Chemistry. — *Coacervation (Partial miscibility in colloid systems).* (Preliminary Communication). By H. G. BUNGENBERG DE JONG and H. R. KRUYT.

(Communicated at the meeting of September 28, 1929).

Introduction.

For the last ten years we have been studying the nature of lyophilic colloids on the ground of the behaviour of sols. The experimental results at which not only we ourselves, but also others, have arrived, have led us to a definite conception about the state in which these systems are. This conception may be schematically expressed in these words : *polymolecular particles stabilized by solvation and capillary-electric charge.*

So far we have confined ourselves to stating that removal of the stabilizing factors leads to an annihilation of the colloid system, to a "flocculation", which in analogy to flocculation in hydrophobic colloids we represented for the sake of simplicity as a combining of the discharged and desolvated particles themselves. But earlier experiences of others as well as our own observation have taught us that we then described only the transition from an initial state (the sol) to a final state (solid precipitant with above liquid), though a very characteristic architectonic intermediate state exists, i.e. an "unmixing", a formation of drops, which may even manifest itself as a separation into two liquid layers.

These phenomena of unmixing have many times been the subject of an investigation, especially in systems in which two colloids are found, which each in itself can give stable sols in water, but which together give rise to phenomena of unmixing. In this connection we may mention the names of several compatriots : BEYERINCK, W. P. A. JONKER, TIEBACKX, REINDERS, but also of many others, of whom we only mention WO. OSTWALD [1]) and his collaborators, in whose treatises the earlier literature is also extensively discussed.

These "phenomena of unmixing" have a superficial resemblance to those in ternary systems ; to give a better survey of the results the current triangle diagrams may be applied, but already in 1911 in a discussion at the 12th Physical and Medical Congress at Groningen one of us pointed out to what errors this analogy may give rise : for the visible separation of a (quasi homogeneous) sol into its components need, for instance, by no means be accompanied with a change of the number of phases.

The word "unmixing" having already a definite meaning, it seems desirable not to use the same word for the phenomenon in colloid systems

[1]) Koll. Z. **43**, 131 (1927); **47**, 258 and 357 (1929).

discussed here. Hence we introduce for this the word *coacervation*; the choice of this word will be discussed presently.

We wish to set forth in this paper how the phenomenon of coacervation fits into the system of our theory for lyophilic colloids. We will briefly mention also investigations which have not yet been published, and which have, indeed, not yet been entirely completed.

Experiments.

When one inquires into causes that call forth coacervation in a lyophilic sol, one is struck by the fact that these causes too bring about flocculation with sometimes very slight modifications of the circumstances, and even unmodified in the case of very analogous systems. Here follows a survey of a number of different procedures, in which (reversible) flocculation may be obtained, and with each procedure we will describe one or more cases, in which we can easily succeed in obtaining coacervation instead of typical flocculation. In what follows we denote by the terms macro- resp. micro-coacervation whether the coacervate forms a coherent liquid layer or is divided in microscopically visible drops.

1. *Flocculation with aliphatic alcohols.* The isostable proteins flocculate at the iso-electric point, when a sufficient quantity of alcohol is added. At 40—50° alcohol very easily brings about micro- and, after standing for a short time, macro-coacervation in an iso-electric gelatin sol.

2. *Flocculation with aliphatic alcohols + little electrolyte.* If to a 1 % gum arabic sol containing 50 m. eq. KCl, alcohol is added till the moment that just a permanent precipitate is formed, after which the substance is boiled up for a moment, one obtains, instead of floccules which are not to be differentiated microscopically, a beautiful coacervated system.

3. *Flocculation with a (poly-) phenol (crystalline tanning materials and tannin included).* In the immediate neighbourhood of the iso-electric point the simple phenols (phenol, pyrocatechin, resorcin, hydroquinone, pyrogallol, oxyhydroquinone, phloroglucine) flocculate isostable proteins. With all the above mentioned phenols [1]) micro- and macro-coacervation with an iso-electric gelatin sol is easily obtained at 40°. One of the factors favourable to micro-coacervation, i.e. the slow action of the flocculating agent, can be realised here by means of slow cooling, as the phenols are not active, or at least much less active at higher temperature. On slow cooling homogeneous liquid drops are formed. They become less and less aqueous, but remain homogeneous. Fig. I shows such drops, which have been formed by the cooling to 42° of a 1.3 % iso-electric gelatin solution, in which 33 % resorcine. The drops are homogeneous at 40°, but in consequence of the relatively rapid cooling during the photographing a

[1]) H. G. BUNGENBERG DE JONG, Rec. trav. chim. **48**, 494 (1929).

