The nature of phase separation in aqueous two-polymer systems

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(Received 10 February 1986; revised 23 April 1986)

Aqueous systems with two soluble polymers often phase-separate into two isotropic solutions. We investigate the molecular mechanism leading to this phase separation. It is concluded that the important factor is the direct molecular interaction between the monomeric units of two polymers, which should be effectively repulsive for phase separation to occur. Previously it has also been suggested that the size and shape of the entire polymer molecules are important factors, but our analysis does not support this view.

(Keywords: phase separation; aqueous two-polymer systems; polymer interaction; aqueous solution; polymer mixing)

INTRODUCTION

It is often found that solutions of two different polymers phase-separate into two isotropic solutions, each enriched in one of the polymer components. This phenomenon, often referred to as polymer incompatibility and most often studied for polymers in an organic solvent, is also observed in aqueous solution. To mix two water-soluble incompatible polymers is thus a convenient way of generating two aqueous phases in equilibrium. This has been used as a very useful separation technique for biological macromolecules[1] and particles, where it is necessary to have an aqueous solvent. In spite of the extensive use of this partitioning technique in biochemical separations, there has been relatively little work on the fundamental physicochemical properties of the system. Even the basic mechanism for the phase separation in the aqueous system is still not fully understood. The present paper deals with this question. There exist in the literature essentially two types of explanation for the occurrence of phase separation[2,3], and one of these has been developed with particular reference to aqueous systems. Thus, the ideas from Ogston's theory are widely used in the biochemical area to explain phase separation and partitioning in aqueous polymer two-phase systems. In the following two sections we discuss these two approaches separately with reference to their virtues and weaknesses. In the final section we combine the arguments to arrive at a qualitative understanding of polymer incompatibility.

PHASE SEPARATION FROM VIRIAL EXPANSION

The only attempt specifically to address the phase separation problem for aqueous polymer systems is that of Ogston and coworkers[3,4]. It is well known that water has much more complex properties as solvent than simple organic solvents have. This apparently led Ogston to approach the phase separation problem in the aqueous system using a formal thermodynamic approach.

A virial expansion including all second-order terms gives for the solvent (water) chemical potential:

$$\mu_1-\mu_1^{\ominus}=-\frac{RTM_1}{1000}\left(m_2+m_3+\frac{c}{2}m_2^2+\frac{d}{2}m_3^2+am_2m_3\right)\quad(1)$$

using the notation of ref. 3. The two polymer components have indices 2 and 3 and c and d are the second virial coefficients of the pure binary systems, while a is the coefficient due to interactions between the two polymers and m_i denotes the molal concentration. By the Gibbs–Duhem relation and the requirement of formal symmetry between the chemical potentials for the two polymers it follows:

$$\mu_2-\mu_2^{\ominus}=RT(\ln m_2+cm_2+am_3)\quad(2)$$

$$\mu_3-\mu_3^{\ominus}=RT(\ln m_3+dm_3+am_2)\quad(3)$$

In equations (1)–(3) higher-order terms in m_i have been neglected in the expansion. This is a valid procedure for dilute polymer solutions[5] but it is difficult to justify *a priori* at concentrations in the semidilute regime, where the phase separation under discussion actually occurs. However, equations (1)–(3) can always be viewed as an ansatz for the chemical potentials of the components in the system. The coefficients a, c and d are then purely empirical and they have no direct molecular interpretation. With such a view the accuracy and the range of validity of the equations can be determined only by comparison with experimental data. It is also clear that the fitted values of the parameters a, c and d obtained

0032-3861/86/111768$03.00

in the semidilute regime will in general not equal the true virial coefficients. Edmond and Ogston[3] showed that they could get a reasonable fit to the observed phase equilibria in poly(ethylene glycol) (PEG) and dextran mixtures using equations (1)–(3). Phase separation occurs if the product cd is larger than a^2. This demonstrates that from an empirical point of view the equations (1)–(3) give a satisfactory description of the phase separation in two-polymer systems. Typical concentrations when the phase separation occur are in the range 5–15% (w/w) polymer[1]. This does not correspond to a dilute polymer solution and one is well outside the regime where the virial expansion to second order can be expected to be valid. It is thus clear that the coefficients a, c and d obtained by fitting the phase behaviour do not correspond to the true second virial coefficients.

To obtain a qualitative molecular understanding of the mechanism behind the phase separation, Edmond and Ogston interpret the coefficients as second virial coefficients in spite of this problem. To simplify the arguments further, they consider only the excluded volume contribution to the virial coefficient. This has the pleasant consequence that the phase separation problem can be discussed in terms of the size and shape of the polymer molecules, but in fact it creates another serious problem. There are a few cases known where pure excluded volume effects lead to phase separation into two isotropic liquids. For example, mixtures of hard spheres of two different sizes do not phase-separate according to the Percus–Yevick equation[6]. Mixtures of hard rods with hard spheres are not expected to phase-separate. The rod–rod excluded volume[7] goes as length^2 × radius while the rod–sphere excluded volume is simply related to the volumes of the objects. It is thus unfavourable to concentrate the rods in one phase.

