in calculating the crossover curve of Fig. 2. The concentrations have been converted to mass fractions w_p according to $w_p \approx M_p c^* / (d_w + M_p c^*)$ where d_w is the mass density of pure water. A similar calculation can be done for dextrans using the data of Senti et al. [11]. However, we did not include it in Fig. 2. The reason is that the resulting curve is uninteresting as it lies well to the right on the plot for all of the dextrans commonly in use due to their relatively high molecular masses.

As pointed out by Abbott et al. [6], the existence of the crossover concentration plays an important
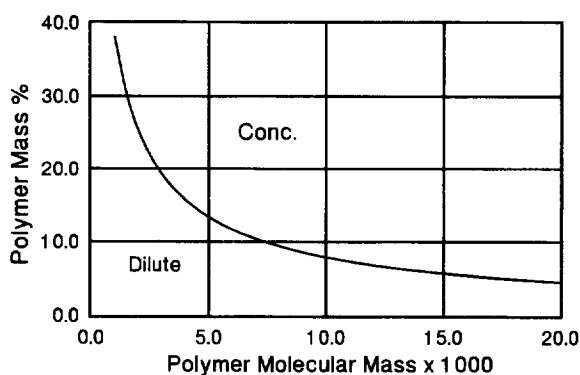

Fig. 2. Theoretically calculated curve for the polymer mass fraction corresponding to the cross over composition, which is the composition at which the solution regime changes from dilute to concentrated in aqueous mixtures of poly(ethylene glycol)s at ambient conditions.

role in the choice of model, and in the physical picture used to represent the state of a particular solution. For example, in a dilute solution the important length scale is R which means that according to the scaling hypothesis [12], the molecular interactions between polymer chains can be represented as functions of R. In a concentrated solution the important length scale is the correlation length of the polymer mesh ξ. Again the scaling hypothesis [12] holds that the molecular interactions can be expressed in terms of ξ, which correlates with the average distance between monomers in the polymer mesh. However, it should again be pointed out that these polymer solutions are very dilute in terms of the number of solute molecules present. For example, the cross-over concentration for PEG 1000 is approximately 0.6 mol l^{-1} and that for PEG 8000 is $2 \cdot 10^{-3}$ mol/l. The fact that the solution is dilute also has important implications. These facts will be referred to repeatedly throughout the rest of this review.

3. Prediction of two-phase formation

If one assumes that the phases in an aqueous two-phase system are at equilibrium, then predicting the formation of two phases and predicting the equilibrium state (composition, density, etc.) can be done from the equality of the component chemical potentials and system mass balances. In performing this calculation we further assume that the polymers are represented by a single component with the average molecular mass of the polymer. Then for a system consisting of a solvent s and two solutes i and j,

$$\mu_s^u(T, P, c_i^u, c_j^u) = \mu_s^l(T, P, c_i^l, c_j^l) \tag{3a}$$

$$\mu_i^u(T, P, c_i^u, c_j^u) = \mu_i^l(T, P, c_i^l, c_j^l) \tag{3b}$$

$$\mu_j^u(T, P, c_i^u, c_j^u) = \mu_j^l(T, P, c_i^l, c_j^l) \tag{3c}$$

where μ_k^i is the chemical potential of solute i in phase k [upper (u) or lower (l)] and c_k^i is the composition (mol volume^{-1} or mol/kg) of solute i in phase k.

The mass balances represent the fact that all material added to the system must be in either the

upper or the lower phase. Thus, the total mass of added solvent W_s, the total mass W_i of added solute i and the total mass W_j of added solute j are distributed according to

$$W_s = w_s^u W^u + w_s^l W^l \tag{4a}$$

$$W_i = w_i^u W^u + w_i^l W^l \tag{4b}$$

$$W_j = w_j^u W^u + w_j^l W^l \tag{4c}$$

where w_i^k is the mass fraction of solute i in phase k defined by $w_i^k = W_i^k / (W_s^k + W_i^k + W_j^k)$, where W_i^k is the mass of solute i in phase k, and W^k is the total mass of all components in phase k. The solute composition in either mol volume^{-1} or mol kg^{-1} can be calculated rather easily from the mass fractions and phase density.

There is ample evidence to the fact that the polymers used in aqueous two-phase extraction are polydisperse [1,3]. There is also ample evidence that the polymers fractionate between the two phases [13], e.g., the higher-molecular-mass PEGs preferentially partition to the PEG phase and so on for the dextrans. Therefore, the molecular mass of the polymer in the upper and in the lower phase is different, and neither is equal to the overall molecular mass of the polymer [13–16]. To portray this effect better, let us consider a two-phase system with a very large PEG phase and a very small dextran phase. For such a system, the average molecular mass of the PEG in the PEG phase would approximately equal the average overall molecular mass of the PEG added to the system. But, the average molecular mass of the dextran in the dextran phase would be greater than the average overall molecular mass of the dextran added to the system. The reason for this effect is that the low molecular mass fractions of the dextran are more soluble in the PEG phase than the high molecular mass fractions.

As illustrated in Fig. 3, the polydispersity and the consequent phase fractionation of the polymers will cause the binodial curve to shift when plotted in a three-component phase diagram. The reason for this effect is that as the overall composition is varied along a fixed tie line, the fractionation of the polymers causes a change in the molecular mass of the polymers actually present in each phase. This shift in the plot would not be observed in a multi-
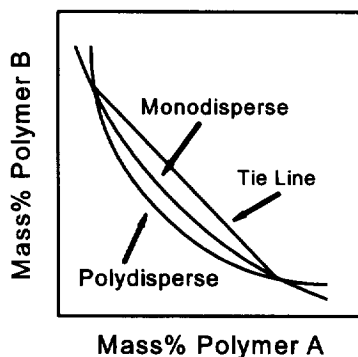
Fig. 3. The effect of the polydispersity of phase forming polymers on the binodial curve of aqueous two-phase systems. The tie line connects the composition of two phases in equilibrium.

dimensional plot where one coordinate was given to each of the polymer molecular fractions present. However, in a three-component plot, this effect gives the impression that the composition of the phases depends on the mass or volume ratio of the phases.

The simplest and possibly the most effective technique to include the effect of polydispersity and phase fractionation into phase diagram calculations is the pseudo-component method [17–19]. Essentially, the method amounts to replacing the many individual components of different molecular masses contained in a polymer with a continuous statistical distribution of molecular masses, and then using the distribution in a mathematically consistent quadrature procedure to choose a limited number of components (say five) of a specified molecular mass which can be used to represent the polydisperse polymer. This technique was adapted for used with two-phase systems in conjunction with the UNIQUAC model [14–16], but it is widely applicable with other models also. A brief summary of the procedure taken from the work of Sandler's group at Delaware [14–16] will be presented here. Thus, for each polydisperse polymer i we assume that the molecular mass distribution is given by the Lansing–Kraemer function

$$F(M_i) = \frac{1}{\beta_i \pi^{1/2}} \exp[-(1/\beta_i^2) \ln^2(M_i/M_{o_i})] \qquad (5)$$

where β_i and M_{o_i} are adjustable parameters, M_i is the molecular mass of polymer fraction i and π is the number pi. Using this distribution one can obtain an

expression for the number average M_{n_i} and the mass average M_{w_i} molecular mass of the polymer for a polymer i

$$\frac{1}{M_{n_i}} = \frac{\int_0^\infty \frac{1}{M_i} F_i(M_i) \, dM_i}{\int_0^\infty F_i(M_i) \, dM_i} = M_{o_i} \exp[-1/\beta_i^4] \qquad (6)$$

$$\frac{1}{M_{w_i}} = \frac{\int_0^\infty M_i F_i(M_i) \, dM_i}{\int_0^\infty F_i(M_i) \, dM_i} = M_{o_i} \exp[1/\beta_i^4] \qquad (7)$$

These relations can be used to obtain values for the distribution parameters β_i and M_{o_i} from experimentally measured average polymer molecular masses M_{n_i} and M_{w_i}. We proceed by stating that the average value of a function θ (M_i) weighted by this distribution is given by

$$\int_0^\infty F_i(M_i)\theta(M_i) \, dM_i =$$

$$\frac{1}{\beta_i \pi^{1/2}} \int_0^\infty \exp[-1/\beta_i^2 \ln^2(M_i/M_o)]\theta(M_i) \, dM_i \qquad (8)$$

Making the substitutions of $x_i = 1/\beta_i \ln (M_i/M_{o_i})$ and $G(x_i) = \theta(M_i)$ one can use Gauss–Hermite quadrature [20] to replace the integral with a sum over n terms, where n is the number of pseudo-components or polymer fractions to be used. The appropriate expression is

$$\int_{-\infty}^{+\infty} \exp[-x_i^2]G(x_i) \, dx_i = \sum_{k=1}^n W_{k_i} G(z_{k_i}) \qquad (9)$$

where the z_{k_i} are the zeros of the Hermite polynomials and W_{k_i} are the weight factors in the expansion. The Hermite polynomials H_n are defined by

$$H_n(x) = n! \sum_{m=0}^{n/2} (-1)^m \frac{(2x)^{n-2m}}{m!(n-2m)!} \qquad (10)$$

The numerical values of the z_{k_i} and of the W_{k_i} are found in the literature [21]. The W_{k_i} can also be calculated from

$$W_{k_i} = \frac{n! \pi^{1/2}(z_{k_i})^{n+1}}{[H_{n+1}(z_{k_i})]^2} \qquad (11)$$

Once the z_{k_i} and the W_{k_i} are found, the pseudo-

component mass fraction of any k of the n pseudo-components of component i can be found from

$$w_{k_i} = W_{k_i} \Big/ \sum_{l=1}^{n} W_{l_i} \qquad (12)$$

and its molecular mass can be calculated from

$$M_{k_i} = M_{k_i} \exp[(\beta_i z_{k_i})] \qquad (13)$$

The above-mentioned mathematical procedure amounts to breaking up each of the polydisperse polymers added to the system into n representative fractions, and treating the system as if one were adding not one but n chemically similar components differing only in molecular mass. After this process is accomplished, the phase equilibrium calculation proceeds as before except that instead of three chemical potential equalities and mass balances, there are $1 + 2n$ equations for a three-component system.

The inclusion of polydispersity into the calculation of phase diagrams makes a small but still significant contribution as illustrated in Fig. 3. It also probably makes an even greater contribution to the partitioning of proteins.

4. Chemical thermodynamics

The objective of this section is to provide a basic review of the most important thermodynamic quantities and concepts to be used in the discussions that follow. Although most of the material is well known, it was felt that this review would aid readers not familiar with the subject in understanding the various thermodynamic concepts. Those wishing further details are referred to any of the many excellent monographs and text books on chemical thermodynamics.

