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An Approach to the Study of Phase Separation in Ternary Aqueous Systems

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1. Simple thermodynamic expressions are used to describe the properties of uncharged binary and ternary polymer solutions, in particular the sedimentation equilibrium of binary systems and the osmotic pressures and 'incompatible' phase separations of ternary systems. 2. Sedimentation-equilibrium experiments were performed on four samples of dextran and two of polyethylene glycol. The critical points of the phase diagrams were determined for the mixed solutions of polyethylene glycol–dextran–water and of polyethylene glycol–bovine serum albumin–0·2 M-sodium chloride solution. Osmotic pressures were measured on a single-phase mixed solution of a polyethylene glycol and a dextran. By use of the simple thermodynamic expressions consistent values of second virial and interaction coefficients for the materials used were obtained from these experiments. 3. The interpretation of the values of the second virial and interaction coefficients, on the basis of three models of molecular interaction, is discussed.

The separation of polymer solutions into two or more liquid phases has been known for a long time, and is discussed in standard works such as Flory (1953), Stuart (1953), Tompa (1956) and Huggins (1958). In systems that contain two polymers and a solvent, two types of phase separation are recognized: 'incompatibility', in which the two polymers are concentrated in different, solvent-rich, phases; and 'complex coacervation', in which the two polymers are concentrated in the same, often solvent-poor, phase. A number of theoretical treatments (see Voorn, 1959) of these phenomena have been given; Albertsson (1960), who has investigated experimentally a number of water-soluble polymer systems that exhibit incompatibility, gives a qualitative account of the factors involved.

Except for the work of Albertsson (1960), whose interest was in finding immiscible aqueous systems for use in the partition separation of cell particles and macromolecules, little attention seems to have been given to the possible significance of incompatibility in biological systems: attention has rather been concentrated on complex coacervation, particularly by Bungenberg de Jong (1936, 1949) and his school. It seemed to us possible that, in the relatively concentrated systems found in the cytoplasm of cells and extracellularly, conditions might exist in which 'incompatible' phase separation could occur and that such separations might account for some of the heterogeneities that are visible in such situations. For example, Ogston (1962) suggested that phase separation might occur in a mixed solution of hyaluronic acid and protein; Ogston (1937) and J. P. Johnston (personal communication) found values of the osmotic pressure in mixtures of proteins in excess of those expected from the sums of the osmotic pressures of the components, and Laurent & Ogston (1963) and Preston, Davies & Ogston (1965) found similar osmotic-pressure excesses in mixed solutions of hyaluronic acid and BSA,* compatible with the possibility of phase separation (which has not, however, been observed in these systems).

With these considerations in mind, we decided to investigate, in the first place, a few ternary systems of uncharged water-soluble macromolecules with the object of finding whether their behaviour could be represented reasonably well by rather simple thermodynamic expressions. Such systems are admittedly over-simplified as models for biological systems; but their study opens the way to an investigation of more complex (quaternary) systems containing polyelectrolytes. The method also provides a means for estimating the thermodynamic interactions between polymers that exhibit incompatibility.

We describe measurements made on the incompatible phase separations of eight ternary

*Abbreviations: BSA, bovine serum albumin; PEG, polyethylene glycol.

systems formed by four samples of dextran and two of PEG in water, and on the pseudo-ternary systems of isoelectric BSA and the two PEG samples in 0·2 M-sodium chloride solution. The sedimentation equilibria of binary solutions of the dextran and PEG samples in water were studied and a few osmotic-pressure measurements on one of the ternary systems of dextran and PEG in water were made.

THEORY

Phase separation in a ternary system. The condition (Prigogine & Defay, 1954) that defines the spinodal curve, separating regions of absolute instability, is:

$$\mu_{22}\mu_{33}-\mu_{23}^2 = 0 \qquad (1)$$

The subscripts 2 and 3 refer to the two solute components. μ_{23} is the partial derivative $(\partial\mu_2/\partial n_3)_{T,p,n_1,n_2}$ of μ_2 (the chemical potential of component 2) with respect to n_3 (the number of moles of component 3) with temperature, pressure and the number of moles of all other components remaining constant. Analogous definitions apply to μ_{22} and μ_{33}. Equivalent expressions to eqn. (1) may be written in terms of the other pairs of components.

The binodal or coexistence curve (on which lie the compositions of immiscible solutions at equilibrium, connected by the tie-lines) is defined by:

$$\begin{aligned}\mu_1' &= \mu_1'' \\ \mu_2' &= \mu_2'' \\ \mu_3' &= \mu_3''\end{aligned} \qquad (2)$$

where prime (′) and double prime (″) indicate pairs of phases in equilibrium.