From this section we conclude that equations (1)–(3) can qualitatively account for the phase behaviour but that the analysis provides little insight into the molecular mechanism leading to the phase separation. Furthermore, this type of analysis cannot explain the experimental data (figure 2.12 in ref. 1) on the phase separation of dextran and poly(ethylene glycol) with hydroxypropyldextrans of different degree of substitution. It is demonstrated here that the number of hydroxypropyl groups on the dextran determines its compatibility. A hydroxypropyldextran with a low content of hydroxypropyl groups is not compatible with poly(ethylene glycol), but when the degree of substitution of hydroxypropyl groups on the dextran is increased it becomes compatible with poly(ethylene glycol).

PHASE SEPARATION USING THE FLORY–HUGGINS THEORY

Already in 1948 Scott[2] presented a quantitative analysis of the polymer incompatibility problem using the Flory–Huggins theory[8] as a basis. This analysis is now generally accepted as giving a reasonable description of polymer phase separation in organic solvents.

The chemical potentials derived by Scott are:

$$\mu_1-\mu_1^{\ominus}=RT[\ln\phi_1+(1-N_2^{-1})\phi_2+(1-N_3^{-1})\phi_3+\chi_{12}\phi_2^2 + \chi_{13}\phi_3^2+(\chi_{12}+\chi_{13}-\chi_{23})\phi_2\phi_3] \tag{4}$$

$$\mu_2-\mu_2^{\ominus}=RT\{\ln\phi_2+(1-N_2)\phi_1+(1-N_2/N_3)\phi_3 + N_2[\chi_{12}\phi_1^2+\chi_{23}\phi_3^2+(\chi_{12}+\chi_{23}+\chi_{13})\phi_1\phi_3]\} \tag{5}$$

$$\mu_3-\mu_3^{\ominus}=RT\{\ln\phi_3+(1-N_3)\phi_1+(1-N_3/N_2)\phi_2 + N_3[\chi_{13}\phi_1^2+\chi_{23}\phi_2^2+(\chi_{13}+\chi_{23}-\chi_{12})\phi_1\phi_2]\} \tag{6}$$

Here ϕ_i is the volume fraction of component i and N_i the degree of polymerization of the polymer. It is supposed here that the two polymers are purely monodisperse. As in (1)–(3), the expressions (4)–(6) contain three parameters, the Flory–Huggins interaction coefficients χ_{12}, χ_{13} and χ_{23}. There are thus clear formal similarities between the two sets of equations, but their theoretical justifications are nevertheless clearly different.

The Flory–Huggins theory is derived using assumptions relevant particularly for the semidilute regime, where the phase separation occurs. It is derived using a microscopic model, albeit simplistic, and essentially a mean field approximation. The interaction parameters χ_{ij} can thus be related to molecular interactions. The basic virtue of the Flory–Huggins theory relative to the virial expansion of equations (1)–(3) is that the entropy of mixing polymers with solvent is described in a much more satisfactory way. The flexibility of the polymers has been taken into account explicitly, while this physical effect is very difficult to catch in a formal virial expansion. To summarize the physical content in equations (4)–(6) one can say that interaction terms have been explicitly related to segment–segment, segment–solvent and solvent–solvent interactions rather than polymer–polymer ones and that the flexibility of the polymer has been explicitly included in the entropy-of-mixing term.

We have tested to what extent equations (4)–(6) can describe the properties of polymer phase separation in aqueous systems. *Figure 1* shows that it is indeed possible to obtain a fit to the experimental data with reasonable