The most fundamental thermodynamic criteria for phase equilibrium in mixtures is the equality of the component chemical potentials in all the phases present in the system. There are, however, a number of auxiliary functions commonly used in both modeling and experiment. The Gibbs free energy G is the most fundamental quantity for phase-equilibrium problems at ordinary conditions, i.e., constant temperature and pressure. The molar Gibbs free energy

of any particular phase (G in J mol^{-1} mixture) is related to the component chemical potential μ_i by

$$G(T, P, x_1, x_2, x_3...) = x_1 \mu_1(T, P, x_1, x_2, x_3...)$$
$$+ x_2 \mu_2(T, P, x_1, x_2, x_3...)$$
$$+ x_3 \mu_3(T, P, x_1, x_2, x_3...) + ...$$
$$\qquad (14)$$

where μ_i is the chemical potential of component i defined by

$$\mu_1(T, P, x_1, x_2, ...) = \left(\frac{\partial NG}{\partial N_1}\right)_{T, P, N_2, N_3,...} \qquad (15a)$$

$$\mu_2(T, P, x_1, x_2,...) = \left(\frac{\partial NG}{\partial N_2}\right)_{T, P, N_1, N_2,...} \qquad (15b)$$

where T is the temperature, P is the pressure, N is the total number of moles in the phase, N_i is the number of moles of component i in the phase, and x_i is the mole fraction of component i in the phase.

The component chemical potential defines a number of related auxiliary functions such as the thermodynamic activity of a component given by

$$\mu_i(T, P, x_1, x_2, \ldots) = \mu_i^o(T, P, x_1^o, x_2^o, \ldots)$$
$$+ RT \ln a_i(T, P, x_1, x_2, \ldots ; x_1^o, x_i^o, \ldots) \qquad (16)$$

where μ_i^o is the reference chemical potential of component i, a_i is the thermodynamic activity of component i, and the x_i^o values are the reference compositions of all the components in the solution. For an ideal solution, the thermodynamic activity ($a_i = x_i$) is equal to the mole fraction.

The reference chemical potential μ_i^o is a subtle matter. The idea is to choose a reference chemical potential that has either very simple or no composition dependence and that is as close to the actual state of the component i in the mixture as possible. For a solvent, component 1, such as water which can exist as a liquid at the usual temperatures and pressures of the experiment, the reference chemical potential $\mu_i^o(T, P, 1, 0, ...)$ is that of pure solvent. The reference composition is $x_1^o = 1$, $x_2^o = 0$, $x_2^o = 0$, etc. For solutes (components 2, 3, etc.) such as polymers, salts, or biomolecules that can not usually exist as a liquid at the same conditions, the reference chemical potential $\mu_i^o(T, P, 1, 0, ...)$ is usually that of the solute at infinite dilution in the solvent, and the reference

composition is again $x_1 = 1$, $x_2 = 0$, $x_3 = 0$, etc. Note that the reference chemical potentials cancel out of the equations when the chemical potentials in different phases are set equal in phase equilibrium calculations. For this reason, one can always use the thermodynamic activities in place of the chemical potentials in phase equilibrium calculations.

In the development of models for the Gibbs free energy of mixtures, an auxiliary function called the excess Gibbs free energy G^e is usually employed. This function is the difference between the Gibbs free energy of the real mixture G and that of an ideal mixture G^{im} at the same temperature, pressure and composition. The defining expression for G^e is

$$G^e(T, P, x_1, x_2, ...) = G(T, P, x_1, x_2, ...)$$
$$- G^{im}(T, P, x_1, x_2, ...)$$
$$= \sum_i x_i \mu_i - \sum_i x_i \mu_i^{im} \tag{17}$$

where the superscript im stands for ideal mixture. Inserting expressions for the Gibbs free energy in terms of chemical potentials and thermodynamic activities gives

$$G^e = \sum_i x_i (\mu_i^o + RT \ln a_i) - \sum_i x_i (\mu_i^o + RT \ln x_i)$$
$$= RT \sum_i x_i \ln\left(\frac{a_i}{x_i}\right) \tag{18}$$

The thermodynamic activities are related to the excess Gibbs free energy by

$$\ln\left(\frac{a_1}{x_1}\right) = \frac{1}{RT}\left(\frac{\partial NG^e}{\partial N_1}\right)_{T,P,N_2,N_3,...} \tag{19a}$$

$$\ln\left(\frac{a_2}{x_2}\right) = \frac{1}{RT}\left(\frac{\partial NG^e}{\partial N_2}\right)_{T,P,N_1,N_3,...} \tag{19b}$$

In some situations such as a polymer solution, the Gibbs free energy of mixing ΔG_{mix} rather than the excess Gibbs free energy G^e is frequently employed. The Gibbs free energy of mixing is the difference between the real solution Gibbs free energy and the free energy of the mixture components as pure liquid for the solvent and as liquids at infinite dilution for the sparingly soluble solutes. The definition for ΔG_{mix} is

$$\Delta G_{mix}(T, P, x_1, x_2, ...) = G(T, P, x_1, x_2, ...)$$
$$- x_1 \mu_1^o(T, P, 1, 0,...)$$
$$- x_2 \mu_2^o(T, P, 1, 0, ...) - ... \tag{20}$$

The Gibbs free energy of mixing is related to the excess Gibbs free energy by the expression

$$\Delta G_{mix} = G^e + RT \sum_i x_i \ln x_i \tag{21}$$

Lastly, I would like to briefly mention the thermodynamic consistency relations given below by the cross-derivative equality for component chemical potentials:

$$\frac{\partial \mu_i}{\partial m_j}\bigg|_{T,P,m_k \neq j} = \frac{\partial \mu_j}{\partial m_i}\bigg|_{T,P,m_k \neq i} \tag{22}$$

and the Gibbs–Duhem equation, which at constant temperature and pressure is

$$0 = \sum_i m_i \, d\mu_i\big|_{T,P} \tag{23}$$

where for both equations m_i is the molality of component i, μ_i is the chemical potential of component i, and where the summation is taken over all components including the solvent. All model expressions and all equilibrium experimental data for the component chemical potentials must satisfy the cross derivative relations and the Gibbs–Duhem equation. Mathematical expressions or experimental data that do not satisfy these requirements violate the laws of thermodynamics.

5. Osmotic viral expansions

The use of osmotic viral expansions to represent the properties of aqueous two-phase systems gained popularity starting with the work of Edmond and Ogston [22,23]. The reasons for this popularity are simply that the resulting expressions model the known experimental behavior with reasonable accuracy, they are mathematically simple, and the physical interpretation of the model parameters is relatively simple. Theory tells us, however, that there are two different osmotic viral expansions, one based on the work of McMillan and Mayer [24] and the other on the work of Hill [25–27]. There are subtle

but very important differences between these two expansions which are often not recognized.

This class of theories gives expressions for the solution thermodynamic properties and, in particular, for the chemical potential of solutes in terms of a power series in solute composition, either mol volume^{-1}, mol kg^{-1} solvent, or mol mol^{-1} solvent. The coefficients in the expansion represent the molecular interactions between small groups of solutes, e.g., pairs, triplets, and so on. The role of the solvent is a very subtle matter which is handled differently in each of the two theories, as will be explained later. Being expansions in composition, these theories are strictly applicable only at very low solute concentration. But, since the concentrations of solutes and in particular polymers are rather low even at the cross-over concentration, it is then fair to say that the theories are at least approximately applicable to the solutions commonly encountered in aqueous two-phase extraction.

In polymer solutions, the interpretation of the coefficients in terms of molecular interactions changes as we move from a dilute solution regime to a concentrated solution regime. The change in the molecular interactions with solution regime implies, that the model for the osmotic viral coefficients must also be changed with solution regime. This introduces complications in the theory which detract from the attractive simplicity of this class of models. Although, the effect is often ignored in practice, perhaps in an effort to preserve the simplicity of the approach. Though, it should be realized that the terms dilute and concentrated have rather a special meaning here because the number of moles of solutes per unit volume is always very small.

5.1. The McMillan–Mayer theory

The theory of solutions derived by McMillan and Mayer [24] gives the thermodynamic properties of a multicomponent system as a power series in solute concentration in moles volume^{-1} or molarity. The coefficients of the series directly represent the interactions between small groups of solute molecules, i.e., pairs, triplets, and so on, in otherwise pure solvent. Thus, the chemical potential μ_i of a solute i in a solution of a solvent s and another solute j is given by

$$\frac{\mu_i(T, \mu_s, c_i, c_j)}{RT} = \frac{\mu_i^\circ(T, P, 0, 0)}{RT} + \ln c_i$$
$$+ 2B_{ii}(T, \mu_s)c_i + 2B_{ij}(T, \mu_s)c_j$$
$$+ \ldots \qquad (24)$$

where μ_s is the solvent chemical potential, μ_i° is the solute chemical potential at zero solute concentration, c_k is a solute concentration in moles of solute per volume and B_{ij} is the McMillan–Mayer osmotic second viral coefficient for the interaction between a molecule of solute i and a molecule of solute j in otherwise pure solvent. It should be noted that the chemical potential of the solvent has to be obtained from the Gibbs–Duhem equation using expressions for the solute chemical potentials. However, the above expression for the solute chemical potentials is given at constant T and μ_s, whereas the Gibbs–Duhem equation is written at constant T and P. It is, therefore, necessary to convert these expressions to constant P as outlined below before the Gibbs–Duhem equation is used.

In the McMillan–Mayer theory the solvent is treated as a featureless background continuum; a stage in which the solute molecules exist and interact with each other. This result is rigorous and it simplifies the statistical mechanics of the problem enormously. It also makes it possible to give a simple interpretation to the McMillan–Mayer osmotic viral coefficients in terms of an energy of interaction between groups of two (u_{ij}), three (u_{ijk}) or more molecules in pure solvent. For example (see Fig. 4), the second viral coefficient is given by
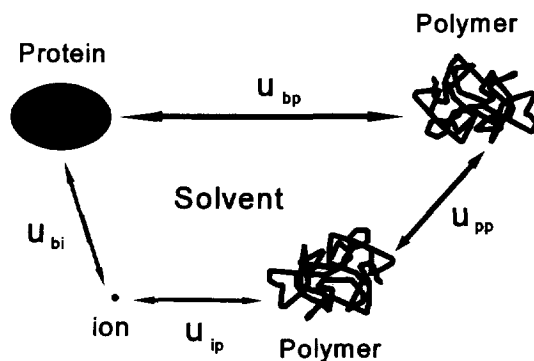

Fig. 4. Relative sizes of molecules and energies of interaction u_{ij} for proteins or biopolymers (b), polymers (p) and ions in a salt i, all in the presence of a solvent.

$$B_{ij} = -2\pi \int_0^\infty [\exp(-u_{ij}/kT) - 1] \, d\omega_i \, d\omega_j \, dr_i \, dr_j$$

$$(25)$$

where k is the Boltzmann constant, and where the integration is performed over all possible orientations and positions of molecules i and j. The important issue here is that B_{ij} involves only interactions between a molecule i and a molecule j, where i and j can be any solute but not the solvent.

