Phase Separation in an Aqueous Quaternary System

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(1) We have measured the incompatible phase separation that occurs in a polyethylene glycol–sodium dextran sulphate–sodium chloride–water system and have determined a critical point. (2) We have measured the activity coefficients of sodium chloride in critical-point concentrations of polyethylene glycol and sodium dextran sulphate respectively, and the osmotic coefficient of sodium dextran sulphate at the critical-point concentration. (3) With use of the relevant thermodynamic equations for a quaternary ionic system, we have determined the interaction coefficients between polyethylene glycol and dextran sulphate and between polyethylene glycol and sodium chloride. The former could be due mainly to volume exclusion, but the latter is too large to be explained on that basis.

Edmond & Ogston (1968) showed that the ‘incompatible’ phase separations that occur in some aqueous polyethylene glycol–dextran and polyethylene glycol–bovine serum albumin systems can be represented by fairly simple thermodynamic equations and that their parameters can be related to the notion of molecular exclusion. In some of the experiments with albumin, 0.2M-sodium chloride was used as solvent: however, since the albumin was at its isoelectric point and so carried no net charge, the salt could be regarded as part of the solvent and the system could be treated as pseudo-ternary. Albertsson (1960) showed that incompatible phase separation occurs also in aqueous systems that contain charged polymers. Since most biological macromolecules carry net charge under physiological conditions, we wished to see whether a similar thermodynamic treatment could be applied to a system of this kind. We have therefore made measurements on a polyethylene glycol–sodium dextran sulphate–sodium chloride–water system.

THEORY AND METHODS

General. The spinodal and binodal curves, tangential at the critical point, which describe the stability properties of a ternary system (Prigogine & Defay, 1954) become, for a quaternary system, spinodal and binodal surfaces tangential along a critical line. A plane of constant composition with respect to one of the solute components (e.g. neutral salt) may cut these surfaces along curves that resemble the ternary diagram in that they are tangential at the point (a critical point) where the plane cuts the critical line. Such a point can be determined experimentally by keeping the whole system at constant composition

with respect to neutral salt. However, such a system cannot be treated theoretically as though it were ternary, with solvent and salt regarded as a single component. Even though the solvent and salt were kept in constant proportions in the whole system, this would not apply to the phases into which it separates; that is, the tie lines would not, in general, lie in planes of constant composition with respect to salt.

By extending the treatment of Prigogine & Defay (1954) for a ternary system to the quaternary case, it can be shown (i) that the most restrictive condition for the boundary between unstable and stable states is that, for example,

$$\begin{vmatrix} \mu_{22} & \mu_{23} & \mu_{24} \\ \mu_{32} & \mu_{33} & \mu_{34} \\ \mu_{42} & \mu_{43} & \mu_{44} \end{vmatrix} = 0 \quad (1)$$

and (ii) that all such conditions of order 3 are equivalent. Taking notice of the condition that all $\mu_{ij} = \mu_{ji}$, eqn. (1) can be written

$$\mu_{22} \mu_{33} \mu_{44} + 2\mu_{23} \mu_{34} \mu_{24} - \mu_{22} \mu_{34}^2 - \mu_{33} \mu_{24}^2 - \mu_{44} \mu_{23}^2 = 0 \quad (2)$$

Eqn. (2) describes the spinodal surface. The position upon this surface of the critical line is described by any one of the equations (if one is satisfied, all are satisfied)

$$\left(\frac{\partial^2 \mu_1}{\partial n_1^2} \right) = 0 \quad (3)$$

at constant T , p , n and all μ other than μ_1 . We therefore have in principle, as in the ternary case, two independent equations for the critical line and we could use these to determine the values of two parameters. For reasons given below, we have in fact made use only of eqn. (2) to determine the value of a single parameter of the system.

Definition of components and concentrations. In an ionic system, there are several possible ways of defining the components. We have chosen (see Ogston, 1962)

component 1, water; component 2, polyethylene glycol; component 3, sodium dextran sulphate; component 4, NaCl. Because of the convenience of replacing the number of moles of a component by its molality, we use molal concentrations: m_3 is then the molal concentration of dextran sulphate polyanion and m_4 that of Cl^- . In calculating molal concentrations, we use the number-average molecular weights of the polymers for reasons given by Edmond & Ogston (1968).

