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, 0.2 M-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,

\[ \begin{bmatrix} \mu_{22} & \mu_{23} & \mu_{24} \\ \mu_{32} & \mu_{33} & \mu_{34} \\ \mu_{42} & \mu_{43} & \mu_{44} \end{bmatrix} = 0 \]  

(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

\[ \mu_{22} \mu_{33} \mu_{44} + 2 \mu_{23} \mu_{34} \mu_{42} \mu_{34} - \mu_{22} \mu_{34}^2 - \mu_{33} \mu_{24}^2 - \mu_{44} \mu_{23}^2 = 0 \]  

(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)

\[ \left( \frac{d^2 \mu}{\partial n_i^2} \right) = 0 \]  

(3)

at constant \( T, p, n \) and all \( \mu \) other than \( \mu_i \). 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

\[
\frac{\mu_2 - \mu_1^0}{RT} = \ln m_2 + cm_2 + a_{23} m_3 + a_{24} m_4
\]

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 activity coefficients of NaCl at the same molality in water and a term which (following Katchalsky, Alexandrowics & Kedem, 1964) treats the sodium dextran sulphate as though only \( \xi \), instead of the stoichiometric number, of its sulphate groups per molecule are ionized. Accordingly we write

\[
\frac{\mu_2 - \mu_1^0}{RT} = 2\ln m_4 + \ln \left( \frac{\xi m_3 + m_4}{m_4} \right) + 2\ln \gamma_4 + a_{24} m_2
\]

\[
- \ln m_4 + \ln \left( \frac{\xi m_3 + m_4}{m_3} \right) + 2\ln \gamma_4 + a_{24} m_2
\]

(5)

Provided that \( \xi \) is independent of \( m_2 \) (\( \gamma_4 \) is so by definition) eqns. (4) and (5) are consistent with \( \mu_{24} = \mu_{43} \) (Table 1).

If \( \xi \) were also independent of both \( m_3 \) and \( m_4 \), we could at once write an equation for \( \mu_2 \) that would satisfy the requirements \( \mu_{23} = \mu_{23} \) and \( \mu_{34} = \mu_{43} \) (cf. Ogston, 1962). However, this cannot be done for the present case, where \( \xi \) is a variable. We therefore write an expression for \( \mu_1 \) (the osmotic-pressure equation)

\[
\frac{\mu_1 - \mu_1^0}{RT} = m_2 + \frac{c}{2} m_2^2 + \phi_3 m_3 + 2\phi_4 m_4
\]

\[
+ a_{23} m_2 m_3 + a_{24} m_2 m_4
\]

(6)

in which \( m_1 \) is the molality of water, \( \phi_3 \) is the osmotic coefficient of the sodium dextran sulphate and \( \phi_4 \) 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 \( \phi_4 \) 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 \( \mu_1 \) \((\mu_{23}, \mu_{34} \) and \( \mu_{43} \) \) in terms of the defined quantities. The remaining \( \mu_{ij} \) are obtained by partial differentiations of eqns. (4) and (5). The resultant

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Source equation</th>
<th>Form</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{33} ) ( RT )</td>
<td>(4), (5), (6)</td>
<td>( \frac{\phi_3}{m_3} + \phi_3 \frac{\xi m_3 m_4}{m_4} \frac{\partial \xi}{\partial m_3} + \phi_4 \frac{\xi m_3 m_4}{m_4} \frac{\partial \xi}{\partial m_4} )</td>
<td>1.70 x 10^{4}</td>
</tr>
<tr>
<td>( \mu_{44} ) ( RT )</td>
<td>(5)</td>
<td>( \frac{1}{m_4} + 2\ln \gamma_4 + \frac{m_4 \xi}{m_3 + m_4} \frac{\partial \xi}{\partial m_4} + 1 )</td>
<td>6.27</td>
</tr>
<tr>
<td>( \mu_{23} ) ( RT )</td>
<td>(4)</td>
<td>( a_{23} )</td>
<td>4.67 or -1.62 x 10^{3}*</td>
</tr>
<tr>
<td>( \mu_{33} ) ( RT )</td>
<td>(4), (5), (6)</td>
<td>( a_{23} )</td>
<td>35</td>
</tr>
<tr>
<td>( \mu_{44} ) ( RT )</td>
<td>(4)</td>
<td>( a_{24} )</td>
<td>35</td>
</tr>
<tr>
<td>( \mu_{43} ) ( RT )</td>
<td>(5)</td>
<td>( a_{124} )</td>
<td>4.42 x 10^{2}</td>
</tr>
<tr>
<td>( \mu_{34} ) ( RT )</td>
<td>(4), (5), (6)</td>
<td>( \phi_3 - m_3 \frac{\partial \phi_3}{\partial m_3} + \phi_4 \frac{\xi m_3 m_4}{m_4} \frac{\partial \xi}{\partial m_4} )</td>
<td>2.73 x 10^{2}</td>
</tr>
</tbody>
</table>