The two curves are tangential at the critical point, which is defined by:

$$\left(\frac{\partial\mu_2}{\partial n_2}\right)_{T,p,n_1,\mu_3} = 0 \qquad (3a)$$

$$\left(\frac{\partial^2\mu_2}{\partial n_2^2}\right)_{T,p,n_1,\mu_3} = 0 \qquad (3b)$$

or by equivalent expressions for component 3 (see Prigogine & Defay, 1954). To proceed further in solving these equations, we need to give algebraic expressions to the chemical potentials of the components as functions of composition. We choose simplified forms of the consistent expressions derived by Ogston (1962) and write:

$$\mu_2 = \mu_2^0+RT(\ln m_2+cm_2+am_3) \qquad (4a)$$

$$\mu_3 = \mu_3^0+RT(\ln m_3+dm_3+am_2) \qquad (4b)$$

$$\mu_1 = \mu_1^0-\frac{RTM_1}{1000}\left(m_2+m_3+\frac{c}{2}m_2^2+\frac{d}{2}m_3^2+am_2m_3\right) \qquad (4c)$$

where m_2 and m_3 are molalities of solutes, and c, d and a are constant coefficients. Eqn. (4c) is obtained from eqns. (4a) and (4b) with the use of the Gibbs–Duhem equation. Since we may use molalities in place of numbers of molecules, and since $m_1 = 1000/M_1$, the latter equation may be written:

$$\frac{1000}{M_1}d\mu_1+m_2\left(\frac{\partial\mu_2}{\partial m_2}dm_2+\frac{\partial\mu_2}{\partial m_3}dm_3\right)+m_3\left(\frac{\partial\mu_3}{\partial m_2}dm_2+\frac{\partial\mu_3}{\partial m_3}dm_3\right) = 0$$

or, from eqns. (4a) and (4b):

$$d\mu_1 = -\frac{RTM_1}{1000}[(1+cm_2)dm_2+(1+dm_3)dm_3+ad(m_2m_3)]$$

whose definite integral is eqn. (4c).

From eqns. (3a) and (3b), with use of eqns. (4a) and (4b), we obtain for the critical point:

$$\left(\frac{1}{m_2^{\text{crit}}}+c\right) = a(m_3^{\text{crit}}/m_2^{\text{crit}})^{\frac{2}{3}} \qquad (5a)$$

$$\left(\frac{1}{m_3^{\text{crit}}}+d\right) = a(m_2^{\text{crit}}/m_3^{\text{crit}})^{\frac{2}{3}} \qquad (5b)$$

where m_2^{crit} and m_3^{crit} are the molalities at the critical point. A merit of the rather simple expressions (4) and (5) is that, given values of m_2^{crit}, m_3^{crit} and one of the coefficients c, d or a, the values of the other two can be determined.

In this respect, the critical point has advantages over the binodal curve (the spinodal curve cannot be determined experimentally) for which eqns. (2) cannot be solved explicitly. It is possible, however, to compute the binodal curve, once values for c, d and a are available (compare Tompa, 1949). From eqns. (2) and (4), two of the quantities m_2', m_2'', m_3' and m_3'' can be eliminated, leaving, for example, a relationship between m_3' and m_3'':

$$\frac{Q}{a}+(m_3'-m_3'')+\frac{cQ^2}{2a^2}\left(\frac{e^P+1}{e^P-1}\right)+\frac{d}{2}(m_3'^2-m_3''^2)+\frac{Q}{e^P-1}(m_3'e^P-m_3'') = 0 \qquad (6a)$$

$$P = \frac{c}{a}ln\left(\frac{m_3'}{m_3''}\right)+\frac{cd-a^2}{a}(m_3'-m_3'') \qquad (6b)$$

$$Q = -\left[\ln\left(\frac{m_3'}{m_3''}\right)+d(m_3'-m_3'')\right] \qquad (6c)$$

$$m_2' = m_2''e^P = \frac{Qe^P}{a(e^P-1)} \qquad (6d)$$

By choosing a suitable value of, say, m_3' the value of m_3'' that satisfies eqn. (6a) can be found, and the corresponding values of m_2' and m_2'' are then obtained from eqn. (6d).