The difficulty with this approach is that in order for the solvent to be treated as background, the thermodynamic state of the solvent must be held constant even as the concentration of solute changes, and this means that μ_s must be held constant, which is why it appears in the functional dependence of μ_i. A consequence of this fact is that one can not use the above expression for μ_i directly in a phase equilibrium or other calculation at a constant pressure, say at laboratory pressure. Rather, the expression for μ_i must be converted from a state at constant μ_s to a state at constant P, i.e., from $\mu_i(T, \mu_s, c_i, c_j)$ to $\mu_i(T, P, c_i, c_j)$ which is what is required for the calculation. The easiest procedure to accomplish this change [28,29] is to consider that μ_s is constant because the solution is in osmotic equilibrium with pure solvent across a semi-permeable membrane as illustrated in Fig. 5. Then the solvent chemical potential $\mu_s(T, P + \pi, c_i, c_j) = g(T, P) = $ constant, since T and P are constant. Constant μ_s then implies that the solution pressure is $P + \pi$. Then constant pressure $\mu_i(T, P, c_i, c_j)$ can be obtained from

$$\mu_i(T, \mu_s, c_i, c_j) = \mu_i(T, P + \pi, c_i, c_j) \qquad (26a)$$

$$\mu_i(T, P, c_i, c_j) = \mu_i(T, P + \pi, c_i, c_j)$$

$$+ \int_{P+\pi}^{P} \bar{V}_i(T, P, c_i, c_j) \, dP \qquad (26b)$$

where π is the osmotic pressure and $\bar{V}_i$ is the partial molar volume of solute i in solution. One can with reasonable accuracy assume that $\bar{V}_i$ is independent of pressure and equal to its room pressure value, and for polymers but not for salts one can further assume that it is independent of composition and equal to its infinite dilution value $\bar{V}_i^o(T)$. There are correlations giving $\bar{V}_i^o(T)$ for PEGs and dextrans [30] as a
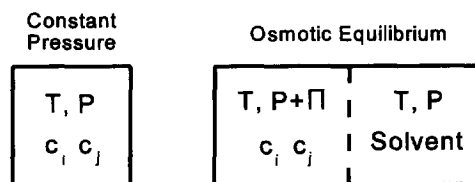

Fig. 5. Contrast of a solution at constant temperature and pressure to a solution in osmotic equilibrium with its own solvent through a membrane permeable to the solvent only. Note that the temperature and compositions of both solutions are the same but that the pressures differ.

function of polymer molecular mass. For salts, the reader is referred to the work of Millero [31]. This osmotic pressure correction is often ignored in phase equilibrium calculations involving aqueous two-phase systems. The reason is that there is a partial cancellation of the correction for the top and bottom phases when the chemical potentials in the top and bottom phases are set equal to each other.

The McMillan–Mayer theory has been used to calculate phase diagrams in aqueous two-phase system by a number of different research groups over the years. The McMillan–Mayer osmotic viral coefficients have been experimentally measured with a variety of techniques. Thus, Edmond and Ogston [22] experimentally determined values for the co-efficients from osmotic pressure, sedimentation equilibria and phase diagram critical point. The Berkeley Group [32,33] experimentally measured the coefficients using low-angle laser light scattering and vapor pressure measurements. The group at Darmstadt [34–36] measured the coefficients by vapor pressure osmometry and membrane osmometry. It should be noted that the calculations of Edmond and Ogston and those of the Berkeley group assume that the polymers are monodisperse while those of the Darmstadt group do include the polydispersity and fractionation of the polymers. To the best of my knowledge, the aforementioned osmotic pressure correction to the component chemical potentials has been neglected in all of these phase diagram calculations.

5.2. The Hill theory: molecular interaction scaling

The theory derived by Hill [25–27] seems superficially similar to that of McMillan and Mayer [24],

and indeed both give the component chemical potentials as a power series in solute composition. However, the theory of Hill gives the component chemical potentials at constant temperature and pressure rather than constant solvent chemical potential μ_s, and it, therefore, requires no osmotic pressure correction. The power series is an expansion in solute molality m_i (mol solute kg^{-1} solvent or mol solute mol^{-1} solvent) rather than concentration (moles of solute per volume). The conversion between these two units of molality is $1000/M_s$, where M_s is the solvent molecular mass. The chemical potential μ_i of a solute i in a solution of a solvent s and another solute j is given by

$$\frac{\mu_i(T, P, m_i, m_j)}{RT} = \frac{\mu_i^o(T, P, 0, 0)}{RT} + \ln m_i$$
$$+ 2\tilde{B}_{ii}(T, P)m_i + 2\tilde{B}_{ij}(T, P)m_j + \ldots$$
$$(27)$$

where P is the system pressure, μ_i^o is the reference solute chemical potential at zero solute concentration, m_k is the solute molality in mol solute mol^{-1} solvent, and $\tilde{B}_{ij}$ is the Hill osmotic second viral coefficient for the interaction between a molecule of solute i and a molecule of j in pure solvent. It should be noted again that the chemical potential of the solvent has to be obtained from the Gibbs–Duhem equation using the above expression for the solute chemical potentials.

Since the solvent chemical potential is not kept constant as solute is added to the solution, the coefficients in the theory of Hill no longer have the simple interpretation given above for McMillan–Mayer osmotic viral coefficients. In addition to including interactions between solute molecules, these coefficients explicitly include interactions between solute and solvent molecules. The Hill osmotic viral coefficients $\tilde{B}_{ij}$ are certainly accessible experimentally by any of the techniques commonly used to measure thermodynamic activities such as vapor pressure lowering, osmometry, isopiestic experiments, light scattering, etc. [37]. Kabiri-Badr [38], for example, used isopiestically measured water activities to evaluate Hill coefficients in several aqueous salt–polymer mixtures. There is also a simple and rigorous thermodynamic relation [27,37] between the McMillan–Mayer coefficients B_{ij} and

the Hill coefficients $\tilde{B}_{ij}$. For the second viral coefficients the relation is

$$\tilde{B}_{ij} = \frac{\rho_s^o}{2}(2B_{ij} - \bar{V}_i^o - \bar{V}_j^o + K_s RT)$$
$$(28)$$

where ρ_s^o is the molar density of pure solvent, $\bar{V}_i^o$ is the partial molar volume of solute i at infinite dilution and $K_s = (-1/V)\partial V/\partial P|_T$ is the isothermal compressibility of pure solvent. The last contribution is negligible at ordinary conditions because the isothermal compressibility is rather small for liquids. The partial molar volumes for the polymers [30] and salts [31] commonly used in aqueous two-phase extraction are available from the literature. For polymers the partial molar volumes are available as correlations of the form $\bar{V}_i^o = \bar{V}_{mi}^o N_i$, where N_i is the degree of polymerization and $\bar{V}_{mi}^o$ is the formal partial molar volume of a monomer. In cases where partial molar volumes are not available in the literature, they are obtainable from solution density data which generally are easy to measure. It should be noted from the above expression that converting a McMillan–Mayer osmotic viral coefficient B_{ij} to a Hill osmotic viral coefficient $\tilde{B}_{ij}$ can not be done by just changing the units from mol volume^{-1} to mol kg^{-1}.

To model the Hill osmotic viral coefficients, one can construct a theoretically simple model for the McMillan–Mayer coefficients and then relate it to the Hill coefficients according to the above relation. Scaling approaches have proven particularly useful in this effort. Again the reader is referred to Fig. 4. One possible approach is to consider that to a first approximation the interaction between two polymer molecules i and j can be treated as that between two rigid spherical blobs of diameter R_i and R_j. Then statistical mechanics [27,39] gives an interaction coefficient of the form

$$B_{ij} = \frac{2\pi}{3}\left(\frac{R_i + R_j}{2}\right)^3.$$
$$(29)$$

To a fair approximation one can assume that the quantities R_i and R_j are equal to the end-to-end distance of the polymer coil. Then, scaling theory based on the renormalization group applied to polymer solutions [40–42] tells us that the end-to-end distances R_i and R_j are related to the degrees of

polymerization (N_i and N_j) for polymers i and j by the expression

$$R_i = b_i N_i^\nu, \qquad R_j = b_j N_j^\nu \tag{30}$$

where ν is a universal exponent valid for all polymers provided they are sufficiently long, and where b_i and b_j are proportionality constants evaluated from experimental data. The value of ν has been calculated by various means; thus, LeGuillou and Zinn-Justin [41] give a value of 0.5885 from a theoretical expansion, Douglas et al. [42] give a value of 0.592 from a different theoretical expansion, and Kabiri-Badr [38] and Cabezas et al. [30] obtained an empirical value of 0.593 from experimental isopiestic data on aqueous solutions of PEG. It should be noted that all of the values are in remarkably good agreement.

When a salt s is present in aqueous solution it dissolves into ions i which are charged. It is then necessary to include an electrostatic term such as that from the Debye–Hückel theory [43] or any of a number of other electrolyte solution models [44] to account for the interactions between the charged ions (also see Section 9). The interaction between the salt and a polymer p or another neutral solute is then given as a sum over the interactions of the ions with the polymer. The McMillan–Mayer viral coefficient B_{sp} for the case of a strong electrolyte is given by

$$B_{sp}(T, \mu_w) = \frac{1}{\nu_s} \sum_i \nu_i B_{ip}(T, \mu_w) \tag{31}$$

where ν_s is the number of ions in the salt, e.g., 3 for Na_2SO_4, ν_i is the number of ions of type i, e.g., 2 for Na^+ in Na_2SO_4, and B_{ip} is the ion-polymer osmotic viral coefficient, and the sum is taken over all the ions of the salt.

A scaling argument similar in spirit to that for polymers can be made for the interaction between a neutral polymer molecule p and an ion or a small molecule i. One assumes that the ion being small can sample the entire length of the polymer chain (see Fig. 4). The McMillan–Mayer osmotic viral coefficient B_{ip} is then approximately proportional to the length L of the polymer chain which is itself proportional to the degree of polymerization N_i. The resulting expression is

$$B_{ip} = \bar{b}_{ip} L = b_{ip} N_p^\tau \tag{32}$$

where b_{ip} is a proportionality constant evaluated from experimental data. The scaling hypothesis indicates that the exponent τ should have a value of about one and be universal for all polymers. Kabiri-Badr [38] found that a value of 1.033 for the exponent τ was adequate to reproduce the phase diagrams for three different molecular masses of poly(ethylene glycol) and three different salts at ambient conditions. Since Kabiri-Badr's value of 1.033 is within 3% from one, his result tends to support the scaling hypothesis.

6. Lattice theory

The idea of modeling macromolecular liquid mixtures in terms of a crystal lattice originated with a number of research groups during the 1930s and 1940s. Guggenheim [45] gives an excellent review of the work from this era in his monograph on mixtures. Ochs [46] reviewed this material in historical perspective and related it to more recent developments. This class of theories is attractive because of simplicity since in a lattice one can distribute and redistribute (see Fig. 6) the macromolecules and the small molecules until all the possible arrangements or configurations have been examined. One can, in fact, easily count or at least estimate the total number of possible configurations Ω for the system. The
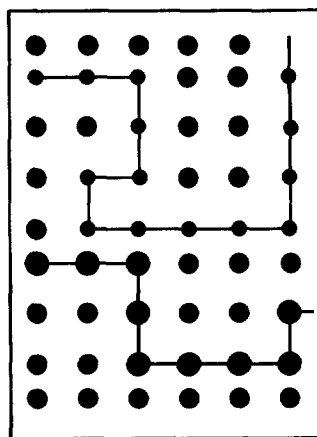

Fig. 6. Illustration of the distribution of chains of two different types polymers and a solvent in a square lattice. Entropies are obtained from lattices by counting all of the possible configurations of the polymers and the solvent on the lattice.

number of configurations together with the famous Boltzmann relation, $-S = k\ln\Omega$, between entropy S and Ω give a combinatorial entropy for the mixture. It is also possible to develop expressions for the enthalpy by counting the number of energetic interactions between solute molecules or solute and solvent molecules. From the entropy and the enthalpy the Gibbs free energy can be calculated which contains all the thermodynamic information for the mixture. The main difficulty with these approaches is that in general, the entropy of a lattice, and, in fact, any entropy obtained by some combinatorial counting procedure can not be made equal to that of a liquid because it has too much order. A second problem particular to aqueous mixtures is that enthalpy effects are usually rather important, whereas the attractiveness of the theory is its ability to give the entropy by a simple procedure. Although it must be accepted that modern developments have ameliorated these difficulties, the fundamental problems still remain.

