

PHASE SEPARATION IN POLYELECTROLYTE  
SOLUTIONS. THEORY OF COMPLEX  
COACERVATION

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FIVE FIGURES

INTRODUCTION

It occurs frequently that solutions (even dilute solutions) of two polymers in the same solvent are immiscible or nearly so. Good examples are rubber and polystyrene dissolved in benzene or methylcellulose and polyvinylalcohol dissolved in water. Dobry and Boyer-Kawenoki ('47) have described a large number of cases of this "incompatibility," where each of the two phases formed is practically a pure solution of one of the two polymers. This phenomenon is explained by a negative heat of mixing of the two polymers, which means that heat has to be supplied for mixing. Even a small negative heat of mixing expressed per gram of polymer becomes a large effect if expressed per mole and can easily overcome the relatively small gain in entropy that may be seen as the "driving force" for mixing.

The phase separation in colloids studied by Bungenberg de Jong ('49a, b) and coworkers is of a fundamentally different type. Mixing of two aqueous solutions of gum arabic and gelatin at a suitable pH produces phase separation but in this type one phase contains most of the two polymers

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combined with a modest amount of solvent, whereas the other phase is a dilute, often very dilute, solution of one or both the polymers. This type of phase separation occurs when the two polymers carry opposite charges. Bungenberg de Jong and Kruyt ('29) introduced the term "coacervation" and more specifically "complex coacervation" for this phenomenon. The Latin word *acervus* being heap, coacervation means literally coming together in a heap (of polymer particles), the prefix complex indicates that coacervation is brought about by a kind of complex formation between oppositely charged particles.

#### PHENOMENOLOGY OF COMPLEX COACERVATION

Complex coacervation has a number of characteristic features. Since naturally occurring polyelectrolytes often are polyampholytes, their charge depends on the *pH* of the solution. Complex coacervation is consequently sensitive to the *pH*. With gum arabic, a carbohydrate carrying carboxyl groups and gelatin, a protein carrying both carboxyl and amino groups, complex coacervation is possible only below *pH* 4.8, the isoelectric point of gelatin and above *pH* 2. Below *pH* 2, the dissociation of the carboxyl groups of the gum arabic is nearly suppressed.

Addition of neutral salts decreases the tendency to coacervation. In the presence of an excess of ions the charged groups on the polyelectrolyte are screened by the formation of dense ionic atmospheres around each charge, thus diminishing their interactions and consequently the mutual attraction between the polycations and the polyanions. It is obvious that this effect will be stronger the higher the valency of the added ions and that the suppression of coacervation by salts therefore should follow a Schulze-Hardy rule both with respect to the cations and to the anions

$$4-1 > 3-1 > 2-1 > 1-1$$

$$\text{and } 1-4 > 1-3 > 1-2 > 1-1.$$

Mention might be made here of the similarity in behavior of a complex coacervate and a globulin, both being "insoluble" in water but soluble in acids, bases, or salt solutions. The behavior of both might be subject to the same explanation.

The definitely liquid character of the complex coacervates was originally explained (Bungenberg de Jong, '49a, b; Bungenberg de Jong and Kruyt, '29) as a consequence of a strong hydration of the polymer particles. The electrostatic attraction that brings the particles together was supposed to be unable to press the water of hydration away, and the remaining hydration presumably allowed the coacervate to flow as a Newtonian liquid. This explanation was corroborated by the ability of alcohol, acetone, and similar "dehydrating agents" to promote coacervation, to make the coacervate more concentrated, and to counteract the influence of salts or of an unsuitable *pH*.

Bungenberg de Jong ('49a, b) gathered a large amount of data on complex coacervation. He varied the polyelectrolyte components and the salts, and sometimes used only one polyelectrolyte combined with oppositely charged polyvalent small inorganic ions (e.g., gum arabic and  $\text{Th}^{4+}$  salts) or replaced the polyelectrolyte by micelle-forming detergents. He pointed out that a far-reaching parallelism exists between complex coacervates and the behavior of biological membranes. Both swell or shrink under the influence of salts. Antagonism between  $\text{Ca}^{++}$  and  $\text{Na}^+$  with  $\text{Ca}^{++}$  as shrinking and  $\text{Na}^+$  as the swelling agent may be compared to the possibility of forming a complex coacervate between a negative polyelectrolyte ion and positive  $\text{Ca}^{++}$  or other polyvalent ions, the coacervation being suppressed by the addition of a sufficient amount of salts with ions of lower valency.