Significance of the coefficients c, d and a. Eqn. (4c), which is in effect the osmotic-pressure equation, shows that c and d are equivalent to the second virial coefficients of components 2 and 3, and a describes the thermodynamic interaction between components 2 and 3, all on the molal scale of concentration. Theoretical treatments of polymer interactions of the type of the Flory–Huggins or Flory 'dilute-solution' theories (Flory, 1953, chapter 12; Tompa, 1956, chapter 4) lead to expressions analogous to eqns. (4) but containing terms in higher powers of the weight/volume concentration. Conversion of eqns. (4) to a basis of weight/volume concentration also yields, on expansion, a series of such terms. The values of these terms, in both expressions, diminish rapidly at small or moderate values of the concentration; and, provided that c, d and a are positive, the signs of comparable terms are the same and their magnitudes not very different. We can therefore provisionally accept eqns. (4) as reasonable approximations.

Determination of the virial coefficients in binary systems by sedimentation equilibrium. The method of Nichol, Ogston &

Preston (1967) was used. Since the molecular weight of PEG 20000 was not known initially, an equation for the estimation of the 'non-ideality coefficents' α_1^* and α_2^* independently of the molecular weight, M, was derived by elimination of M from the differential (eqn. 4 of Nichol *et al.* 1967) and integrated forms (their eqn. 8) of the equilibrium sedimentation equation, to give:

$$Y = \frac{\omega^2 \partial\rho/\partial c}{RT}\left(\frac{r^2}{2} - \frac{rc\ln c}{dc/dr}\right)$$
$$= \alpha_1^* c(1-\ln c) + \alpha_2^* c^2(1-2\ln c) + I \qquad (7)$$

(where I is an integration constant, and c, in this instance, refers to concentration), from which α_1^* and α_2^* were estimated by least-squares treatment.

The coefficients α_1^* and α_2^* are (subject to the limitations discussed by Nichol *et al.* 1967) equivalent to the second and third virial coefficients of the solute, on a weight/volume concentration basis; in particular, α_1^* is equal to $2A_2$, where A_2 is the conventional second virial coefficient (see Nichol *et al.* 1967, eqn. 1). For reasons given above we have assumed that, to a sufficient degree of accuracy, the contribution of α_2^* can be included in a single second virial coefficient on the molal scale; accordingly we identify:

$$\alpha_1^* = 2A_2 = \frac{10^3 c}{M_2^2} \text{ or } = \frac{10^3 d}{M_3^2} \qquad (8)$$

EXPERIMENTAL AND RESULTS

Materials

Dextrans. Four dextran samples with $\bar{M}_w/\bar{M}_n$ less than 1·5 were supplied to us by Dr K. Granath (see Table 1). These are referred below to by 10^{-3} times their $\bar{M}_n$ values, i.e. dextran 52·8, 37·5, 27·2 and 19·7.

PEG samples. PEG 6000 and PEG 20000 were supplied by Carbide and Carbon Chemicals Co., New York, N.Y., U.S.A.

BSA. This was lot 86B–1830 and lot 17B–0530 obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A.

Table 1 summarizes the physical data for these materials.

Stock solutions. Dextran stock solutions were made up to approx. 15–20% (w/w) (the dextran was dissolved by heating to 100°). The concentrations of all dextran solutions were determined polarimetrically.

The PEG samples were assumed to be dry. Stock solutions of about 10% (w/w) were prepared. Solutions of unknown concentration were analysed refractometrically.

The BSA stock solution in 0·2 M-NaCl was of concentration about 30% (w/w). The concentrations of BSA solutions were determined spectrophotometrically.

All solutions were diluted by weight to suitable final concentrations before analysis.

Methods

Partial specific volumes ($\bar{v}$). These were determined for the dextrans by measuring the pycnometric density at 25° of solutions of concentration about 4×10^{-2} g./ml. The partial specific volume was used to derive the density increment $\partial\rho/\partial c$ (Casassa & Eisenberg, 1964) for analysis of equilibrium-sedimentation results. The value of $\partial\rho/\partial c$ in water for dextrans was 0·397 and for the PEG samples was 0·165.

Specific refractive increments. These were measured with a differential refractometer (Cecil & Ogston, 1951).

Optical rotation (α). This was measured in a 1 dm. cell with a Perkin–Elmer model 141 polarimeter. Values of $[\alpha]_{25}^{578}$ and $[\alpha]_{25}^{436}$ used for the determination of dextran concentrations were derived by assuming $[\alpha]_{25}^{D} = +199°$, given by Albertsson (1960).