Description of chemical potentials. Following Edmond & Ogston (1968) we consider first that there will be only exclusion interactions of polyethylene glycol with sodium dextran sulphate and with NaCl, and we therefore write

$$\frac{\mu_2 - \mu_2^0}{RT} = \ln m_2 + c m_2 + a_{23} m_3 + a_{24} m_4 \quad (4)$$

where c represents the self-interaction (second virial coefficient) of polyethylene glycol and a_{23} , a_{24} are its interaction coefficients with components 3 and 4 respectively.

Considering next the chemical potential of NaCl, we have to take notice of the contribution that the sodium dextran sulphate makes to the total Na^+ concentration, of the exclusion interaction between NaCl and polyethylene glycol (a_{24}) and of non-ideal ionic interactions. We can arbitrarily write the last as the product of the value (γ_4) of the activity coefficients of NaCl at the same molality in water and a term which (following Katchalsky, Alexandrowicz & Kedem, 1964) treats the sodium dextran sulphate as though only ζ , instead of the stoichiometric

number, of its sulphate groups per molecule are ionized. Accordingly we write

$$\begin{aligned} \frac{\mu_4 - \mu_4^0}{RT} &= 2 \ln m_4 + \ln \left(\frac{\zeta m_3 + m_4}{m_4} \right) + 2 \ln \gamma_4 + a_{24} m_2 \\ &= \ln m_4 + \ln (\zeta m_3 + m_4) + 2 \ln \gamma_4 + a_{24} m_2 \end{aligned} \quad (5)$$

Provided that ζ is independent of m_2 (γ_4 is so by definition) eqns. (4) and (5) are consistent with $\mu_{24} = \mu_{42}$ (Table 1).

If ζ were also independent of both m_3 and m_4 , we could at once write an equation for μ_3 that would satisfy the requirements $\mu_{32} = \mu_{23}$ and $\mu_{34} = \mu_{43}$ (cf. Ogston, 1962). However, this cannot be done for the present case, where ζ is a variable. We therefore write an expression for μ_1 (the osmotic-pressure equation)

$$\begin{aligned} -\frac{m_1}{RT} (\mu_1 - \mu_1^0) &= m_2 + \frac{c}{2} m_2^2 + \phi_3 m_3 + 2\phi_4 m_4 \\ &\quad + a_{23} m_2 m_3 + a_{24} m_2 m_4 \end{aligned} \quad (6)$$

in which m_1 is the molality of water, ϕ_3 is the osmotic coefficient of the sodium dextran sulphate and ϕ_4 is the osmotic coefficient of NaCl at the same molality in water. With the assumptions that c , a_{23} and a_{24} are constants and that ϕ_4 depends only on m_4 , eqn. (6) can then be completely differentiated and the result put, together with the complete differentials of eqns. (4) and (5), into the Gibbs-Duhem equation. This yields the three partial differential coefficients of μ_3 (μ_{32} , μ_{33} and μ_{34}) in terms of the defined quantities. The remaining μ_{1j} are obtained by partial differentiations of eqns. (4) and (5). The resultant

Table 1. *Forms and values of differential coefficients, μ_{1j} in the system water (1), polyethylene glycol (2), sodium dextran sulphate (3) and sodium chloride (4)*