On account of the possible interest of complex coacervation to biology and because it is an interesting phenomenon per se, it seemed important that we look for a more quantitative theory.

## GENERAL THEORETICAL CONSIDERATIONS

The problem is how to estimate the electrostatic attraction both with and without added salts, and how to find a suitable formulation for this rather mystical hydration.

*Hydration or entropy.* We approached the problem of hydration by considering phase separations for noncharged polymers. If a "bad solvent" is added to a solution of a nonpolar polymer, e.g., alcohol to a solution of polystyrene in benzene, a separation into two liquid phases occurs, one containing a high and the other a low concentration of the polymer. The solvent content of the concentrated phase has been explained as an entropy effect. With increase of the solvent content, the number of possible arrangements of this phase increases tremendously, with a corresponding increase in entropy; this is more important than the simultaneous (small) loss in energy of mixing. Quantitative treatments of this entropy of mixing effect have been given by Flory ('41, '42, '44) and Huggins ('42a, b). Tompa ('49) and Bamford and Tompa ('50) have developed the theory for phase separation in multicomponent systems. The same approach can be used for polyelectrolyte systems.

*Electrostatic attraction.* The electrostatic attraction seems to be difficult to evaluate for two polyelectrolyte coils. However, the coacervate is after all a rather concentrated solution and instead of containing clearly separated individual coils, it will be just a mass of intertwined coil molecules with the small ions distributed between them. The interaction between individual charged spots and ions will be much more pronounced than the interaction of complete polyelectrolyte molecules. The electrical free energy of the whole coacervate, therefore, may suitably be approximated in the same way as the electrical free energy of an electrolyte solution, according to the Debye-Hückel theory (Debye and Hückel, '23).

We thus shall calculate the electrical free energy by assuming that the influence of the polyelectrolytes on it is the same as that of an equivalent number of monovalent

ions. In the coacervate this picture will not be too unreasonable. It may be rather unsatisfactory in very dilute equilibrium liquids, but then the contribution of the polyelectrolytes to the electrical free energy is small anyway and an error in the calculation will not be very important.

If we use these approaches for the entropy of mixing and the electric free energy and neglect the possible contribution of short-range (van der Waals) forces to the energy of mixing, it is not difficult to formulate a quantitative theory of complex coacervation.

## QUANTITATIVE FORMULATION OF THE ENERGY

Consider a volume  $V$  of solution containing  $n_i$  molecules (or ions) of species  $i$ , the molecular partial volume of each species being  $r_i v$ , where  $v$  is the molecular partial volume of the solvent. The charge per ion will be given by  $z_i e$ ,  $e$  being the elementary charge. In order to keep the equations as simple as possible, we will assume that the molecular volumes of all small ions are also equal to  $v$ , and that of the polyions to  $r v$ , the charge of the polyions being either  $+ze$  or  $-ze$ . For convenience, the ratio  $z_i/r_i = \sigma_i$  will be introduced and called charge density. For the solvent it will be zero, for the small ions 1, and for the polyions a number between 0 and 1.

According to the theory of Debye and Hückel ('23), the electrical free energy of the solution in the volume  $V$  is given as follows:

$$F_{el} = - \frac{e^2}{3\epsilon} \kappa^2 N_z \quad (1)$$

with

$$\kappa^2 = \frac{4\pi e^2 N_z}{\epsilon k T V} \quad (2)$$

where  $\epsilon$  is the dielectric constant of the solvent,

$e$  the elementary charge,

$N_z = \sum n_i |z_i| =$  the total number of elementary charges (+ and -),

$k =$  Boltzmann's constant,

$T =$  absolute temperature.

Introducing volume fractions  $\phi_i = \frac{n_i r_i v}{V}$  and the ratio  $N = V/v$  of the total volume  $V$  to the elementary volume  $v$ , the equation for  $F_{el}$  can be written:

$$\frac{F_{el}}{NkT} = -\alpha [\sum \sigma_i \phi_i]^{3/2} \quad (3)$$

with

$$\alpha = \left( \frac{e^2}{\epsilon kT} \right)^{3/2} \cdot \frac{2}{3} \sqrt{\frac{\pi}{v}} \quad (4)$$

For water as a solvent and room temperature,  $\alpha$  is about 3.5.