Extinction coefficient ($E_{1\,cm.}^{1\%}$). Spectrophotometric measurements on BSA solutions were made in a Beckman DU spectrophotometer at 280 mμ.

Intrinsic viscosity [η]. Intrinsic viscosities were measured with a 10 ml. N.S. capillary viscometer of water flow time 70 sec. over a concentration range $0–1\cdot5\times10^{-2}$ g./ml. at 25°.

Equilibrium sedimentation. The method was that of Nichol *et al.* (1967). The experimental details are set out in Table 2. The values for the non-ideality coefficients α_1^* and α_2^* of the dextrans were estimated from eqns. (6a) and (7a) and from eqn. (9) (the equations are those of Nichol *et al.* 1967). These values were reasonably consistent, as was to be

Table 1. *Summary of constants for the materials used*

Material	$10^{-3}\bar{M}_w$	$10^{-3}\bar{M}_n$	Partial specific volume (ml./g.)	Specific refractive increment (ml./g.)	Intrinsic viscosity (ml./g.)	Optical rotation (1 g./ml.; 1 dm.) 436 mμ	578 mμ	$E_{1\,cm.}^{1\%}$ at 280 mμ
Dextran 52·8	77·5*	52·8*	0·60	0·153	24·1	390	207	—
Dextran 37·5	50·5*	37·5*	0·60	0·153	21·1	390	207	—
Dextran 27·2	36·5*	27·2*	0·60	0·153	19·5	390	207	—
Dextran 19·7	26·5*	19·7*	0·60	0·153	16·0	390	207	—
PEG 6000	—	8·0†	0·837‡	0·137	20·4	—	—	—
PEG 20000	—	19·4¶	0·837‡	0·136	38·8	—	—	—
BSA in 0·2M-NaCl	—	73·2§	—	0·188	—	—	—	6·6‖

* K. Granath (personal communication).
† Alexandrowicz (1959).
‡ Nichol *et al.* (1967).
§ Davies (1966).
‖ Kronman & Foster (1957).
¶ See text.

Table 2. *Details of sedimentation-equilibrium experiments on dextrans and PEG* 20000, *and values of coefficients* α_1^* *and* α_2^* (*Nichol* et al. 1967)

Times (hr.) are given in parentheses after speeds.

Material.....	Dextran 52·8*	Dextran 37·5	Dextran 27·2	Dextran 19·7	PEG 20000	PEG 20000
$10^3 \times$ Initial concn. (g./ml.)	29·5	33·9	26·0	37·5	29·6	2·0
$10^3 \times$ Final concn. at meniscus (g./ml.)	0	0	0	0	0·26	—
$10^3 \times$ Final concn. at bottom (g./ml.)	132	156	115	164	61	—
Column height (cm.)	0·28	0·38	0·30	0·46	0·30	0·15
Cell thickness (cm.)	0·15	0·15	0·15	0·15	0·30	3·0
$10^{-3} \times$ Initial speed (rev./min.)	59·78 (3)	59·78 (1·5)	59·78 (2)	59·78 (2·5)	59·78	33·45
$10^{-3} \times$ Final speed (rev./min.)	39·46 (6)	39·46 (5·5)	39·46 (5·5)	39·46 (5)	59·78 (20)	33·45 (24)
$10^3\ \alpha_1^*$	1·02†	1·11†	1·09†	1·17†	5·18‡	—
$10^3\ \alpha_1^*$	1·09§	1·09§	1·08§	1·23§	—	—
$10^3\ \alpha_2^*$	2·99†	2·71†	2·89†	2·88†	20·2‡	—
$10^3\ \alpha_2^*$	2·10§	2·53§	2·92§	2·45§	—	—
$10^{-3}\ M_w$	—	—	—	—	—	19·4

* Values quoted from Edmond, Farquhar, Dunstone & Ogston (1968).
† Calculated by eqn. (9) of Nichol *et al.* (1967).
‡ Calculated by eqn. (7) of this paper.
§ Calculated by eqns. (6a) and (7a) of Nichol *et al.* (1967).

expected from the low degree of polydispersity of the dextrans. For reasons given by Nichol *et al.* (1967) the values obtained by eqn. (9) were preferred.

The 'non-ideality coefficients' of PEG 20000 were estimated by eqn. (7) of this paper and a further equilibrium run at low concentration (2 mg./ml.) was made to determine M under conditions where the effects of non-ideality were negligible. The value of M was obtained from the slope of the plot of $\log\left(\frac{1}{r}\frac{dc}{dr}\right)$ versus r^2, which was linear throughout the range of concentration.