From a practical point of view, this class of theories is formulated assuming concentrated polymer solutions, and the expressions are not expansions in solute concentration. Therefore, the question of applicability as the solution becomes more concentrated that arose in connection with osmotic viral expansions is avoided entirely. There is, however, serious doubt that the same lattice expressions are applicable in very dilute solutions where there exists large regions of pure solvent between polymer coils. Extension of these ideas, particularly in their more modern version, to aqueous two phase systems is natural, and some of the more important recent developments in this area are summarized below.

6.1. The Flory–Huggins theory and its extensions

The classical polymer solution theory of Flory [47,48] and Huggins [49], or Huggins and Flory, depending on the source, and its various modifications have been the basis of several efforts [50,51] to model the phase behavior of two-polymer aqueous two-phase systems. Benge [52] gives an excellent critical review of the application of the Flory–Huggins theory to polymer solutions and two-phase systems covering work up to about 1986. Benge notes that the Flory–Huggins interaction parameters

χ_{ij} show composition dependence in two-polymer aqueous two-phase systems. More recently, the research group at Lund [53–55] has modified the Evers et al. [56] extension of the Flory–Huggins theory by allowing the monomers to have internal degrees of freedom. This modification gives an interaction parameter χ_{ij} which is temperature and composition dependent addressing some of the problems pointed out by Benge. This approach gives reasonably correct phase behavior with temperature and composition for two-polymer aqueous two phase systems.

The attractiveness of the Flory–Huggins and related theories include their relative simplicity, the fact that they give qualitatively good predictions or at least correlations for phase behavior, and their ability to give mechanistic insight into the phase formation process. For example, in the original form of the Flory–Huggins theory a combinatorial expression is obtained for the entropy of mixing ΔS_{mix} by considering all the ways of distributing molecules of macromolecular solutes and molecules of solvent on a lattice (see Fig. 6). The expression for the entropy is

$$\Delta S_{\mathrm{mix}} = -k \sum_i N_i \ln \Phi_i \qquad (33)$$

where k is the Boltzmann constant, N_i is the number of moles of component i, Φ_i is the volume fraction of component i, and where the summation is performed over all the components present including the solvent. The internal energy of mixing ΔU_{mix} is then given by

$$\Delta U_{\mathrm{mix}} = kT \sum_i \sum_j N_i \Phi_j \chi_{ij} \qquad (34)$$

where χ_{ij} is an interaction parameter between components i and j. Finally, these two expressions are combined to give the Helmholtz free energy of mixing ΔA_{mix} which is assumed to be equal to the Gibbs free energy of mixing ΔG_{mix} from which all of the necessary solution thermodynamics can be obtained. The expression for the free energy is

$$\frac{\Delta G_{\mathrm{mix}}}{RT} \approx \frac{\Delta A_{\mathrm{mix}}}{RT} = \Delta U_{\mathrm{mix}} - T \, \Delta S_{\mathrm{mix}} \qquad (35)$$

The approximate equality above indicates that this relation is strictly valid only for situations where the

volume change of mixing is zero or at least negligibly small. This is usually the case for liquid mixtures away from the critical region.

6.2. The UNIQUAC model: polydispersity

The research group at Delaware [14–16,57,58] has developed a model for the phase behavior of two-polymer aqueous two-phase systems based on the UNIQUAC [59] equation. The UNIQUAC model is a lattice theory which incorporates the concept of local composition and Guggenheim's quasi-chemical approximation. Incidentally, UNIQUAC is an acronym for UNIversal QUAsi-Chemical. The Delaware group also included the effect on the phase diagram due to the polydispersity of the polymers. This was done using the pseudo-component procedure already outlined in the section on prediction of two-phase formation. Since the solute mole fractions in polymer solutions are rather small, the UNIQUAC equation was rewritten [60] in terms of mass fractions w_i.

As originally developed by Wilson [61], the local composition concept was used to evaluate the volume fractions in the Flory–Huggins theory. The local composition concept simply states that due to the differences in the molecular interactions between dissimilar types of molecules, the composition in the neighborhood of any molecule is different from the overall bulk composition in the mixture. This "local composition" around a molecule implies that there is a deviation from the random mixing of dissimilar molecules in a mixture. It further states that "local compositions" are proportional to a Boltzmann type factor where the argument in the exponential is a difference in interaction energies. The quasi-chemical approximation of Guggenheim [45] treats the differences in interaction between similar and dissimilar molecules as a quasi-chemical reaction. This can be illustrated by considering the interactions between molecules as a molecular exchange, $(a-a)+(b-b)\Rightarrow2(a-b)$, in a mixture of molecules of type a and b. Guggenheim gives expressions for the enthalpy and the entropy of the mixture from these considerations.

The UNIQUAC expression for the thermodynamic activity a_j of a component j in a mixture is

$$
\ln a_j = \ln \phi_j' = \frac{z}{2} M_j q_j' \ln\left(\frac{\theta_j'}{\phi_j'}\right) + M_j l_j'
$$
$$
- M_j \frac{\theta'}{w_j} \sum_k l_k' w_k + M_j q_j'\left(1 - \ln \sum_k \theta_k' \tau_{kj}\right.
$$
$$
\left. - \sum_k \frac{\theta_k' \tau_{jk}}{\sum_m \theta_m' \tau_{mk}}\right),
\tag{36}
$$

where w_k is the mass fraction of component k and ϕ_j' is the mass-based volume fraction of component j given by

$$
\phi_j' = \frac{r_j' w_j}{\sum_j r_j' w_j}
\tag{37}
$$

where r_j' is a mass based volume parameter for component j which is fitted to data, z is the lattice coordination number usually set equal to 10, M_j is the molecular mass of component j, q_j' is a mass based surface area parameter for component j that is fitted to data, θ_j' is the mass-based surface area fraction of component j given by

$$
\theta_j' = \frac{q_j' w_j}{\sum_j q_j' w_j}
\tag{38}
$$

l_j' is given by

$$
l_j' = \frac{z}{2}(r_j' - q_j') - \left(r_j' - \frac{1}{M_j}\right)
\tag{39}
$$

and τ_{jk} is given by

$$
\tau_{jk} = \exp\left(\frac{U_{jk} - U_{kk}}{RT}\right)
\tag{40}
$$

where the difference $U_{jk} - U_{kk}$ is a molecular interaction parameter that is fitted to data.

This model, particularly when polymer polydispersity is included, reproduces phase diagrams for two-polymer aqueous two-phase systems accurately. These good results are in part due to careful fitting of the model parameters using modern maximum likelihood techniques, and to inclusion of the effect of polymer polydispersity. Some of the difficulties with this class of approaches include assigning clear physical significance to the parameters and their values, and the lack of uniqueness of the parameter

values, i.e., the fact that there probably exist more than one set of values which give equally good results.

6.3. Polymer blob rescaling

The blob rescaling approach originated at the Korean Advanced Institute of Science and Technology [62–64]. It is based on De Gennes' concept [12] that a long polymer molecule can be considered a sequence of regions or blobs which have essentially independent motions. As illustrated in Fig. 7, these blobs may consist of several monomers and attached solvents. These assumptions have the collective effect of transforming a polymer molecule into a collection of small pseudo- independent blobs which can be approximately treated as small molecules. These blobs, however, are not exactly independent, since the number of blobs is related to the number of polymer and solvent molecules by mass conservation equations. This means that the number of blobs can not be varied independently of solvent and polymer. The thermodynamic implications are that the chemical potential equalities which define phase equilibrium still have to be stated in terms of polymers and solvent not blobs, although the molecular thermodynamic or statistical mechanical model for the chemical potentials may be constructed in terms of blobs and their interactions. As illustrated in Fig. 8 for the case of lattice based theories, this involves renormalizing the lattice, i.e., redefining the original lattice where the fundamental length is the size of a
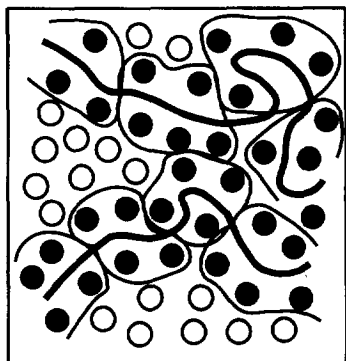

Fig. 7. Splitting of polymer chains in solution into a series of statistically independent or semi-independent blobs each consisting of several monomers with attached solvent molecules.
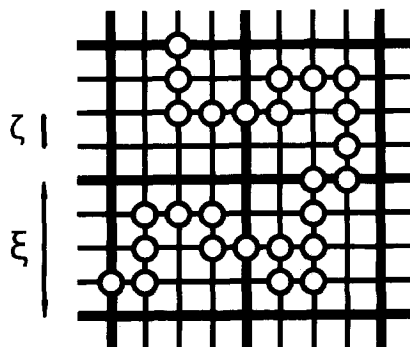

Fig. 8. Renormalization of a lattice with characteristic size ξ into a lattice with characteristic size ξ, where ξ is a multiple of ζ. A polymer chain with monomers of size ζ distributed on this lattice can be redefined as a chain of blobs of size ξ each consisting of several monomers.

monomer ζ into a new lattice where there is a new fundamental length ξ which is the size of a blob. Then, a small molecule lattice-based model such as the UNIQUAC [59], the NRTL [65] or some other model can be used to represent the polymer and solvent chemical potential in terms of interactions between blobs and blobs and solvent. Incidentally, NRTL is an acronym for Non-Random Two-Liquid. For purposes of illustration, the UNIQUAC equation for a mixture of components 1 and 2 in this case is written as

$$\frac{G^{e}}{RT} = x_1 \ln\frac{\Phi_1}{x_1} + x_2 \ln\frac{\Phi_2}{x_2}$$

$$+ \frac{z}{2}\left(q_1 x_1 \ln\frac{\theta_1}{\Phi_1} + q_2 x_2 \ln\frac{\theta_2}{\Phi_2}\right) - q_1 x_1 \ln(\theta_1$$

$$+ \theta_2 \tau_{21}) - q_2 x_2 \ln(\theta_2 + \theta_1 \tau_{12}) \qquad (41)$$

where x_i is the mol fraction of blob i, Φ_i is the volume fraction of blob i, θ_i is the area fraction of blob i, z is the coordination number of the lattice set equal to 10, q_i is the surface area parameter of blob i and τ_{ij} is defined by

$$\tau_{ij} = \exp\left(-\frac{U_{ij} - U_{jj}}{RT}\right) \qquad (42)$$

where $U_{ij} - U_{jj}$ represents the difference between the energy of interaction of a molecule of type i and a molecule of type j and two molecules of type j. Notice that $\tau_{ij} \neq \tau_{ji}$.