Typical features of  $F_{el}$  to which we shall return presently are:

(1) It is proportional to the 3/2 power of the concentration of the total charge; (2) it is independent of the distribution of the charge between polyelectrolyte and small ions; and (3) it is inversely proportional to the 3/2 power of the dielectric constant.

These three features will be preserved in other more refined models although presumably in a less simple form. The electrical free energy will be:

(1) Proportional to a power higher than the first power of the concentration of charges, (2) not very dependent on the distribution of charges, and (3) increasing strongly with decreasing dielectric constant.

Turning now to the entropy of mixing, we shall use the Flory-Huggins expression (Flory, '41, '42, '44; Huggins, '42a, b),

$$TS_{mix} = -kT \sum n_i \ln \phi_i \quad (5)$$

Dividing by  $NkT$ , we find

$$\frac{TS_{mix}}{NkT} = -\sum \frac{\phi_i}{r_i} \ln \phi_i \quad (6)$$

The most characteristic feature of the entropy of mixing is the relatively small contribution of the polyions. This is caused by the factor  $1/r$ . This feature will also be preserved in more refined models.

The complete expression for the free energy of a polyelectrolyte solution that will be used in further derivations is thus

$$\frac{F}{NkT} = -\alpha [\sum \phi_i]^{3/2} + \sum \frac{\phi_i}{r_i} \ln \phi_i \quad (7)$$

#### TWO-COMPONENT SYSTEMS

As a rule, complex coacervating systems are multicomponent systems containing water, two polymers, one or more salts, and possibly nonionic substances such as alcohol.

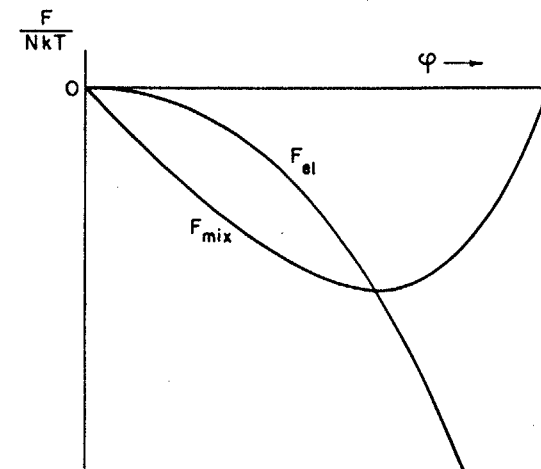


Fig. 1 Electrical part ( $F_{el}$ ) and entropy of mixing parts ( $F_{mix} = -TS_{mix}$ ) of free energy of mixing plotted against volume fraction of polymer PQ.

The most simple system is evidently a two-component system consisting of water and a polymer salt PQ composed of  $z$ -valent polycations  $P^{z+}$  and  $z$ -valent polyanions  $Q^{z-}$ , both with the same molecular volume  $rv$ .

In this way a system is obtained with only one concentration variable, for which we take

$$\phi = \phi_P + \phi_Q.$$

Equation (7) can then be written

$$\frac{F}{NkT} = -\alpha (\sigma \phi)^{3/2} + (1-\phi) \ln (1-\phi) + \frac{\phi}{r} \ln \frac{\phi}{2}. \quad (8)$$

Figure 1 shows the electrical part and the entropy of mixing part of  $F/NkT$  plotted separately against  $\phi$ . As is normal

in this kind of representation, the curvature in the entropy of mixing curve is positive. The electrical free energy has a negative curvature. The combined curve, representing the total free energy can have either a positive curvature for all values of  $\phi$  or a negative curvature in the middle part and positive curvatures at the two ends as shown in figure 2. In the latter, phase separation will occur in the system, the composition of the two coexistent phases is given by the  $\phi$  values of the tangent points of the bitangent to the curve.