* Calculated from eqn. (2) with use of \( \mu_{42} \) (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)

<table>
<thead>
<tr>
<th></th>
<th>Polyethylene glycol</th>
<th>Sodium dextran sulphate</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight $M_n$</td>
<td>$19.4 \times 10^3$</td>
<td>$77 \times 10^3$</td>
<td>58.44</td>
</tr>
<tr>
<td>Molality</td>
<td>$5.46 \times 10^{-3}$</td>
<td>$1.63 \times 10^{-3}$</td>
<td>0.2013</td>
</tr>
<tr>
<td>Self-interaction coefficient, $c$</td>
<td>$1.95 \times 10^3$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Molal activity coefficient, $\gamma_4$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\partial m_{\text{g}}/\partial m_4$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\phi_4$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>—</td>
<td>$155^\dagger$</td>
<td>—</td>
</tr>
<tr>
<td>$\partial \phi_3/\partial m_3$</td>
<td>—</td>
<td>$-1.9 \times 10^4^\dagger$</td>
<td>—</td>
</tr>
<tr>
<td>$\partial \phi_3/\partial m_4$</td>
<td>—</td>
<td>zero $^\dagger$</td>
<td>—</td>
</tr>
<tr>
<td>$\partial \phi_3/\partial m_2$</td>
<td>—</td>
<td>$125^\dagger$</td>
<td>—</td>
</tr>
<tr>
<td>$\partial \phi_3/\partial m_4$</td>
<td>—</td>
<td>$-2.5 \times 10^4^\dagger$</td>
<td>—</td>
</tr>
<tr>
<td>$\partial \phi_3/\partial m_4$</td>
<td>—</td>
<td>approx. $10^3^\dagger$</td>
<td>—</td>
</tr>
</tbody>
</table>

* From Edmond & Ogston (1968).
† From Robinson & Stokes (1955).
‡ This work; see the text.

**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 ($M_w = 77.5 \times 10^3$) this analysis predicts a value of $M_w$ of $113 \times 10^3$ for the sodium dextran sulphate; light-scattering measurements, kindly made for us by Dr G. Granath, gave a value of $M_w$ of $117 \times 10^3$, in satisfactory agreement. The corresponding value of $M_n = 77 \times 10^3$ 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 $m_1$; in calculating
the final molarities 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 $\zeta$ 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 $\phi_3$,
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 $\zeta$) 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

$$-\frac{m_4}{RT} (\mu - \mu_4^0) = \phi_3 m_3$$

$$+ 2\phi_4 \{m_4 - [m_4(\zeta m_3 + m_4)]^l\}$$

(8)

where $m_4$ is the molality of sodium chloride in the
outer solution. The value of $\phi_3$ 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 $\alpha_{23}$.** The values of $\mu_{23}$ and $\mu_{43}$ (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 $\mu_{43}$ because this was based
upon potentiometric measurements which were of
higher precision than the osmotic measurements.
Since eqn. (2) is quadratic in $\mu_{33}$, alternative values
for $\alpha_{23}$ 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 $\alpha_{24}$.** 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 sur-
prising. This conclusion does not invalidate the
forms of the equations used.

**Values of $\zeta$ and of $\phi_3$.** The value of $\zeta$, representing
the apparent molecular charge of the dextran
sulphate anion in its interaction with sodium
chloride, is substantially lower than the stoicheio-
metric value of the molecular charge. This is in
accordance with the 'cell' theory of polyelectrolyte
solutions, as is its decrease with increasing con-
centration of polyelectrolyte (Katchalsky et al.
1964). However, the values of $\phi_3$ and $\zeta$ are not
identical, as the latter authors suggest. This points
to a fundamental thermodynamic inconsistancy 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 $\zeta$ and $\phi_3$ 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 $\phi_3$.
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 $\phi_3$.

**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 $\zeta$ and
$\phi_3$. With this reservation, the occurrence of incompat-
ible 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 'a' of $4.8 \times 10^3$ which agrees well with that of $a_{23}$ (Table 1). The value of 'd' obtained is $1.0 \times 10^4$, which is far higher than the value $(4.4 \times 10^3)$ that would be expected from the intrinsic viscosity on the basis of simple volume-exclusion; it is however, in fair agreement with the value $1.24 \times 10^4$ 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.

We are grateful to Dr J. E. Fildes for sulphur analysis; to Dr K. Granath for measurement of light scattering; to Dr D. J. Brown for advice on the sulphation of dextran; to Dr A. Rescigno and Dr J. D. Abernethy for discussion, and to the Australian National University for a Research Scholarship held by E. Edmond.

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