Determination of phase diagrams. All measurements were made at $28 \pm 0{\cdot}1°$. A preliminary idea of the form of each binodal curve was obtained by titration to points of turbidity with the stock solutions of known concentration (Albertsson, 1960). As a result of this experiment, a series of mixtures (at least three) that would fall within the binodal curve and separate into phases of approximately equal volume were prepared. The mixtures were made up by weight, thoroughly mixed and allowed to separate into phases in test tubes. In experiments with PEG and dextran samples (the materials being plentifully available) the total weight of each mixture was approx. 10g. With the BSA and PEG samples the total weight was 5g. After the two phases had settled for several hours the tubes were centrifuged at about 700*g* for 10 min. and samples of the upper phase were withdrawn. The remaining upper phase was removed together with some of the lower phase, and a sample of the lower phase was obtained. The samples of upper and lower phases were diluted by weight and analysed. The results were plotted on the phase diagrams on a weight/weight concentration basis.

Mixtures of PEG and dextran were analysed polarimetrically to obtain the dextran concentration and then refractometrically to determine the PEG, allowance being made for the refraction due to the known concentration of dextran. In phases containing only a small proportion of PEG it was not possible to determine the concentration of PEG sufficiently accurately by this method. On the phase diagram a straight line can be drawn (the tie-line) joining the compositions of the two phases in equilibrium and passing through the original concentration of the mixture (before phase separation occurred), provided that the concentrations are plotted on a weight/weight basis (Albertsson, 1960). This tie-line can be constructed if only the original composition and that of one of the phases is known. The position of the point corresponding to the composition of the other phase will be where the tie-line crosses the measured dextran concentration.

Mixtures of BSA and PEG were analysed in a similar way except that the determination of the BSA was made spectrophotometrically. The specific refractive increments of the PEG were unchanged in 0·2 M-NaCl.

The critical point, where the line passing through the midpoints of the tie-lines cuts the binodal curve, was obtained from each phase diagram. The concentrations of the components at the critical point are given, both as weight/weight concentrations and molalities, in Table 3.

Osmotic pressure. The method was that of Edmond *et al.* (1968). The shrinkage of a Sephadex G-50 bead was calibrated by photographic measurements in series of solutions of PEG 20000 and of dextran 52·8, of known osmotic pressures (calculated from the concentration, w/v, and second virial coefficients). Solutions with osmotic pressures less than 0·6 kg./cm.^{2} were used. Below this pressure the curves of bead diameter against osmotic pressure coincided for both materials, indicating that no penetration of the bead by the solute occurred. A series of homogeneous mixtures (i.e. whose compositions lay outside the binodal curve) of PEG 20000 and dextran 52·8 was made. The total osmotic pressures were less than 0·6 kg./cm.^{2}. The excess of shrinkage due to the components at their concentrations in the mixture over that calculated from the sum of their individual osmotic pressures is a measure of the

Table 3. *Composition of solutions at critical point*

System		$10^2 \times$ Concn. (g./g.)		$10^3 \times$ Molality	
2	3	c_2^{crit}	c_3^{crit}	m_2^{crit}	m_3^{crit}
PEG 6000	Dextran 52·8	4·1	6·0	5·7	1·3
PEG 6000	Dextran 37·5	4·2	7·0	5·9	2·1
PEG 6000	Dextran 27·2	5·2	8·2	7·5	3·5
PEG 6000	Dextran 19·7	4·8	8·5	6·9	5·0
PEG 20000	Dextran 52·8	2·8	5·2	1·6	1·1
PEG 20000	Dextran 37·5	2·8	5·8	1·6	1·7
PEG 20000	Dextran 27·2	3·1	6·1	1·8	2·5
PEG 20000	Dextran 19·7	3·3	7·0	1·9	4·0
PEG 6000	BSA in 0·2 M-NaCl	3·7	16·7	5·9	2·9
PEG 20000	BSA in 0·2 M-NaCl	2·7	15·2	1·7	2·6

Table 4. *Osmotic-pressure excesses and corresponding interaction coefficients, a, observed with mixtures of PEG* 20000 *and dextran* 52·8 *in aqueous solution*

π is the osmotic-pressure excess (dynes/cm.^{2})

$10^2 \times$ Concn. (g./g.)			
PEG 20000	Dextran 52·8	$10^8 \pi/RT$	Interaction coefficent (a)
1·5	6·0	340	4000
2·1	5·1	350	3770
2·3	4·1	280	3680
3·0	3·0	275	3480
3·7	1·9	260	4210
1·6	2·2	180	5680
1·9	3·1	190	3540

excess of osmotic pressure (proportional to am_2m_3, eqn. 4c) due to the interaction between the two polymers. The osmotic-pressure excesses and the compositions of the solutions are shown in Table 4.