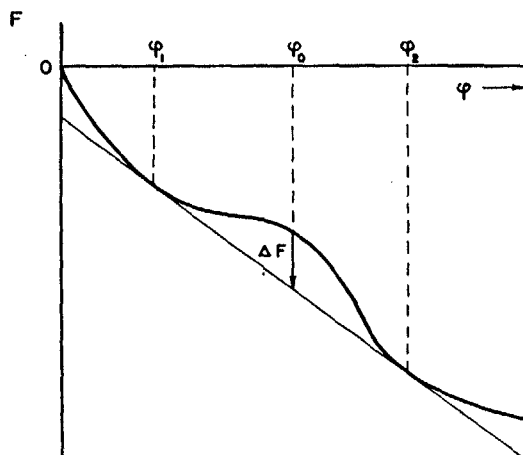


Fig. 2 Free energy versus volume fraction in a system showing demixing.  $\Delta F$  is the decrease in free energy when a mixture of composition  $\phi_0$  is split into two phases with compositions  $\phi_1$  and  $\phi_2$ .

For any mixture between the compositions  $\phi_1$  and  $\phi_2$  the free energy of the two phase system, as given by a point on the bitangent, is lower than that of the one-phase system represented by the corresponding point on the curve.

Obviously, a curve of the type represented in figure 2 has two inflection points. In a critical curve, representing a situation just on the verge of unmixing, these two inflection points have merged. This leads to the mathematical condition for the critical point:

$$\frac{\partial^2 F}{\partial \phi^2} = 0 \quad \text{and} \quad \frac{\partial^3 F}{\partial \phi^3} = 0. \quad (9)$$

Application of these conditions as expressed by equation (9) to equation (8) shows that the critical condition is obtained with good approximation if

$$\sigma^2 r \approx 0.5. \quad (10)$$

This shows that for complex coacervation to occur, either the charge density  $\sigma$  or the molecular weight  $r$  or both have to be sufficiently large.

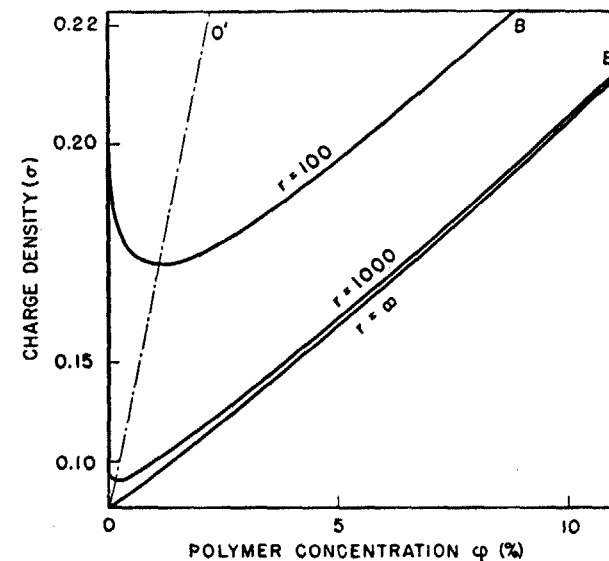


Fig. 3 Influence of charge density  $\sigma$  and chain length  $r$  on phase separation. The figure has been constructed from equation (8) with  $\alpha = 3.655$ . The drawn lines represent the composition of the two phases, the dotted line  $00'$  is the locus of the critical points.

Figure 3 shows lines representing the two coexistent phases for combinations of  $\sigma$  and  $r$ . The critical points are found on the line  $00'$ , the right-hand branches of the curves represent the coacervates, the left-hand branches the equilibrium liquids. It is gratifying that for reasonable molecular weights ( $r=1000$  would correspond to  $M \sim 20,000$ ); the charge density should exceed 0.08 (i.e., the equivalent weight should be below about 250) and that the concentrations of the co-

acervates are of the order of a few per cent, which is in reasonable agreement with actual experimental figures.

Calculations on these and on the more complicated systems are given in greater detail in the thesis of the second author (Voorn, '56). The equilibrium concentrations are derived from the condition that the chemical potential for each individual species has to be the same in the two phases. The chemical potentials are obtained by differentiation of the free energy with respect to the concentrations.

### THREE-COMPONENT SYSTEMS

More important conclusions can be drawn from the study of symmetrical three-component systems, i.e., systems that can be considered to consist of the solvent, a polymer-polymer salt PQ, and a simple mono-monovalent salt KA.