Coefficient	Source equation	Form	Value
$\frac{\mu_{22}}{RT}$	(4)	$\frac{1}{m_2} + c$	2.13×10^3
$\frac{\mu_{33}}{RT}$	(4), (5), (6)	$\frac{\phi_3}{m_3} + \frac{\partial \phi_3}{\partial m_3} - \frac{m_4(m_3 \frac{\partial \zeta}{\partial m_3} + \zeta)}{\zeta m_3 + m_4}$	1.70×10^4
$\frac{\mu_{44}}{RT}$	(5)	$\frac{1}{m_4} + 2 \frac{\partial \ln \gamma_4}{\partial m_4} + \frac{m_3 \frac{\partial \zeta}{\partial m_4} + 1}{\zeta m_3 + m_4}$	6.27
$\frac{\mu_{23}}{RT}$	(4)	a_{23}	4.67 or $-1.62 \times 10^{3*}$
$\frac{\mu_{32}}{RT}$	(4), (5), (6)	a_{23}	
$\frac{\mu_{24}}{RT}$	(4)	a_{24}	35
$\frac{\mu_{42}}{RT}$	(5)	a_{24}	
$\frac{\mu_{34}}{RT}$	(4), (5), (6)	$\frac{\partial \phi_3}{\partial m_4} + \frac{\zeta - m_4 \frac{\partial \zeta}{\partial m_4}}{\zeta m_3 + m_4}$	4.42×10^2
$\frac{\mu_{43}}{RT}$	(5)	$\frac{m_3 \frac{\partial \zeta}{\partial m_3} + \zeta}{\zeta m_3 + m_4}$	2.73×10^2

* Calculated from eqn. (2) with use of μ_{43} (see the text).

Table 2. Values of parameters relevant to the measured critical point of the system water (1), polyethylene glycol (2), sodium dextran sulphate (3) and sodium chloride (4)

	Polyethylene glycol	Sodium dextran sulphate	NaCl
Molecular weight $\bar{M}_n$	$19.4 \times 10^3^*$	$77 \times 10^3^\dagger$	58.44
Molality	5.46×10^{-3}	1.63×10^{-3}	0.2013
Self-interaction coefficient, c	$1.95 \times 10^3^*$	—	—
Molal activity coefficient, γ_4	—	—	0.735†
$\partial \ln \gamma_4 / \partial m_4$	—	—	-0.45†
ϕ_4	—	—	0.935†
ζ	—	155†	—
$\partial \zeta / \partial m_3$	—	$-1.9 \times 10^4 \dagger$	—
$\partial \zeta / \partial m_4$	—	zero†	—
ϕ_3	—	125†	—
$\partial \phi_3 / \partial m_3$	—	$-2.5 \times 10^4 \dagger$	—
$\partial \phi_3 / \partial m_4$	—	approx. $10^2 \dagger$	—

* From Edmond & Ogston (1968).

† From Robinson & Stokes (1955).

‡ This work; see the text.

expressions are given in Table 1. It is seen that all $\mu_{ij} = \mu_{ji}$ provided that (for $\mu_{34} = \mu_{43}$)

$$\frac{\partial \phi_3}{\partial m_4} = \frac{m_3 \left(\frac{\partial \zeta}{\partial m_3} \right) + m_4 \left(\frac{\partial \zeta}{\partial m_4} \right)}{\zeta m_3 + m_4} \quad (7)$$

Procedure. At first we accepted the conclusion of Katchalsky *et al.* (1964) that, at least approximately, ϕ_3 can be equated with ζ . However, direct measurement of ϕ_3 showed that this approximation does not apply to our system. Accordingly, we made use of independent estimates (see below) of ϕ_3 and ζ and of their differential coefficients, as well as of c and of a_{24} . With the experimental values of m_2 , m_3 and m_4 at the critical point, this leaves only a_{23} to be determined. Since the critical point necessarily lies upon the spinodal surface, the value of a_{23} was determined from eqn. (2).

Determination of parameters other than a_{23} . The values adopted are given in Table 2; the resultant values of μ_{ij} are given in Table 1. The value of c was known from the sedimentation-equilibrium measurements of Edmond & Ogston (1968). The critical point values of m_2 and m_3 were determined experimentally; that of m_4 was known by keeping the whole system 0.2013 *m* with respect to NaCl. Values of γ_4 and ϕ_4 were those quoted by Robinson & Stokes (1955). Values of ζ and of a_{24} were obtained by comparing the e.m.f. values of a cell without liquid junction, between reversible Na^+ and Cl^- electrodes, containing 0.2 *m*-NaCl and the critical-point concentrations, respectively, of sodium dextran sulphate and polyethylene glycol in 0.2 *m*-NaCl, with use of eqn. (4) and the relationship

$$\mu_4 - \mu_4^0 = (E - E_0)F$$

The differential coefficients of ζ were similarly obtained by varying m_3 and m_4 from their critical-point values each by about 5%. The values of ϕ_3 and its derivatives were obtained from osmotic measurements with a single bead of Sephadex G-50, as described by Edmond, Farquhar, Dunstone & Ogston (1968).