DISCUSSION

We have accepted the value of $5{\cdot}0 \times 10^{-3}$ for α_1^* of PEG 6000 from Nichol *et al.* (1967) and a value of 8000 for M from Alexandrowicz (1959). Eqn. (8) then gives $c = 320$. Since most of the polymeric solutes are not completely homogeneous, a choice had to be made of which average molecular weight to use. Because $\bar{M}_n$ is the appropriate value in the osmotic equation (4c), we have used this throughout. Accordingly all quantities (c, d, a and molalities) are consistently expressed in terms of $\bar{M}_n$. The only value available for the PEG 20000 was that from equilibrium sedimentation; but the measurements of Alexandrowicz (1959) on PEG 6000 suggest little difference between its $\bar{M}_n$ and $\bar{M}_w$ values, and we have assumed that this holds good for PEG 20000.

The adequacy of eqns. (5a) and (5b) to describe the critical-point data was tested in two ways: (i) the values for c for each PEG, obtained by equilibrium sedimentation, were used in eqns. (5) to calculate the value of d for each dextran and these values are compared, in columns 2–4 of Table 5, with each other and with the values of d obtained by equilibrium sedimentation of the dextrans; (ii) pairs of values of a, for the interaction of each dextran with each PEG, were calculated by use of the equilibrium-sedimentation values respectively of c for the PEG and d for the dextran, by use of eqns. (5); these comparisons are shown in columns 5–8 of Table 5. Table 5 includes the less complete results obtained with BSA, for which an independent value of d was not available.

For comparison Table 6 shows values calculated from the results of Albertsson (1960) on mixtures of various dextrans with PEG 6000 at 20°. The values for the conventional second virial coefficients, A_2, of dextrans obtained from our measurements (Table 2) and from Albertsson's measurements (Table 6) show satisfactory agreement with values quoted in the literature. Thus Senti *et al.* (1955) obtained values between $1{\cdot}25 \times 10^{-4}$ ($\bar{M}_w$ $2{\cdot}7 \times 10^6$) and $6{\cdot}5 \times 10^{-4}$ ($\bar{M}_w$ $18{\cdot}4 \times 10^3$); the values that they quote are for $2A_2$, as usually defined (Tompa, 1956, p. 296), and have been halved. Mariani, Ciferi & Maraghini (1955) obtained $1{\cdot}95 \times 10^{-4}$ ($\bar{M}_n$ 171×10^3) to $2{\cdot}68 \times 10^{-4}$ ($\bar{M}_n$ $93{\cdot}2 \times 10^3$), and Chernyak & Polushina (1961) (their values have also been halved) obtained $4{\cdot}5 \times 10^{-4}$ ($\bar{M}_w$ 108×10^3) to $6{\cdot}25 \times 10^{-4}$ ($\bar{M}_w$ $42{\cdot}2 \times 10^3$). The extensive values of Granath (1958) show a similar trend with molecular weight.

The values of d for isoelectric BSA give values of A_2 (eqn. 8) of $1{\cdot}0 \times 10^{-4}$ and $0{\cdot}86 \times 10^{-4}$, in reasonable agreement with the value $0{\cdot}8 \times 10^{-4}$ obtained from the results of Scatchard, Batchelder & Brown (1946). The values of a obtained for the dextran 52·8–PEG 20000 system by osmotic-pressure measurement (Table 4) are in approximate agreement with those obtained by phase separation.

In spite of the simplicity of the equations used and the approximations to which attention has been drawn, it appears that they lead to reasonable and

Table 5. *Comparison of the values of molal thermodynamic coefficients obtained by sedimentation equilibrium, and the critical points of phase separation between PEG* 6000 ($c = 320$) *or PEG* 20000 ($c = 1950$) *and dextrans or BSA (see the text)*

	Second virial coefficient (d)			Interaction coefficient (a) with			
		From interaction with		PEG 6000		PEG 20000	
Material	From sedimentation	PEG 6000	PEG 20000	From c	From d	From c	From d
Dextran 52·8	2840	2800	3420	1350	1330	3360	2920
Dextran 37·5	1560	1470	1770	980	1020	2470	2250
Dextran 27·2	810	990	1200	760	650	2010	1520
Dextran 19·7	450	520	680	580	530	1520	1150
BSA	—	900	1050	900	—	1910	—