A graphical representation of the free energy against the concentrations now demands a three-dimensional figure with  $\phi_{\text{polymer}}$  and  $\phi_{\text{salt}}$  along two axes and  $F$  along the third (see fig. 4). Increasing the salt content will bring the composition of the two coexistent phases closer together. This can be explained qualitatively in the following way. The decrease in free energy in phase separation is due to the more than proportional increase in the electrical interaction ( $\phi^{3/2}$ ) in accumulating the polymer in a concentrated phase or, in other words, by the fact that in concentrating the polymers the radius of the ionic atmosphere is decreased, which increases the electrical interaction. Now if salt is present, it will be distributed nearly equally over the two phases because any markedly uneven distribution would give rise to an intolerably high decrease in the entropy of mixing. Consequently, there is already a considerable and nearly equal amount of ions in the two phases and accumulation of the polymer in one of the two phases will only change the radius of the ionic atmospheres to a smaller extent, thus making the gain in free energy smaller than in the absence of salt. The compositions of the two phases, therefore, come closer

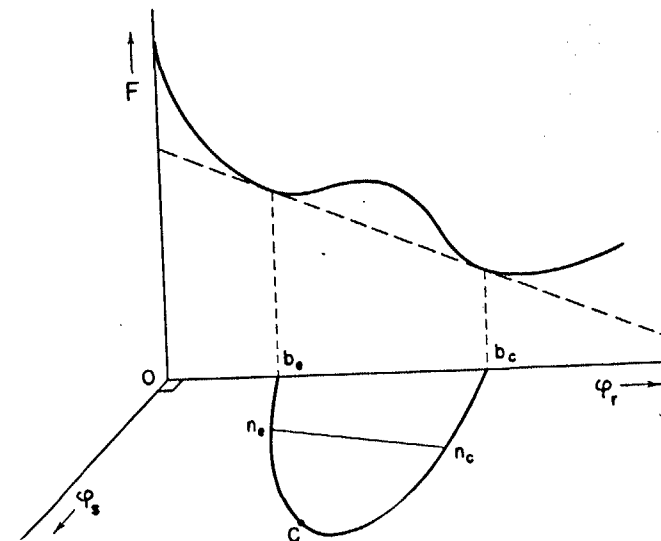


Fig. 4 Free energy versus concentrations in a three-component system.  $\phi_r$  is the volume fraction of the polymer PQ and  $\phi_s$  that of the salt KA. The line  $b_e C b_c$  is the projection of tangent points of the bitangents to the  $F$  plane;  $n_e n_c$  is a node line, connecting coexistent phases.

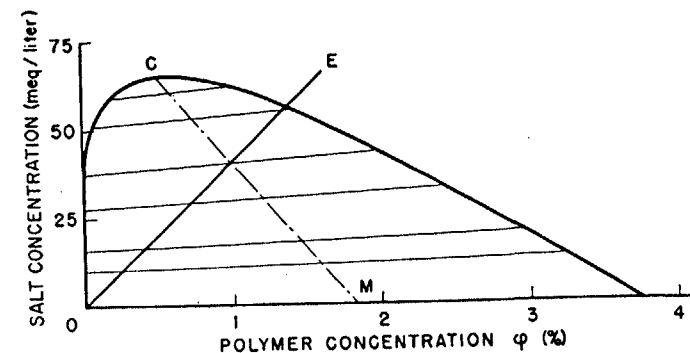


Fig. 5 Phase diagram for complex coacervation in the system solvent, polymer PQ and univalent salt KA. The figure has been constructed for  $r = 1000$ ,  $\sigma = 0.15$ , and  $\alpha = 3.655$ . Polymer concentrations are expressed in volume per cent. In the conversion of the salt concentration to milliequivalents per liter it has been assumed that the molar volume of the ions is  $18 \text{ cm}^3$ , the same as that of water.

together with increasing salt content, and finally reach a critical point, beyond which no phase separation occurs. The curve  $b_n c_n c_n b_c$  in figure 4 represents the projection onto the  $\phi_1-\phi_2$  plane of the tangent points of the bitangents of the  $F$  plane.  $C$  is the critical point. Figure 5 gives an actual example of the calculated equilibrium figure for the case  $r = 1000$ ,  $\sigma = 0.15$ , and  $\alpha = 3.655$ .