The NRTL equation defined below has a simpler

mathematical structure as compared to the UN-IQUAC model. It predates the UNIQUAC by nearly a decade. The expression is

$$\frac{G^e}{RT} = x_1 x_2 \left(\frac{\tau_{21} G_{21}}{x_1 + x_2 G_{21}} + \frac{\tau_{12} G_{12}}{x_2 + x_1 G_{12}} \right) \qquad (43)$$

where x_i is the mol fraction of blob i and τ_{ij} is defined by

$$\tau_{ij} = \frac{U_{ij} - U_{jj}}{RT} \qquad (44)$$

where $U_{ij} - U_{jj}$ is again the difference in the energy of interaction between a molecule of type i and a molecule of type j and two molecules of type j. Again note that $\tau_{ij} \neq \tau_{ji}$. Finally, G_{ij} is defined by

$$G_{ij} = \exp(-\alpha_{ij} \tau_{ij}) \qquad (45)$$

where α_{ij} is the non-randomness parameter and $\alpha_{ij} = \alpha_{ji}$.

The model parameters, whether the UNIQUAC or the NRTL equation is used, are all fitted to experimental data, usually phase diagram data. The approach does seem to reproduce with reasonable accuracy the composition dependence and the temperature dependence of mixtures of one and two polymers in a solvent including aqueous mixtures of PEG and dextran. The main difficulties with the approach are again difficulty in assigning physical significance to the parameter values, and the fact that the parameter values are not unique.

7. Integral equation theory

A very recent and unique approach is that of the Berkeley group [66]. This approach combines integral equation theory, a hard sphere equation of state, perturbation theory, the McMillan–Mayer osmotic viral expansion and other elements. All of these are brought together to derive a very general expression for a modified excess Helmholtz free energy, A'_{Ex}, for an aqueous mixture of polymers, salts and proteins. A'_{Ex} is defined as $A'_{Ex} = A - N_w \mu_w^o$, where A is the Helmholtz free energy of the mixture, N_w is the number of moles of water and μ_w^o is the chemical potential of pure water. The complexity of this method prevents the inclusion of a complete

description within the space of this review. Instead, a description of the underlying principles will be attempted. This modified excess Helmholtz free energy is divided into five additive contributions.

$$A'_{Ex} = A'_{Ex,hs} + A'_{Ex,na} + A'_{Ex,ic} + A'_{Ex,cc} + A'_{Ex,ve} \qquad (46)$$

where $A'_{Ex,hs}$ is the contribution from repulsive molecular forces which are modeled here as hard sphere interactions, $A'_{Ex,na}$ is a first-order correction for non-additivity of molecular sizes, $A'_{Ex,ic}$ represents the contribution from ionic charging, $A'_{Ex,cc}$ embodies the interactions between all charged species in the mixture and $A'_{Ex,ve}$ contains the effect from short-range non-electrostatic interactions such as dipoles, dispersion, etc. The non-additivity correction, $A'_{Ex,na}$, is necessary because the expression for the hard sphere contribution, $A'_{Ex,hs}$, assumes that the polymer coils and the proteins interact as impenetrable spheres which is not exactly true. For instance, a small protein can penetrate inside the polymer coil, and the polymer coil at any rate is not a rigid body. This model also assumes that the liquid mixture is incompressible, i.e., it has no pressure dependence, which is essentially equivalent to assuming that the Helmholtz and Gibbs free energies are equal, i.e., $G \approx A$. This means that we can use A in place of G in all of the expressions previously developed in the section on chemical thermodynamics. In this way one can easily obtain expressions for chemical potentials and, in principle, all other thermodynamic properties except volumes.

The model development then proceeds by constructing expressions for the five contributions to A'_{Ex} by various appropriate methods. For example, the expression for the hard sphere contribution, $A'_{Ex,hs}$, is developed from the Boublik [67] and Mansoori et al. [68] equation of state for hard spheres. The Boublik and Mansoori et al. equation of state is obtained from the solution of the Ornstein–Zernike integral equation [69] for the case of hard spheres. The expression for the non-additivity correction, $A'_{Ex,na}$, to the hard sphere contribution is obtained from a perturbation theory [70,71]. The contribution from ion charging, $A'_{Ex,ic}$, is calculated from the expression of Born [72]. The expression for the contribution from charge–charge interaction between the

ions, $A'_{Ex,cc}$, is obtained from the solution to the Ornstein–Zernike integral equation [69] using the mean spherical approximation [73–77]. Application of the mean spherical approximation to electrolytes is further discussed in the section on electrolyte solutions. Lastly, the contribution from short-range interactions between molecules in solution, $A'_{Ex,ve}$, is obtained from the McMillan–Mayer [24] osmotic viral expansion. All of the contributions to the modified Helmholtz free energy are carefully constructed to be consistent with the McMillan–Mayer system $(T, \mu^o_w, c_1, c_2, ...)$, and the resulting expressions for the chemical potentials need to be converted to constant pressure $(T, P, c_1, c_2, ...)$ for use in calculations as pointed out earlier. Unfortunately, no effort is made to include this rigorous but simple conversion in the model. The reason that the model still works adequately is probably due to: [1] the partial cancellation of the conversion in phase equilibrium calculations as discussed in the section on McMillan–Mayer theory, and [2] absorption of the conversion by the empirically adjusted model parameters.

The resulting overall model is, perhaps, the most complete and sophisticated available in the literature except as noted above. It is, however, a fairly complex model. Although, it has to be admitted that the model does accurately represent the behavior of rather a complex system so that the model's complexity is not unjustified. The model parameters are varied including species sizes, osmotic viral coefficients, etc. Overall, the model represents the phase behavior of polymer–polymer and salt–polymer aqueous two-phase systems, and the partitioning of proteins quite adequately which is a very significant accomplishment.

8. Other models

These are models which do not easily fall into one of the categories outlined above. Although they may well have elements derived from them. For example, the free energy equations for the group contribution model are like osmotic viral expansions, and the concepts underlying the excluded volume theory are similar to those in lattice theories. Still the new and important element in each case, e.g., group contribu-

tion or excluded volume, does not easily fall into the category of a viral expansion or a lattice theory. For purposes of this article, Therefore, these approaches have been classified under a separate category.

8.1. Group contribution

A very recent development is the molecular thermodynamic model developed by the group at Kaiserslautern [78]. The model expressions give the excess Gibbs free energy G^e of an aqueous mixture of two polymers or a polymer and a salt as a power series in relative surface fraction Θ_i of the solutes i where the coefficients A_{ij}, A_{ijk} and A_{ijk} represent the energy of interaction per contact between pairs and triplets of solute molecules respectively. As explained in the section on chemical thermodynamics, the excess Gibbs free energy is defined as the difference between the free energy of a real mixture and that of an ideal mixture both at the same temperature, pressure and composition. It is usually assumed that the contribution of the solvent to the ideal mixture free energy is given by the Lewis–Randall rule while the contribution from solutes is assumed to be given by Henry's law. This method is essentially a viral expansion using relative surface fraction as a concentration measure. An electrostatic expression $G^{e,LR}$ is added to the above when ions or other charged species are present. This may seem rather an unusual idea to those normally accustomed to dealing with molality and molarity as a measure of concentration. However, one must consider that interactions between molecules do occur through the surface of the molecules so that surface fraction is indeed a logical and valid means of measuring concentration. For a multicomponent mixture, the expression for the excess Gibbs free energy is

$$\frac{G^e}{n_w RT} = \left(\frac{1000}{M_w}\right)^2 \sum_i \sum_j \frac{\Theta_i \Theta_j}{\Theta_w \Theta_w} A_{ij}$$

$$+ \left(\frac{1000}{M_w}\right)^3 \sum_i \sum_j \sum_k \frac{\Theta_i \Theta_j \Theta_k}{\Theta_w \Theta_w \Theta_w} A_{ijk}$$

$$+ \frac{G^{e,LR}}{n_w RT} \tag{47}$$

where the sums are taken over all the components present, n_w is the number of moles of water, M_w is

the molecular mass of water, and the relative surface fraction Θ_i is calculated from

$$\Theta_i = \frac{m_i q_i}{\sum\limits_j m_i q_i} \tag{48}$$

and the sum is again taken over all the components present, m_i is the molality of component i and q_i is the surface parameter of component i. The molecules of each of the components is then broken down into groups (methyls, ethers, ethyls, etc.), and the interaction parameters A_{ij} and B_{ijk} are expressed in terms of summations over the groups making up the molecules of each of the components. The expression for A_j is

$$A_{ij} = \sum_k \sum_l \Theta_k^{(i)} \Theta_l^{(1)} a_{kl} \tag{49}$$

where a_{kl} is an interaction parameter between groups k and l that depends on temperature, and where the weighing factor $\Theta_k^{(i)}$ is the surface fraction of group k in molecule i which is calculated from

$$\Theta_k^{(i)} = \frac{\nu_k^{(i)} Q_k}{\sum\limits_l \nu_l^{(i)} Q_l} \tag{50}$$

and where the summation is taken over the groups in molecule i, $\nu_k^{(i)}$ is the number of groups of type k in molecule i and Q_k is the surface area of group k. The model parameters are the surface area of each group Q_k and the temperature-dependent group interaction a_{kl}, which have to be fitted to experimental data. The principal practical advantage of this method is that the model parameters are formulated in terms of the groups making up the component molecules, and, since most molecules are made up from a relatively small number of groups, it is then possible to include a very large number of components with a small number of parameter values.

8.2. Excluded volume theory

The group at Sheffield [79,80] has developed a very simple model for the binodial curve in polymer–polymer aqueous two-phase systems based on statistical geometrical arguments [81]. This approach represents a significant departure from the models presented above. The theory is based on the follow-

ing arguments: (1) the two phases are saturated with respect to each of the polymers, i.e., all of the volume is occupied by molecules of either one of the polymers including perhaps the water of hydration; (2) the concentration of each polymer in each phase is determined by how many molecules of each polymer fit into the volume of the phase. Fig. 9 gives a sketch representing the physical situation. According to the theory, however, the volume occupied by a polymer molecule is an effective volume rather than a molecular volume. This accounts for the fact that the polymer molecule is likely to have a shell of hydrated water around it.

From a practical point, the most important result from the theory is the following simple one parameter expression for the binodial curve. This result is in principle applicable over the entire span of the phase diagram from the top to the bottom phase. The expression is

$$\ln\left(V_{ijo}^* \frac{w_j}{M_j}\right) + V_{jio}^* \frac{w_i}{M_i} = 0 \tag{51}$$

This expression relates the mass fractions w_i and w_j of solutes i and j to each other in terms of the solute molecular masses M_i and M_j and one fitted parameter V_{jio}^* which represents the effective excluded volume of molecules i and j in the mixture. To use this expression, for example, assume that $w_i = w_{peg}$ and $w_j = w_{dex}$ and simply insert a value for w_{dex} and
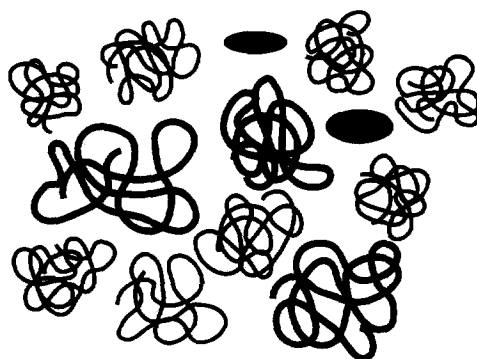

Fig. 9. The concept of excluded volume for a solution of two different types of polymer coils and proteins (solid ellipses). Note that large coils require more volume than an equivalent mass of the small coils. The proteins require even less volume than the same mass of either coils.

calculate w_{peg} from the equation. One should carefully note, however, that the expression is applicable to only one phase at a time, e.g., if w_{peg} is a top phase composition then w_{dex} must also be a top phase composition. This result has been extended [80] to include the polydispersity of the polymers by using averaged molecular masses and an averaged effective excluded volume.