Table 6. *Values of thermodynamic coefficients calculated from the measurements of Albertsson* (1960) *of the critical points of various dextrans with PEG* 6000

Dextran (Albertsson, 1960)	$10^{-3}\bar{M}_w$	$10^{-3}\bar{M}_n$	a	d	$10^4 A_2$
68	2200	280	5330	45900	2·9
48	460	180	3650	21400	3·3
37	179	83	1940	5800	4·4
24	—	405	1040	1720	5·2
17	30	23	640	650	6·1

fairly consistent values for second virial and interaction coefficients in these systems.

A more severe test is the comparison between the observed coexistence curve and that calculated from c, d and a by use of eqns. (6) (Fig. 1). The general agreement is fairly good; as would be expected, increasing deviations occur at points distant from the critical point.

Interpretation of the thermodynamic coefficients. Though there is no need to interpret thermodynamic quantities in terms of molecular properties, to do so may assist mechanistic insight, and may make possible the reverse process of predicting thermodynamic behaviour from molecular properties. Such interpretation requires a model. Though both realism and refinement of the model are desirable, it may be necessary to sacrifice both to some extent in the interests of tractability.

The refined treatments by the Flory–Huggins and Flory 'dilute-solution' theories involve the calculation of the integrated interactions between segments of overlapping molecules, taking into consideration both the entropy of interaction and the heat of interaction expressed by the quantity χ_1 (Flory, 1953, pp. 508–514). Since these treatments require a detailed statistical model of the molecules, they can be used here only in connexion with our results on the PEG samples; they are not

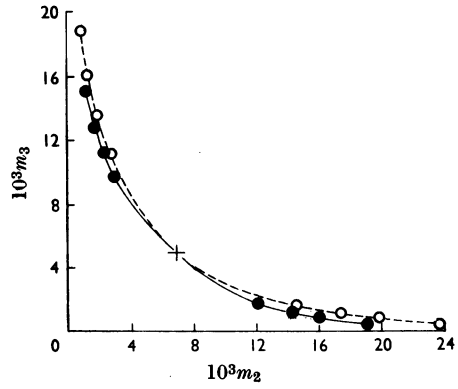

Fig. 1. Comparison of the experimental binodal curve (●) and that calculated from eqns. (6) (○) for the system PEG 6000–Dextran 19·7. + is the critical point.

applicable to the highly branched dextran structure. Calculation of χ_1 from the second virial coefficients of PEG 6000 and PEG 20000 (Table 2) and their intrinsic viscosities (Table 1), with use of eqns. XII-31″, XII-60, XII-70 and XIV-23 of Flory (1953), taking the value of Φ as $2{\cdot}3 \times 10^{21}$ (Flory, 1953, Table 38), gives values of χ_1 of 0·436 and 0·434 by the Flory–Huggins treatment, and 0·420 and 0·408 by the Flory 'dilute-solution' treatment. All that can be concluded is that these are reasonable values for a linear polymer in a 'good' solvent.

However, Flory (1953, chapter 12) shows, by his 'dilute-solution' treatment, that the second virial and interaction coefficients can be regarded as expressing the volumes mutually excluded by neighbouring molecules. We might therefore adopt a simplified view, that the molecules are behaving as equivalent impenetrable spheres, and so identify:

$$U_{22} = 10^3 c = \frac{4\pi N}{3}(2r_2)^3 \qquad (9a)$$

$$U_{33} = 10^3 d = \frac{4\pi N}{3}(2r_3)^3 \quad (9b)$$

$$U_{23} = 10^3 a = \frac{4\pi N}{3}(r_2+r_3)^3 \quad (9c)$$

where U_{22}, U_{23} and U_{33} are the molar excluded volumes for pairs of spherical molecules 2–2, 2–3 and 3–3, whose equivalent radii are r_2 and r_3. Table 7 shows a comparison between values of r_2 and r_3 calculated from eqns. (9a) and (9b) and the values calculated (on the same molecular model) from the intrinsic viscosities (Table 1). Table 8 shows a similar comparison between values of r_2+r_3 calculated from eqn. (9c) and the corresponding values taken from Table 7. The agreements shown in Tables 7 and 8 are surprisingly good considering the crudity of the model; however, it is clear that since eqns. (9) require the cubes of radii only very rough estimates of the thermodynamic coefficients could be obtained from, for example, intrinsic viscosities on this basis.