The conversion of volume fractions  $\phi$  to concentrations in milliequivalents per liter is effected by assuming the molar volume of the solvent and that of the monovalent ions to be  $18 \text{ cm}^3$ . The line OE corresponds to equivalent amounts of polymer and salt. If the coacervating system is prepared from the two polyelectrolytes each neutralized with monovalent ions (i.e., from PA and QK) with the possible addition of monovalent salt KA, the initial systems are all on or to the left of the line OE.

The line MC is the locus of the middles of the node lines. It is very close to a straight line. Coacervating systems of this type follow, therefore, the law of the rectilinear diameter first found by Cailletet and Mathias (1886) in gas liquid equilibria.

The node lines do not run parallel to the polymer axis but they indicate a slight excess of salt in the coacervate. This is again in agreement with qualitative expectation because the coacervate contains more charges per unit volume and consequently has stronger interaction among the charges, thus lowering the free energy. The effect however can only be small on account of the large entropy contribution of the small ions.

This consequence of the theory had never been tested. We did some experiments on complex coacervates of the K salt of gum arabic and gelatin chloride and found indeed that the coacervate contained about 10% more KCl than the equilibrium liquid, the concentrations being expressed per unit volume of the phases (see table 1).

#### FOUR-COMPONENT SYSTEMS

Dropping the condition of symmetry and allowing systems in which the amounts of polycation and polyanion are no longer equivalent, we arrive at four-component systems. Ex-

perimentally, the choice of components will probably be water, KA, QK, and PA, but other possibilities exist to describe the same systems. A choice in which the four-component systems can be more easily derived from the three-component ones is water, PQ, KA, and PA (or QK, depending on which of the polymers is in excess).

As compared to the symmetric systems, a new variable appears, i.e., the potential difference between the two phases. If the polycations are in excess, the potential of the coacervate is positive with respect to the equilibrium solution.

TABLE 1  
*Distribution of KCl in a coacervated system of gelatin-chloride and potassium arabinatate*

40°C.; pH, 3.75; polymer concn. in coacervate, 14% by wt., in equil. liquid, 0.5% by wt.									
EXPT. NO. →	1a	1b	2a	2b	3a	3b	4a	4b	MEAN
<i>concn. of KCl in meq/liter</i>									
In coacervate ( $C_c$ )	9.2	8.7	9.3	9.3	8.8	8.9	8.7	9.0	9.0
In equil. liquid ( $C_p$ )	8.24	8.29	8.25	8.25	8.34	8.33	8.32	8.29	8.29
$C_c/C_p$	1.08		1.12		1.06		1.06		1.08

Although this potential difference does not occur explicitly in the expression for the free energy of the phases [eq. (7)], because the phases are considered to be electroneutral, it cannot be neglected when equilibrium conditions are derived by use of equality of the electrochemical potentials of each species in the two phases.

If PA is in excess, it will accumulate like any other salt in the coacervate. However, because of the large number of small ions A, this is unfavorable from an entropy point of view and therefore the coacervate will be more symmetric than the original mixture, whereas the equilibrium liquid will be more asymmetric, containing a good many  $P^+$  ions but very few  $Q^-$  ions. Table 2 shows the calculated distribution for the polymer-polymer salt PQ, the excess species

PA and the electrolyte KA between the two phases for a representative case. It is very striking how close the distribution coefficient of KA remains to unity, how extreme that of PQ may be, and how that for PA is intermediate never exceeding 10.

If the asymmetry is too large, coacervation is completely prevented. One might say that the excess polymer PA acts as added electrolyte to the polymer-polymer salt PQ.