EXPERIMENTAL AND RESULTS

Sodium dextran sulphate. This was prepared from 'Dextran 52.8' (Edmond & Ogston, 1968) by the method of Ricketts (1952). The sodium salt, finally precipitated with ethanol, was thoroughly washed with ethanol and ether, dried *in vacuo* at 75°C and ground. It was free from chloride. A representative sample, dried to constant weight *in vacuo*, gave a water content of 7.45%. Its specific rotation at 436nm, measured in a Perkin-Elmer model 141 polarimeter, was 247° (1g/ml; 1dm). Analysis gave S, 11.0%. From the molecular weight of the original dextran ($\bar{M}_w = 77.5 \times 10^3$) this analysis predicts a value of $\bar{M}_w$ of 113×10^3 for the sodium dextran sulphate; light-scattering measurements, kindly made for us by Dr G. Granath, gave a value of $\bar{M}_w = 117 \times 10^3$, in satisfactory agreement. The corresponding value of $\bar{M}_n = 77 \times 10^3$ was therefore adopted. The number-average of sulphate groups per molecule is then 244.

Polyethylene glycol 20000. This was the same sample as was used by Edmond & Ogston (1968). It contained a negligible amount of chloride.

Determination of the phase diagram. This was done as described by Edmond & Ogston (1968). Stock solutions of polyethylene glycol and sodium dextran sulphate were made up in 0.2013 *m*-NaCl (final concn.).

Potentiometric measurements. These were made with a Radiometer (Copenhagen, Denmark) pH meter in a cell between a Beckmann 39278 Sodium Ion Electrode and a lightly chloridized silver wire, at 25°C. The system was calibrated with sodium chloride solutions of known molality. Solutions of polyethylene glycol and of sodium dextran sulphate were made by weight, dissolved in sodium chloride

of known molality, near 0.2 *m*; in calculating the final molalities of sodium chloride, allowance was made for the water content of the sodium dextran sulphate. Measurements were accurate to 0.3 mV. The critical-point value of ζ was 155 ± 1 apparent charges per molecule.

Osmotic measurements. A single bead of Sephadex G-50 was calibrated with solutions of dextran 500 (Pharmacia, Uppsala, Sweden; batch 5406) and polyethylene glycol 20000, as described by Edmond *et al.* (1968). The points obtained with the two solutes lay well on a smooth curve. The bead diameter was then determined with the same solutions of sodium dextran sulphate in sodium chloride as were used for the potentiometric experiments, and their net osmotic pressures were read from the calibration curve. To obtain ϕ_3 , allowance has to be made for the partition of sodium chloride between the outer solution and the interior of the Sephadex; since its activity coefficient in sodium dextran sulphate (expressed by ζ) had been measured and that in the Sephadex differs negligibly (on a molal scale) from its value in water (J. D. Wells, personal communication), the net osmotic pressure is expressed by

$$-\frac{m_1}{RT}(\mu_1 - \mu_1^0) = \phi_3 m_3 + 2\phi_4 \{m_4 - [m_4(\zeta m_3 + m_4)]^{\frac{1}{2}}\} \quad (8)$$

where m_4 is the molality of sodium chloride in the outer solution. The value of ϕ_3 at the critical point was 125 ± 3 .

Intrinsic viscosity of sodium dextran sulphate. This was measured in 0.2 *m*-NaCl, as described by Edmond & Ogston (1968). Its value was 37.7 ml/g.