Alternatively, one could view the PEG as a thread or fibre rather than as a sphere, while continuing to regard the dextran or BSA molecules as spheres. This is the model used by Ogston (1958), Ogston & Phelps (1960) and Laurent (1963, 1964) to account for the interactions between hyaluronic acid (a chain polymer) and compact molecules. On this basis, if a molecule of PEG has length l and cylindrical radius r_f, the molar excluded volume is:

$$U_{23} = \pi(r_f+r_3)^2 lN = 10^3 a \quad (10)$$

This may also be expressed as ϵ, the excluded volume per g. of fibre. Since:

$$\epsilon = \frac{U_{23}}{M_2} = \frac{10^3 a}{M_2} \quad (11)$$

and

$$M_2 = \frac{\pi r_f^2 lN}{\bar{v}_2} \quad (12)$$

where $\bar{v}_2$ is the partial specific volume of the PEG, it follows from eqns. (10), (11) and (12) that:

$$\epsilon = \frac{\bar{v}_2(r_f+r_3)^2}{r_f^2} \quad (13)$$

Table 9 shows values of ϵ calculated from values of a (Table 5) by eqn. (11). As would be expected, the values for each dextran or BSA with the two PEG samples agree well; also, the value of ϵ increases with the molecular size of the dextran. Calculation of r_f from eqn. (13), as shown in the last column of

Table 7. *Comparison between values of the effective molecular radii calculated from the second virial coefficient by eqns. (9a) and (9b) and those calculated from other data*

Material	Molal second virial coefficient c or d (values from equilibrium sedimentation)	r (Å) From c or d	r (Å) From other data
Dextran 52·8	2340	52	59*
Dextran 37·5	1560	43	50*
Dextran 27·2	810	34	44*
Dextran 19·7	450	29	37*
PEG 6000	320	25	30*
PEG 20000	1950	46	49*
BSA	980†	36	35‡

* Calculated from the intrinsic viscosities (Table 1).

† Mean value from Table 5.

‡ Calculated from the diffusion coefficient (Laurent, 1964).

Table 8. *Comparison of the values of sums of effective molecular radii calculated from the interaction coefficients a (eqn. 9c), from the second virial coefficients c and d (eqns. 9a and 9b) and from other data*

Values of a are means from Table 5.

Interacting solutes		a	r_2+r_3 (Å) From a	From c and d (Table 7)	From other data (Table 7)
PEG 6000	Dextran 52·8	1340	81	77	89
PEG 6000	Dextran 37·5	1000	73	68	80
PEG 6000	Dextran 27·2	700	65	59	74
PEG 6000	Dextran 19·7	560	60	54	67
PEG 6000	BSA	900	71	61	65
PEG 20000	Dextran 52·8	3140	107	98	108
PEG 20000	Dextran 37·5	2360	98	89	99
PEG 20000	Dextran 27·2	1770	89	80	93
PEG 20000	Dextran 19·7	1340	81	75	86
PEG 20000	BSA	1910	91	82	84

Table 9. *Excluded volumes (ml./g. of PEG) for dextrans and BSA by PEG samples and values of the fibre radius of PEG, calculated from the fibre–sphere model (see the text)*

Spherical molecule	ϵ(ml. excluded/g. of PEG) PEG 6000	PEG 20000	rf(Å)
Dextran 52·8	168	162	4·5
Dextran 37·5	125	122	4·5
Dextran 27·2	88	91	4·7
Dextran 19·7	69	69	4·6
BSA	112	110	3·3

Table 9, gives values from the series of dextrans that are reasonable and satisfactorily constant, though that from BSA is somewhat lower. It is noteworthy that the value of ϵ for BSA with PEG samples is similar to the value obtained by light-scattering for its exclusion by hyaluronic acid (Ogston & Preston, 1966). It appears, then, that the last model is a reasonably satisfactory one for the cases studied here.

Note on the term 'precipitation'. The increase of the chemical potential of one solute in the presence of another, such as is expressed by eqns. (4a) and (4b), may lead to its value exceeding that of the corresponding solid phase; if this occurs within the region of the phase diagram that is stable with respect to the separation of liquid phases, then precipitation of solid phase will occur before liquid-phase separation. For example, Laurent (1963) has shown the effect of dextran in lowering the solubilities of solid proteins. The use of the term 'precipitation' by Iverius & Laurent (1967) (and by others whom they quote) to describe what in our experience are likely to have been incompatible liquid-phase separations seems to us to be undesirable.

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