Although a mixture of a single polyelectrolyte PA with water is not expected to show coacervation, and indeed does

TABLE 2

Calculated distribution coefficients of salt KA, polyelectrolyte PA, and polymer-polymer salt PQ in nearly symmetric systems (very small excess of PA)  
 $r = 1000, \sigma = 0.15, \alpha = 3.655$

SALT CONCN. IN EQUILIBRIUM LIQUID	DISTRIBUTION COEFFICIENT OF:		
	KA	PA	PQ
<i>meq/liter</i>			
16.0	1.26	9.76	6130
21.9	1.20	2.09	402
51.3	1.10	1.18	14
56.3	1.07	1.12	6
59	1.05	1.08	4
62.7*	1.025	1.04	2
64.35*	1.00*	1.00*	1.00*

\* Critical point.

not show it, exchange of the monovalent counter ions for divalent ones (lowering of entropy contributions) or addition of such substances as alcohol or acetone that decrease the dielectric constant (increase of the electrical interaction) may bring the system to coacervation. I. Michaeli (private communication) has found this behavior in the sodium and calcium salts of polymethacrylic acid. Bungenberg de Jong ('49a, pp. 384ff) has described a number of cases of so-called "auto-complex-coacervation" in systems of one polyelectrolyte with bi- or polyvalent gegenions.

## RELATIONS TO BIOLOGY

Complex coacervation is believed to be important for biology in several respects.

The parallel with the behavior of biological membranes and with the solubility of globulins has already been mentioned in the Phenomenology section.

Interaction of two proteins or of protein and nucleic acid in nucleoproteins may very often contain, in addition to a specific part, an important interaction of the complex coacervate type, and consequently may be sensitive to salts and pH changes.

Also, in a preparative sense, complex coacervation may find its uses in treatment of mixtures and possibly in processes of fractionation.

## CONCLUSION

The main object of this paper is to draw attention to the very general nature of complex coacervation, to show that it can be explained theoretically by simple general principles, and in particular to show that the difference in behavior between large molecules and small ones is mainly due to entropy effects, whereas the energies of interaction, when expressed per unit weight, are of the same order for large and for small molecules.

## SUMMARY

A brief phenomenological description is given of complex coacervation, the phase separation occurring in polyelectrolyte systems due to interaction of the electric charges of the polyions. Complex coacervation is treated theoretically as a competition between electrical attraction tending to accumulate the charged particles and entropy which tends to disperse them. The treatment is put on a quantitative basis by use of the Debye-Hückel equations for the electrical interaction and the Flory-Huggins theory for the entropy. The properties of free energy versus composition curves or planes are used to treat two-phase equilibria.



Systems of different complexity of composition are treated briefly, the most complicated one consisting of water, two polymers of opposite charge, an electrolyte and a nonionized substance of low dielectric constant.

It is shown experimentally and theoretically that not only the polymers but also the low molecular weight salt is accumulated in the coacervate.

Possibilities of application in systems of biological interest are mentioned.

#### GENERAL DISCUSSION

SCHERAGA<sup>2</sup>: In your free-energy diagram you have a range of compositions in which the system of two polymers and one solvent can minimize its free energy by splitting into two phases. Doesn't this imply that the ratio of one polymer to the other is fixed?

OVERBEEK: Here I assumed that the two polymers were present in equivalent amounts, but it is not necessary to assume this. One can use the same treatment for nonequivalent amounts of polymer, but then one has to add one other axis. Nonequivalent amounts of polymer can occur only in systems containing three or more components.

MEL<sup>3</sup>: Do you consider the main difference between the coacervation complex here and, say, an ordinary crystal process where you have an included solvent to be the amount of solvent actually trapped there?

OVERBEEK: Not only that. The complex coacervate is really liquid. It may be a viscous liquid; the viscosity may be 50 or 100 times that of water. But there is no X-ray diffraction pattern. There is no ordered arrangement of the water molecules, nor is the water content of the coacervate a constant.

SZENT-GYÖRGYI<sup>4</sup>: Would not the behavior be modified considerably if the two charges of the macromolecular chromosomes were the bivalent kind?

<sup>2</sup> Harold A. Scheraga, Cornell University.

<sup>3</sup> H. C. Mel, University of California.

<sup>4</sup> Albert Szent-Györgyi, Marine Biological Laboratory, Woods Hole.

OVERBEEK: I am sure that has an influence. You may remember from the Introduction that Bungenberg de Jong has also studied complex coacervates formed from only one polyelectrolyte. If the same approach is applied to a potassium salt or a sodium salt of a polymer, no phase separation is found, but as soon as the calcium salt is taken, coacervation may occur because the contribution of the bivalent calcium ion to the entropy is less important than that of an equivalent amount of monovalent ions.