DISCUSSION

Value of a_{23} . The values of μ_{34} and μ_{43} (Table 1) are not identical, as they should be. Therefore eqn. (7) is not satisfied. Since the thermodynamic treatment is itself internally consistent, this discrepancy must be due to experimental error. We preferred the value of μ_{43} because this was based upon potentiometric measurements which were of higher precision than the osmotic measurements. Since eqn. (2) is quadratic in μ_{23} , alternative values for a_{23} are obtained (Table 1), of which the positive value is obviously the relevant one. This quantity represents the volume-exclusion interaction between sodium dextran sulphate and polyethylene glycol. By equation (9c) of Edmond & Ogston (1968) and with 49 Å as the effective radius of the polyethylene glycol molecule, the effective radius of the dextran sulphate molecule is 74 Å, which agrees satisfactorily with the value 69 Å calculated from the intrinsic viscosity.

Value of a_{24} . The experimental value, though low in absolute magnitude, is far higher than would be expected on the basis of volume-exclusion between sodium chloride and polyethylene glycol, and must represent an additional interaction of a salting-out character. In view of the chemical nature of polyethylene glycol and the likely effects of it and of salt on the structure of water, this is not surprising. This conclusion does not invalidate the forms of the equations used.

Values of ζ and of ϕ_3 . The value of ζ , representing the apparent molecular charge of the dextran sulphate anion in its interaction with sodium chloride, is substantially lower than the stoichiometric value of the molecular charge. This is in accordance with the 'cell' theory of polyelectrolyte solutions, as is its decrease with increasing concentration of polyelectrolyte (Katchalsky *et al.* 1964). However, the values of ϕ_3 and ζ are not identical, as the latter authors suggest. This points to a fundamental thermodynamic inconsistency in the treatment of Katchalsky *et al.* (1964) which is not affected by the degree of sophistication with which the 'cell' theory is applied. Only under very particular conditions (e.g. if both were constant) could both the equality of ζ and ϕ_3 and the necessary thermodynamic condition stated by eqn. (7) hold good.

Because there seemed to be no way of determining its value, we have not included in our formulations a term to represent the self-interaction of the dextran sulphate ion due to volume-exclusion but have included the expression of this effect in ϕ_3 . Such a term would be of the form $dm_3^2/2$ in the osmotic-pressure eqn. (6) (cf. Edmond & Ogston, 1968). Its value at the critical-point value of m_3 would be expected, on the basis of the effective radius of the dextran sulphate ion, to be only a few per cent of ϕ_3 .

General. In the neutral ternary systems discussed by Edmond & Ogston (1968) only volume-exclusion interactions appeared to be of importance and this made possible a very simple description of their thermodynamic properties in relation to phase separation. The ionic nature of the sodium dextran sulphate and sodium chloride in the quaternary system considered here introduces considerable complications, making it necessary to perform independent measurements of the parameters ζ and ϕ_3 . With this reservation, the occurrence of incompatible phase separation is adequately described, and similar measurements should make it possible to predict phase separation in other systems of this type.

It is, however, noteworthy that treatment of the system as if it were a ternary solution of two uncharged polymers in a single solvent, with use of equations (5a) and (5b) of Edmond & Ogston (1968)

gives a value for ' α ' of 4.8×10^3 which agrees well with that of α_{23} (Table 1). The value of ' d ' obtained is 1.0×10^4 , which is far higher than the value (4.4×10^3) that would be expected from the intrinsic viscosity on the basis of simple volume-exclusion; it is however, in fair agreement with the value 1.24×10^4 obtained from the osmotic measurements, again assuming that the dextran sulphate is behaving as a neutral substance.

In discussing the possible occurrence of phase separation in the system sodium hyaluronate-bovine serum albumin-sodium chloride-water, Ogston (1962) treated this system as one of non-electrolytes, and Preston, Davies & Ogston (1965) and Nichol, Ogston & Preston (1967) made similar assumptions with regard to the osmotic pressure and sedimentation equilibrium of hyaluronic acid. These assumptions appeared to lead to adequate descriptions of the behaviour of the systems in the latter two cases. The question of how far such simplifying assumptions are justified, particularly in the context of phase separation, requires further investigation.

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