The expression can be used to calculate a binodial curve but not the equilibrium composition of the phases. To calculate the equilibrium compositions, the authors use the empirical osmotic viral expansion given below in conjunction to the above expression for the binodial curve applied to both the top and the bottom phases. This gives three equations to solve for the four unknown compositions m_1^T, m_2^T, m_1^B and m_2^B so that a value must be chosen for one of the four variables. The osmotic viral expansion is

$$m_1^T + m_2^T + \frac{a_{2,0}}{2}(m_2^T)^2 + \frac{a_{0,2}}{2}(m_2^T)^2 + a_{1,1}m_1^T m_2^T$$
$$= m_1^B + m_2^B + \frac{a_{2,0}}{2}(m_2^B)^2 + \frac{a_{0,2}}{2}(m_2^B)^2 + a_{1,1}m_1^B m_2^B$$

(52)

where m_1^T, m_2^T, m_1^B and m_2^B are the molalities of components 1 and 2 in the top and bottom phases, respectively, and $a_{0,1}$, $a_{1,0}$ and $a_{1,1}$ are empirical interaction coefficients related to osmotic viral coefficients. The interaction coefficients are determined from other independent measurements.

With regards to osmotic viral expansions, the authors note that some of the mathematical forms for these expansions commonly in use violate thermodynamic consistency requirements. In particular, mention is made of violating the cross-derivative tests for the chemical potentials and the Gibbs–Duhem equation. However, it must be kept in mind here that although a set of empirical expressions for the chemical potentials in a mixture could indeed violate thermodynamic consistency, this problem does not exist for theoretically derived osmotic viral expansions from statistical mechanics.

9. Electrostatic forces

Many of the species commonly found in aqueous two-phase systems are electrostatically charged, e.g.,

ions from phase-forming salts, ions from buffers used to control the pH, proteins and occasionally phase-forming polyelectrolytes. The effect of electrostatic forces can not be represented by any of the methods previously reported here. Rather, it is generally assumed that a term incorporating the effect of electrostatic forces can be added on to the chemical potential or free energy expression representing the other non-electrostatic forces. For example,

$$A = A^{NE} + A^E \quad \text{or} \quad \mu_i = \mu_i^{NE} + \mu_i^E \qquad (53)$$

where A is the Helmholtz free energy, A^{NE} is the contribution from non-electrostatic forces, A^E is the contribution from electrostatic forces, and where the expression for the chemical potential, μ_i, of a component i has a similar interpretation. Again note that one can transform an expression for A into an expression for μ_i and vice versa by the methods outlined in the section on chemical thermodynamics.

The expressions for A^E or μ_i^E are constructed by numerous means, and rather than attempt to catalog all of them here, I will focus on the underlying theoretical ideas which are fewer in number. Excluding the numerous empirical approaches, there are basically four distinct theoretical methods to treat the effect of electrostatic forces in solution: (1) the theory of Debye and Hückel [82] and its various modifications [83,28,84], (2) the mean spherical approximation [73–77], (3) numerical integral equation solutions [85] and (4) molecular simulations [86]. I will concentrate on No. 1 and No. 2 because they give closed-form analytic expressions which are relatively easier to use in separation applications. Note that, while No. 3 and No. 4 yield more rigorous results, they require a numerical solution for each set of conditions and are, therefore, not widely used in modeling aqueous two-phase systems.

9.1. The Debye–Hückel theory and its modifications

The earliest and simplest theory capable of accounting for the observable behavior of solutions containing charged species is that of Debye and Hückel [82]. They assumed that the electrostatic potential (ψ_i) due to the distribution of ions i around

a central ion j in a solution is given by the Poisson equation of electrostatics given below. This assumption replaces the solvent with a continuous medium having no molecular characteristics in which the ions are immersed. The Poisson equation is

$$\nabla^2 \psi_j = \frac{-4\pi e}{\epsilon} \sum_i z_i c_i \hat{g}_{ij} \tag{54}$$

where e is the electronic charge, ϵ is the dielectric constant of the pure solvent, Z_i is the valence of ion i, ρ_i is the number density of ion i and $\hat{g}_{ij}$ is the Ansatz or assumption for the distribution of ions i around central ion j. Debye and Hückel further assumed that the ansatz for the distribution $\hat{g}_{ij}$ is given by a Boltzmann-type exponential factor as shown below.

$$\hat{g}_{ij}(r) = \exp\left(\frac{-z_i e \psi_j(r)}{kT}\right) \approx 1 + \frac{-z_i e \psi_j(r)}{kT} \tag{55}$$

where r is the distance radially outwards from the central ion, k is the Boltzmann constant, and the other symbols have their previously defined interpretation. The exponential in the ansatz in then linearized as shown above, and the result inserted into the Poisson equation. This transforms the equation from a non-linear to a linear differential equation achieving an enormous simplification in the mathematics. This simplification, however, makes the theory strictly applicable to very dilute solutions only. Finally, a boundary condition is imposed which says that the electrostatic potential is zero very far away from the central ion. The equation is then solved for the electrostatic potential, ψ_i, giving,

$$\psi_j(r) = \frac{z_j e}{\epsilon r} \exp[-Kr] \tag{56}$$

where K is the inverse Debye length with units of reciprocal length and defined by

$$K^2 = \frac{4\pi e^2}{\epsilon kT} \sum_i c_i z_i^2 \tag{57}$$

where all the symbols have the usual interpretation. The quantity $1/K$ approximately represents the maximum distance at which two charged species are correlated, i.e., the maximum distance at which ions can electrostatically "see" each other in a dilute solution.

The details of the theoretical development from here on are simple but rather tedious and probably beyond the scope of this review. Thus, I refer the reader to any of number of excellent monographs on this subject [39,85]. Perhaps it should suffice to say that the electrostatic potential, ψ_i, is integrated over distance, used in charging process, and the result summed over all of the ions in the salt s to give an expression for the electrostatic contribution to the chemical potential μ_s^E of the salt. The resulting expression for μ_s^E is widely called the Debye–Hückel limiting law for electrolyte solutions.

$$\frac{\mu_s^E}{kT} = -S_\gamma I^{1/2} [\nu_+ Z_+^2 + \nu_- Z_-^2] \tag{58}$$

where ν_i is the number of positive ($+$) or negative ($-$) ions in the salt, $I = (c_s/2) \sum_i \nu_i Z_i^2$ is the ionic strength, the other symbols have the usual interpretation, and S_γ is the Debye–Hückel slope defined by

$$S_\gamma = \sqrt{\frac{2\pi e^6}{\epsilon^3 k^3 T^3}} \tag{59}$$

The Debye–Hückel limiting law captures the most important physics of electrostatic interactions in very dilute ionic solutions. It is a well-established and rigorous result for dilute solutions of ions in a solvent. Although, again one should note that due to the linearization outlined above, it is strictly speaking applicable only to ionic concentrations approaching those in ordinary tap water! The theory, however, is commonly used for solutions at reasonably high ionic concentrations of up to 0.1 M or so with good results. The deviation between theory and experiment steadily increases with ion concentration as expected. The reason for this seemly good fortune is that the theory is accurate at dilute conditions where electrostatics dominates behavior. At higher ion concentrations where the assumptions in the theory are starting to fail, the importance of electrostatics in determining behavior is steadily decreasing because the presence of so many ions tends to shield the electrostatic charges from each other.

There have been a number of reasonable efforts to develop improved theories based on the concepts of Debye and Hückel. These endeavors which fall under the category of extended Debye–Hückel theories usually follow the same development method outlined for the limiting law, but they include the

effect of ionic size or other interactions on the electrostatic potential (ψ_i). Without exception, all of these models reduce to the Debye–Hückel limiting law as the ionic strength approaches zero. None of these approaches have the rigor of the limiting law but they are necessary to extend the treatment of electrolytes to higher ionic concentrations. Typically, these extended electrostatic potentials have the following functional form,

$$\psi_j(r) = \frac{z_j e}{\epsilon r (1 + K a_{ij} + \cdots)} \exp[K(a_{ij} - r)] \qquad (60)$$

where a_{ij} is the distance of closest approach between ions i and j.

Pitzer [83] used a statistical mechanical procedure together with the electrostatic potential given above to develop a semi-theoretical extended Debye–Hückel model for electrolyte solutions. The parameter values for Pitzer's model are available for many different salts in water. Pailthorpe et al. [28] used the basic development strategy of Debye and Hückel starting with the Poisson equation, but they included a non-electrostatic interaction between ions in their model for the electrostatic potential ψ_i. They then used a statistical mechanical procedure similar to Pitzer's to arrive at an expression for μ_s^E. Perry et al. [84] and Cabezas [87] used a different statistical mechanical method and the electrostatic potential given above to arrive at a model for ionic solutions. Their expression for μ_s^E from this model has approximately the following form:

$$\frac{\mu_s^E}{kT} = \frac{-S_\gamma I^{1/2}}{\sum_i \nu_i z_i^2} \left[\frac{\nu_+^2 z_+^4}{1 + a_{++} B_\gamma I^{1/2}} \right.$$

$$\left. + \frac{\nu_-^2 z_-^4}{1 + a_{--} B_\gamma I^{1/2}} + \frac{2\nu_+ z_+^2 \nu_- z_-^2}{1 + a_\pm B_\gamma I^{1/2}} \right] \qquad (61)$$

where B_γ is a solvent parameter defined by

$$B_\gamma = \sqrt{\frac{8\pi e^2}{\epsilon kT}} \qquad (62)$$

and where ν_i is the number of ions of type i in salt s, a_{ij} is the distance of closest approach of ions i and j, and where the other symbols have the usual interpretation.

9.2. Mean spherical approximation

The mean spherical approximation or MSA [73,74] provides a general statistical mechanical method for the development of models for the behavior of solutions including ionic solutions [75–77]. It does not make use of the Poisson equation and it is not as strictly limited to dilute solutions. The method consists of solving the Ornstein–Zernike integral equation [69] with suitable boundary conditions, also called closure conditions, and using the energy equation to obtain an expression for the internal energy of the solution. From the internal energy one can obtain the following expression [88] for μ_s^E.

$$\frac{\mu_s^E}{kT} = \left[\frac{-e^2}{kT\epsilon} \left(\Gamma \sum_i \frac{c_i Z_i^2}{1 + \gamma a_i} \right) + \frac{\pi}{2\Delta} \Omega P_n^2 \right.$$

$$\left. - \frac{\alpha^2}{8} \left(\frac{P_n}{\Delta} \right)^2 \right] \Big/ \xi_0 \qquad (63)$$

and where

$$P_n = \frac{1}{\Omega} \sum_k \frac{c_k a_k Z_k}{1 + \Gamma a_k} \qquad (64)$$

$$\Omega = 1 + \frac{\pi}{2\Delta} \sum_k \frac{c_k a_k^3}{1 + \Gamma a_k} \qquad (65)$$

$$\Delta = 1 - \frac{\pi}{6} \zeta_3 \quad \zeta_n = \sum_k c_k a_k^n \quad \alpha^2 = \frac{4\pi e^2}{kT\epsilon} \qquad (66)$$

where Γ is the inverse MSA shielding length playing a role analogous to K in the Debye–Hückel theory, n is the number of ionic species present, e.g., two for NaCl, and the other symbols have the usual interpretation. There is no explicit equation for Γ, rather it is obtained by numerically solving the non-linear equation,

$$\Gamma = \frac{\alpha}{2} \sqrt{\sum_i c_i \left[\frac{\left(Z_i - \frac{\pi}{2\Delta} a_i^2 P_n \right)}{1 + \Gamma a_i} \right]^2} \qquad (67)$$

The MSA represents the next step in sophistication from the Debye–Hückel theory and its extensions. It does capture more of the physics of ionic solutions. Although, this comes at the price of a significantly more complex theory lacking the simple intuitive sense of the former approach. One specific issue is the need for solving the implicit equation for Γ at

each concentration of interest. Another particularly troubling aspect of the MSA is that it does not reduce to the limiting law as the ionic concentration approaches zero. Still, the MSA is a reasonable and relatively simple means of representing the properties of ionic solutions.