It is easy to predict that if on the polymer elementary charges are present in pairs, so close together that they should be considered as bivalent ions, this should be a factor in favor of coacervation.

SZENT-GYÖRGYI: This is because many of our biological systems do form coacervates when they should not.

OVERBEEK: When ATP is present quite a number of charges are concentrated and that will enhance the coacervation effect.

RANDALL<sup>5</sup>: A solution of gelatin consists of a system of random coils according to the evidence of light scattering and there are charges of both signs on any one molecule. Your analysis, so far as I understand it, completely avoids the shape factor in the configuration of the molecule. Is that so?

In solutions of such molecules as collagen the process of precipitation may occur. Does your analysis in any way cover the case where the molecule has both signs of charge on it and, secondly, does it cover the case that Flory has dealt with in a rather general way, in two recent papers, of the molecules coming together to form fibrils?

Of course, in collagen, which is a more complex system than those dealt with by Dr. Overbeek, molecules come together in what amounts to a process of crystallization; and all the structures that Dr. Schmitt and his colleagues and others have shown to exist in these precipitates take time to form.

<sup>5</sup> J. T. Randall, University of London King's College.

I will be interested to know if your analysis throws any light on these problems.

OVERBEEK: I would say that the presence of charges of two signs on one molecule can be easily included in the treatment. It is just a question of considering the charge density factor in a little more general way. So far, in our calculation on gelatin, we have considered the charge density as representing the net charge of the gelatin molecule. But we might, of course, also say there is a larger positive charge and a smaller negative charge and they should be included independently. That would not make the treatment more complicated. It would favor coacervation.

Specific forms and the fitting of two molecules more or less onto each other are certainly not included and I should like to see other people work on this aspect.

EDSALL<sup>6</sup>: I presume there are systems that are still outside the critical point where a phase separation does not occur, and by the use of methods such as light scattering one would detect changes caused by the interactions that would vary somewhat with the ionic strength or the dielectric constant and other factors that might be picked up.

OVERBEEK: I don't think there have been any serious experiments in this field, but it would be interesting to go close to the critical point and see whether interactions could be discerned.

EDSALL: I have a particular case in mind, a system that was investigated by light scattering in our laboratory about 10 years ago by Peter Morrison, a system composed of  $\gamma$ -globulin at a pH of about 5.6 and that was negatively charged — and most of the  $\gamma$ -globulin is positively charged — where at low ionic strength there was a lot of precipitation that, in the absence of either component, was perfectly soluble. But even apart from this, where there was no actual precipitation there was a marked increase in the turbidity of the system that could be followed very nicely and varied in a

<sup>6</sup>J. T. Edsall, Harvard University.

striking fashion with a variation in the ionic strength of the system. Here, of course, there are two molecules that are more or less globular proteins. They do not correspond to the particular model that you have assumed, but I think that qualitatively there must be a general resemblance.

OVERBEEK: May I make just a short remark here? One should be careful in considering the turbidity that is obtained in this way as an equilibrium property. It may be that very small droplets of the complex coacervate are formed, and if the situation is such that these droplets carry, e.g., a negative charge, if the coacervate is unsymmetrical, then by the repulsion between the negative charges the formation of one continuous phase may be prevented. We have seen such cases very often.

ANDERSON<sup>7</sup>: I was interested in the comment about the interaction between proteins, for example, in the nucleus and the deoxyribonucleic acid. As I understand it, coacervates are equilibrium systems. One would therefore not be able to isolate a little coacervate globule, whereas a nucleus can be taken out of the cell and will exist without dissolving in solutions of ordinary ionic strength such as probably obtain in cells. But some idea of the lability of the bond between the protein and nucleic acid can be gotten from the fact that if one puts in a large molecule with a higher charge density than either the protein present or the nucleic acid present, such as heparin or protamine, one can displace out the substance of the same charge.

OVERBEEK: I would just like to remark that it would be rather difficult to understand, if the nuclei were just a complex coacervate, why the two nuclei do not melt together. On the other hand, the equilibrium concentration of the coacervate may be so low that even a single nucleus in a comparatively large amount of liquid will not dissolve readily. The distribution ratio can be very extreme.

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