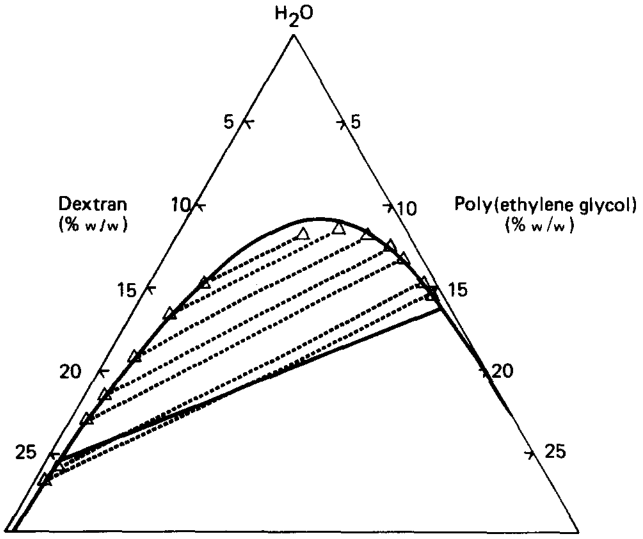

Figure 1 Calculated binodal curve (———) with tieline (———) and experimental points (△) with tielines (-----) for the system dextran D17/PEG 6000/H$_2$O at 20°C (see ref. 1 for experimental values). The degrees of polymerization used in the calculations were 128 for dextran and 140 for PEG. The interaction parameters were 0.3758 for dextran–water interactions, 0.5353 for PEG–water interactions and 0.4795 for dextran–PEG interactions

accuracy. The dextran used by Albertsson in his study comes from *Leuconostoc mesentroides* strain B-512, which produces a fairly linear dextran[1]. One can note that equations (4)–(6) give a direct prediction on how the chemical potentials, and thus the phase equilibria, depend on the degree of polymerization of the polymers.

The mechanistic interpretation of the polymer incompatibility that follows from the Flory–Huggins description is as follows: The main cause of the solubility of a polymer in solution is the increase in entropy due to the increase in allowed polymer configurations in the presence of a solvent. Normally there is an effectively attractive interaction between the polymer segments but, as long as the interaction parameters χ_{12} and χ_{13} are smaller than 0.5, the entropy-of-mixing terms are strong enough to ensure complete miscibility between solvent and polymer. The entropy of mixing of the polymer molecules as such gives an increasingly negligible contribution to the free energy as the degree of polymerization increases. When two different soluble polymers are mixed the entropy of the chain conformation basically depends only on the overall solvent volume fraction. The entropy lost in demixing into one solution rich in polymer 2 and one in polymer 3 is then due only to the entropy of mixing the polymer molecules, which is only a very small term, particularly for long polymers. Thus only a weak effective repulsion between the segments of the two different polymers is sufficient to generate a phase separation.

From equations (4)–(6) one obtains a clear qualitative interpretation of the reason for the phase separation and in addition a reasonable quantitative description of the phenomena. The probable reason that this approach has not been used explicitly for aqueous two-phase systems is that the Flory–Huggins theory shows problems when applied to polymer–water systems[9].

These problems have two main sources. In the original derivation of the Flory–Huggins theory a solvent molecule was assumed to occupy the same volume as a monomer unit of the polymer. For a dextran in water this is not even approximately true. Introducing the effect of unequal sizes makes the determination of the entropy of mixing more involved but the main result is qualitatively the same[10,11] as one would expect. The second complication with the Flory–Huggins theory when applied to aqueous systems creates more difficulties. Water is a structural solvent with, even from a thermodynamic point of view, complicated behaviour. The interpretation of the interaction parameter χ has to be modified when one is dealing with an aqueous system. In molecular terms one can say that the solvation of a monomer unit involves many water molecules and it is associated with changes in the internal degrees of freedom of these water molecules. Primarily this implies that χ, which in the conventional Flory–Huggins theory is purely enthalpic, has the character of a free energy and by comparison with experiments one finds that χ is in fact strongly temperature-dependent[10,12]. Secondly, the fact that many solvent molecules are involved with the interaction of one monomer unit implies that at low solvent contents conditions change in a more complex manner than accounted for in the Flory–Huggins theory based on pair nearest-neighbour interactions only.

CONCLUSIONS

In spite of the difficulties associated with the application of the Flory–Huggins theory in a quantitative way to aqueous polymer systems, the theory still describes many of the characteristic properties in a qualitatively correct way. Thus, when applied to the polymer incompatibility problem, it is clear to us that the basic mechanism leading to the separation of two polymer solution phases in aqueous systems is the same as in other solvents. The main free energy term favouring mixing of the polymers is the entropy term associated with the mixing of the polymer molecules, while the dominating entropy term from the configurations of the polymer is unaffected by demixing. Phase separation is generated by an effectively unfavourable interaction between the units of the two polymers in the sense that it is energetically more favourable to form one 2:2 and one 3:3 pair than two 2:3 pairs.

The alternative description to the phase separation using equations (1)–(3) is less satisfactory. Interpreting these as a truncated true virial expansion is associated with the difficulty that such an expansion is not valid in the concentration range of interest. Alternatively treating the coefficients a, c and d as parameters provides no insight.

When considering the phase separation problem, for example in attempts to design new suitable two-phase systems[13], the central concept is the interaction between the polymer units rather than the excluded volume and the shape and size of the polymers.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Per-Åke Albertsson for helpful suggestions and discussions. This work was supported by grants from the National Swedish Board for Technical Development (STU).

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