9.3. Application to calculations

There is a last subtle point that needs to be considered when using either the Debye–Hückel theory, one of its modifications, or a MSA model for calculations at ordinary laboratory conditions, i.e., constant pressure and temperature [37]. The problem is that all of these models do not treat the solvent as a molecular fluid, rather they treat it as a background continuum in which the ions exist. These models are then in the same category as the McMillan–Mayer viral expansion discussed earlier, and it is necessary to convert these expressions to constant pressure by a procedure similar to that used for the McMillan–Mayer viral expansion. In fact, what the models give is an expression for $\mu_s^{E,model}$ at a pressure of $P + \pi$ where π is the osmotic pressure of the solution, while the required property for calculations is μ_s^E at the pressure of the solution or the laboratory, P. The conversion expression is

$$\mu_s^E(T, P, c_s) = \mu_s^{E,model}(T, P + \pi, c_s)$$
$$+ \int_{P+\pi}^{P} \left[\bar{V}_s^E - \bar{V}_s^{E\infty} \right] dP \qquad (68)$$

where $\bar{V}_s^E$ is the electrostatic part of the salt partial molar volume, $\bar{V}_s^{E\infty}$ is the electrostatic part of the salt partial molar volume at zero salt concentration and Π is the osmotic pressure. The conversion given by the integral above can often be neglected particularly if the ionic concentration is low so that π is small and then $\bar{V}_s^E \approx \bar{V}_s^{E,model}$. However, this can not be automatically done under all circumstances.

10. Summary

10.1. Why do we have so many models?

The state of knowledge of the theory of phase formation and phase equilibria in aqueous two-phase systems is populated by a veritable menagerie of ideas, models and methods. This diversity seems to have sown a fair degree of confusion with many workers. Part of the difficulty here lies in the fact that most of the models generally do represent the experimental phase diagrams reasonably well. This situation arises from the fact that the model parameters are frequently but not always fitted either to the data being modeled or to a closely related experimental measurement. In the hope of clarifying these issues, I have already classified some of the main ideas and offered some recommendations.

Still, why do we have so many models? The existence of so many apparently reasonable models is simply a reflection of our relatively poor understanding of liquids and liquid mixtures. The fact is that a good comprehensive theory of liquids and liquid mixtures is not available at the present time. In the absence of a good fundamental theory, models are developed by various combinations of theory and empiricism yielding a wide range of possible outcomes.

We can illustrate the reason for the existence of so many models by revisiting osmotic viral expansions and noting the many possibilities. These models are based on the theory of McMillan and Mayer [24] or on the theory of Hill [25,26]. Both are widely used for aqueous two-phase systems [30,32,33,35]. The parameters on these models are osmotic viral coefficients. These can either be treated as experimentally measured quantities [32] or they can be modeled. Models for the osmotic viral coefficients can be based on scaling arguments [37] or other approaches [35]. The models for the osmotic viral coefficients contain molecular parameters whose values must be evaluated from experimental data. The data used in the parameter evaluation can be from laser light scattering [32,33], vapor pressure measurements [33], osmotic pressure measurements [34,36], isopiestic experiments [38], or other sources. Each decision that is made in the above sequence of choices will in general yield a subtly different model. If we also consider other fundamentally different approaches such as lattice theories, the number of possible outcomes grows very quickly.

To contrast this situation in liquid state theory, we can consider the theory of the motion of large rigid bodies. Here we do not see a myriad of models because Newton's equations quite adequately repre-

sent the observed behavior. The reason is that the motion of large rigid bodies is a relatively simple physical phenomena for which a very good model has been developed. The behavior of molecules in the liquid state is, unfortunately, not as simple. Although, it should be noted that the aforementioned theoretical deficiency in liquid and liquid mixture theory is not the case for all areas of physical chemistry. We have for example a fairly good theory of low pressure gases in the form of the viral equation of state [89]. We also have good theories for crystalline solids [90] based on perfect lattice statistics. For low pressure gases and crystalline solids, we have succeeded in developing good theories because we have the ideal gas model and the perfect lattice as simple and very well understood reference models to start from. These reference models allow us to treat the behavior of real gases and real crystalline solids as perturbations on the behavior of the reference models.

10.2. Some recommendations for the present

Given the present state of liquid and liquid mixture theory and the existence of various approaches to modeling the phase behavior of aqueous two-phase systems, can some guidance be offered to the user of these models? Rather than offer a preference for any particular approach, perhaps my own, I would first prefer to answer the question by discussing how models are constructed and illustrating how one chooses or develops a model. Later, I will give some more specific suggestions.

As already discussed, in the case of liquids and liquid mixtures we do not have a good reference model from which to start the development of a theory. Consequently, all current liquid and liquid mixture models are based on the approximate application of either gas theory or crystalline solid theory along with varying degrees of empiricism. The choice of either starting point depends on individual views and the ultimate purpose of the model. For example, if we base a model on gas theory, we are lead to an osmotic viral expansion. This implies that the component chemical potentials can be calculated from the energies of interaction between small numbers of solute molecules and solute–solvent interactions as contained in solute partial molar

volumes. The resulting models are simple and do provide some insight into the interaction between solute molecules. However, they do not elucidate the structure of the solution, i.e., how the solute and solvent molecules are arranged, and they do not easily gives familiar quantities such as the solution entropy and enthalpy. Models based on crystalline lattice theories imply that the component chemical potentials can be calculated from some combinatorial entropy expression with energetic corrections plus an enthalpic contribution. They do provide the solution entropy and enthalpy and, perhaps, some insight into the structure of the solution. But, the model parameters are macroscopic quantities that do not have a clear molecular interpretation. It should also be mentioned that these are not the only problems with the liquid-state theory, but they are the most relevant to the issues discussed here.

With these arguments in mind, one is then led to conclude that liquid mixtures seem to have some aspects which are like those of a gas and some which are like those of a crystalline solid. Our choice of a theoretical basis in our modeling work will give results that will invariably represent some aspects of the mixture better than others. Much as we may want, these strengths and weaknesses can not be driven out of our present models like evil spirits exorcized by the incantation of clever parametrization schemes. Therefore, the modeler choosing a theoretical basis has to decide at the beginning exactly what will be expected from the resulting model and be realistic about it. For the user of models, the question is one of choosing a model having the appropriate theoretical basis which allow it to adequately represent the behavior that the user is looking for. For example, a Flory–Huggins theory would be unlikely to give good mixture volumes because the volume change on mixing is assumed to be zero in the theory, but enthalpies of mixing would likely be well represented. Specific recommendations are given for each individual model and the strengths and weaknesses of each is discussed in the appropriate sections above. However, the differences among the various models are sufficiently subtle that it would be inappropriate to try to condense them here in a brief table format, however desirable that may seem.

In addition to the aforementioned issues regarding

the theoretical basis of models, the user of a particular model is confronted with several very practical questions: (1) are the values for the model parameters for the specific mixture of interest available? (2) If the parameter values are not available, can they be measured easily? (3) Is there experimental data available from which values for the model parameters could be obtained? (4) How difficult is it to fit values for the model parameters? (5) Is the model available in the form of a computer program or must the program be generated? Quite frankly, the answer to each of these questions will vary widely from one model to another, and it will often ultimately determine the choice of model.

10.3. Some recommendations for future work

The various models presently available for the phase behavior of polymer–polymer and salt–polymer aqueous two-phase systems do adequately represent experimentally observed phase behavior in general. Although, it is quite true that all models are not exactly equal. For example, some reproduce phase diagrams more accurately than others, some have a physical basis which is firmer than that of others, and there are still other differences such as the fact that some include the polymer molecular mass dependence while others include the temperature dependence, etc. However, from a practical point of view any of these models could with varying degrees of difficulty be used to calculate reasonably accurate a priori phase diagrams for most aqueous two-phase systems. Therefore, it seems to me that the principal practical value of the modeling work up to the present has been to reduce the amount of required experimental data to a minimum. Regardless of my previous criticism, minimizing the need for experimental data is by itself quite an important contribution.

However, there are a number of fundamental deficiencies in all current models. The first one is that each model is a theoretically or empirically based correlation valid over a limited range of conditions such as temperature, polymer molecular mass, composition, etc. If a model is used outside the range of conditions under which its parameters were evaluated, the results may or may not be accurate, i.e., our present models are not nearly as general as

we would like. This difficulty can be addressed by establishing a critically evaluated data set for well characterized two-phase systems over a wider range of conditions and using this data in the evaluation of model parameters in the future. The characterization should include temperature, pressure, phase composition of all components including residual salts, molecular mass and polydispersity of the polymers used and fractionation of the polymers between the phases. The second deficiency is that the information that the models provide regarding molecular interactions and solution structure is limited, although well appreciated. Solution structure information, in particular, is going to be essential for the field to move forward. For example, solution structure would be extremely important if we were to further pursue the analogy between phase separation in cytoplasm and phase formation in aqueous two-phase systems [91]. There are number of steps that can be taken to address this deficiency: (1) include more realistic models for the molecular interactions in our models, (2) expand neutron [6] and light scattering studies [32,33] on some selected aqueous two-phase systems with the objective of providing some real structure information on mixtures of water and phase forming polymers and salts, (3) take a fresh look at either Monte Carlo or molecular dynamic computer simulations [86] for providing information on the structure of the phases or perform studies based on integral equations [92] as a simpler alternative. The third deficiency is that there is not a single model in existence which incorporates all of the knowledge that we now have about phase behavior in aqueous two-phase systems. For example, one model will include polymer molecular mass dependence but neglect polydispersity, and yet another one will include temperature dependence but neglect other issues and so on. The fact is that the field is now "ripe" for a collaborative effort to assemble a state-of-the-art model incorporating all of the best ideas that have been developed over the last decade. It would be impossible to prescribe all of the details of this model, but the basic elements that need to be included can be easily listed here, and these are: (1) the effect of polydispersity and the fractionation of the polymers between the phases, (2) the polymer molecular mass dependence of the phase diagram, (3) the temperature dependence of the phase diagram

and (4) combining an osmotic viral expansion for the dilute regime and some form of a lattice theory for the more concentrated regime. These are subtle but very important issues if we are to make the resulting models widely useful. It is not very difficult to include polymer polydispersity, fractionation, and polymer molecular mass dependence using the methods previously outlined in the present article. For example, the phase diagram dependence on polymer molecular mass can be well represented by use of scaling arguments. The other items, however, require further thought and study but they are still well within the realm of the possible on a short term basis. For example, a lattice theory could be modified to incorporate a reasonable representation of temperature dependence and to yield a theoretically correct osmotic viral expansion at low solute concentration.

Lastly, it should be mentioned that the development of theoretical models for the phase behavior of aqueous two-phase systems formed with polyelectrolytes as phase-forming polymers seems to have attracted little attention. However, if we are to fully explore the analogy between phase separation in cytoplasm and phase formation in aqueous two-phase systems [91], then phase-forming polyelectrolytes will become important. The reason is that the phase-forming macromolecules in cytoplasm are likely to be polyelectrolytes. These may be linear such as DNA and RNA or compact bodies such as most proteins. The starting point for the construction of such models would be to incorporate known results for linear polyelectrolyte solutions [93–95] into the theory of aqueous two-phase systems. For compact polyelectrolytes it may well be possible to use the theories that have been developed for protein partitioning as a starting point [66,96–98]. The actual model could be built by adding appropriately modified polyelectrolyte terms to component chemical potential expressions in a manner similar to that presented in the section on electrostatic forces.

In summary, aqueous two-phase systems exhibit rather interesting and often very complex behavior. This has led to a wide range of very exciting applications in both science and technology which continue to expand. We now have a basic understanding of some but not all of this behavior. There is presently an opportunity to make significant progress in our ability to model and predict the phase behavior of aqueous two-phase systems, and there are exciting new applications on the horizon.

11. List of symbols

A	Helmholtz free energy (J mol^{-1})
A^{NE}	Contribution to the Helmholtz free energy from non-electrostatic interactions (J mol^{-1})
A^{E}	Contribution to the Helmholtz free energy from electrostatic interactions (J mol^{-1})
A'_{Ex}	Modified excess Helmholtz free energy defined by $A'_{Ex} = A - N_W \mu^o_W$
$A'_{Ex,hs}$	Contribution to the modified excess Helmholtz free energy from hard sphere interactions (J mol^{-1})
$A'_{Ex,na}$	Contribution to the modified excess Helmholtz free energy from non-additivity of hard sphere molecular diameters (J mol^{-1})
$A'_{Ex,ic}$	Ion charging contribution to the modified excess Helmholtz free energy (J mol^{-1})
$A'_{Ex,cc}$	Contribution to the modified excess Helmholtz free energy from charge–charge interactions (J mol^{-1})
$A'_{Ex,ve}$	Contribution to the modified excess Helmholtz free energy from short range interactions (J mol^{-1})
ΔA_{mix}	Helmholtz free energy of mixing (J mol^{-1})
A_{ij}	Interaction constant for solutes i and j
A_{ijk}	Interaction constant for solutes i, j, and k
a_i	Mole fraction scale thermodynamic activity of component i (mol mol^{-1}) or size parameter of ion i (m or Å)
a_{ij}	Interaction parameter between group i and group j or distance of closest approach of ions i and j (m)
$a_{i,j}$	Empirical interaction coefficients between component i and j (kg^2 mol^{-2})
B_{ij}	McMillan–Mayer second osmotic viral coefficient for components i and j (l mol^{-1})

$\tilde{B}_{ij}$	Hill theory second osmotic co-efficient for components i and j (mol mol^{-1} or kg mol^{-1})		in Lansing–Kraemer function, $F(M_i)$ (kg mol^{-1})
B_{sp}	McMillan–Mayer second osmotic viral coefficient for salt s and polymer p (l mol^{-1})	M_{n_i}	Number averaged molecular mass of polymer i (kg mol^{-1})
B_{γ}	Debye–Hückel solvent parameter (l$^{1/2}$ m mol$^{-1/2}$)	M_{w_i}	Mass averaged molecular mass of polymer i (kg mol^{-1})
b_i	Scaling proportionality constant for polymer end to end distance (m)	M_{k_i}	Molecular mass of the k fraction of polymer i (kg mol^{-1})
$\tilde{b}_{ip}$	Scaling proportionality constant for the interaction between ion i and polymer p (l mol^{-1})	m_i	Molality of component i (mol mol^{-1}) or (mol kg^{-1}) where the two units are related by $1000/M_s$
c_i	Concentration of component i (mol l^{-1})	N	Total number of moles in a phase (mol)
c^*	Cross-over concentration at which polymer chains start to overlap in solution (mol l^{-1})	N_i	Moles of component i in a phase, mol or degree of polymerization of polymer i
c_i^j	Concentration of component i in phase j (mol l^{-1})	N_w, n_w	Number of moles of water (mol)
d_w	Mass density of pure water (kg l^{-1})	P	Pressure of the system (Pa)
e	Electronic charge (J$^{1/2}$ m$^{1/2}$)	Q_k	Surface area parameter for group k (m^2)
$F(M_i)$	Lansing–Kraemer function for the molecular mass distribution of poly-disperse component i	q_i	Surface area parameter of component or polymer blob i (m^2)
$G(x_i)$	Arbitrary function of x_i	q_i'	Mass-based surface area parameter of component i (m^2)
G	Molar Gibbs free energy of a mixture (J mol^{-1})	R	Universal gas constant (J mol^{-1} K^{-1})
G^e	Molar excess Gibbs free energy of a mixture (J mol^{-1})	R_i	End-to-end distance of a polymer chain in solution (m or Å)
$G^{E,LR}$	Electrostatic excess Gibbs free energy of a mixture (J mol^{-1})	r	Distance radially outwards from the central ion in the Debye–Hückel theory (m or Å)
G^{im}	Molar Gibbs free energy of an ideal mixture (J mol^{-1})	R_g	Radius of gyration of a polymer chain in solution (m or Å)
ΔG_{mix}	Molar Gibbs free energy of mixing (J mol^{-1})	r_i	Symbol for the position vector of the center of mass of a molecule i
$\hat{g}_{ij}$	Ansatz for the distribution of ions i around a central ion j	r_i'	Mass-based volume parameter for component i (m^3)
$H_n(x)$	Hermite polynomial	S	Entropy of a mixture (J mol^{-1} K^{-1})
I	Ionic strength (mol l^{-1})	ΔS_{mix}	Entropy of mixing (J mol^{-1} K^{-1})
k	Boltzmann's constant (J K^{-1})	S_{γ}	Debye–Hückel slope (l$^{1/2}$ mol$^{-1/2}$)
L	Length of a polymer chain along the backbone (m)	T	Temperature of the system (K)
M_p	Molecular mass of a polymer p (kg mol^{-1})	ΔU_{mix}	Internal energy of mixing of a mixture (J mol^{-1})
M_i	Molecular mass of component i or of polymer fraction i (kg mol^{-1})	$U_{ij} - U_{jj}$	Molecular interaction parameter for components i and j (J mol^{-1})
M_{o_i}	Adjustable parameter for component i	u_{ij}	Energy of interaction between a pair of molecules i and j (J)
		$\bar{V}_i$	Partial molar volume of component i (l mol^{-1})

$\bar{V}_i^{\mathrm{E}}$ Electrostatic contribution to the partial molar volume of component i ($1\,\mathrm{mol}^{-1}$)

$\bar{V}_i^{\mathrm{E}\infty}$ Electrostatic contribution to the partial molar volume of component i at infinite dilution or zero concentration of component i ($1\,\mathrm{mol}^{-1}$)

$\bar{V}_i^{\mathrm{o}}$ Partial molar volume of component i at zero concentration of component i ($1\,\mathrm{mol}^{-1}$)

$\bar{V}_{\mathrm{m}i}^{\mathrm{o}}$ Partial molar volume of a monomer of polymer i at zero polymer concentration ($1\,\mathrm{mol}^{-1}$)

V_{ijo}^{*} Effective excluded volume parameter (m^3)

W_i Total mass of solute i in all phases (kg)

W_i^j Mass of component i in phase j (kg)

W^j Total mass of all components in phase j (kg)

W_{k_i} Weight factor in expansion for fraction k in polymer i

w_i^j Mass fraction of component i in phase j ($\mathrm{kg\,kg}^{-1}$)

w_p Mass fraction of component p in solution ($\mathrm{kg\,kg}^{-1}$)

w_{k_i} Mass fraction of the k fraction of polymer i ($\mathrm{kg\,kg}^{-1}$)

x_i Mol fraction of component i ($\mathrm{mol\,mol}^{-1}$) or variable for component i

x_i^{o} Reference mol fraction of component i ($\mathrm{mol\,mol}^{-1}$)

Z_i Valence of ion i

z Lattice coordination number (i.e., number of nearest neighbors to a molecule

z_{k_i} Zero of Hermite polynomial for fraction k of polymer i

α_{ij} Non-randomness NRTL parameter

β_i Adjustable parameter for component i in Lansing–Kraemer function, $F(M_i)$

Γ MSA inverse length (m^{-1})

ϵ Dielectric constant of pure solvent

ζ Monomer size (m)

Θ_i Relative surface fraction of solute i

$\Theta_k^{(i)}$ Surface fraction of group k in molecule i

K Debye–Hückel inverse length (m^{-1})

K_{s} Isothermal compressibility of pure solvent (Pa^{-1})

ξ Correlation length of polymer in a concentrated solution or polymer blob size (m)

$\theta(M_i)$ An arbitrary function of polymer molecular mass, M_i

θ_i' Area fraction of polymer blob i

θ_i Mass based surface area fraction of component i

Φ_i Volume fraction of component or polymer blob i in a mixture, $x_i \bar{V}_i / \sum_j x_j \bar{V}_j$

ϕ_i' Mass based volume fraction of component i

ρ_s^o Molar density of pure solvent ($\mathrm{mol\,l}^{-1}$)

π The number pi or the osmotic pressure of a solution (Pa)

τ Scaling exponent for salt–polymer interactions

μ_i Chemical potential of component i ($\mathrm{J\,mol}^{-1}$)

μ_i^j Chemical potential of solute i in phase j ($\mathrm{J\,mol}^{-1}$)

μ_i^{o} Reference chemical potential of solute i ($\mathrm{J\,mol}^{-1}$)

μ_i^{im} Chemical potential of component i in an ideal mixture ($\mathrm{J\,mol}^{-1}$)

μ_i^{NE} Contribution to the chemical potential of component i from non-electrostatic interactions ($\mathrm{J\,mol}^{-1}$)

μ_i^{E} Contribution to the chemical potential of component i from electrostatic interactions ($\mathrm{J\,mol}^{-1}$)

$\mu_{\mathrm{w}}, \mu_{\mathrm{w}}^{\mathrm{o}}$ Chemical potential of water ($\mathrm{J\,mol}^{-1}$)

ν Universal scaling exponent for polymer end to end distance

ν_s Number of ions in salt s

ν_i Number of ions of type i in a salt s

$\nu_k^{(i)}$ Number of groups of type k in molecule i

X_{ij} Flory–Huggins interaction parameter between components i and j

Ω Number of possible configurations available to a system

Ψ_i Electrostatic potential around ion (i)

ω_i Symbol for the orientation angles of molecule i

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Disclaimer

Certain commercial materials are identified in this article to adequately specify the experimental procedures and conditions. Such identification does not imply recommendation by NIST or US EPA, nor does it imlpy that the materials are necessarily the best available for the purpose.

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