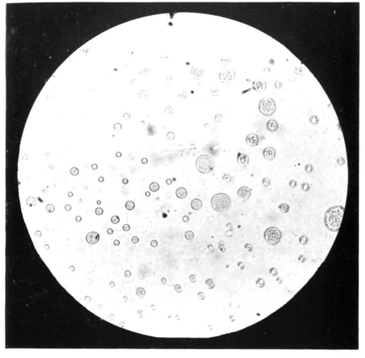

Fig. I. Gelatin + Resorcin; 65×

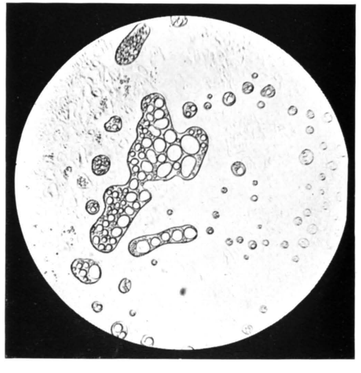

Fig. II. Gelatin + Resorcin; 110×

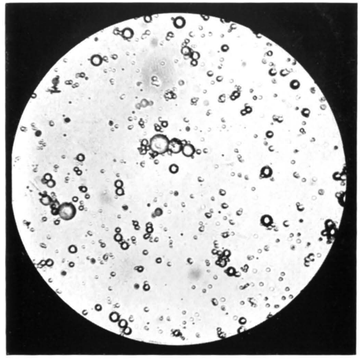

Fig. III. Gelatin + Tannin; 270×

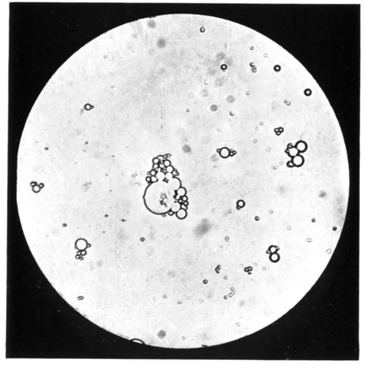

Fig. IV. Gelatin + Tannin; 270×

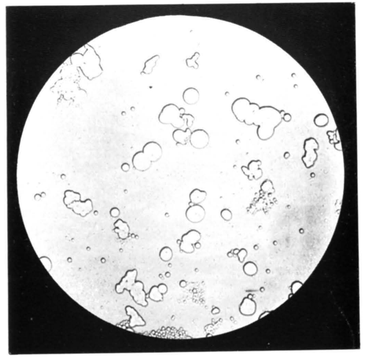

Fig. V. Casein; 220×

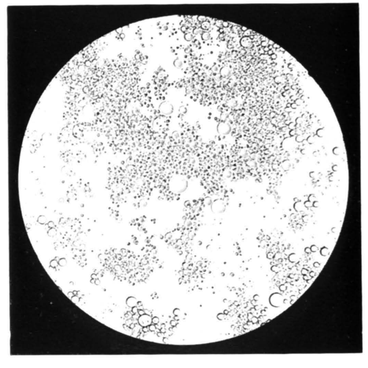

Fig. VI. Amandin; 245×

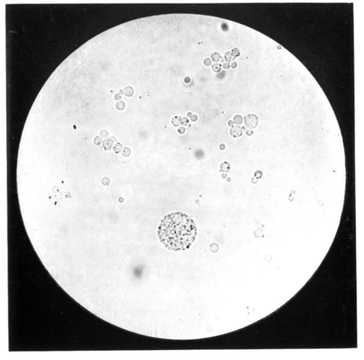

Fig. VII. Gelatin + $K_4Fe(CN)_6$; 310×

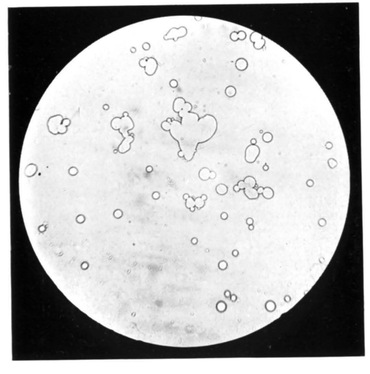

Fig. VIII. Serum Albumen + Gum Arabic; 120×

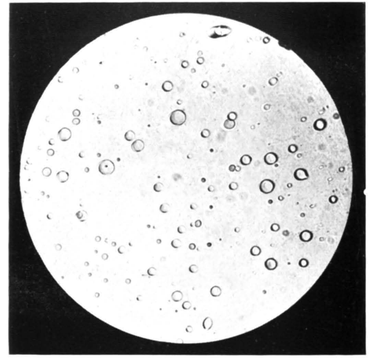

Fig. IX. Zeiss; 340×

vacuolisation has taken place. The same preparation was now rapidly heated and rapidly cooled, and then photographed (fig. II).

Just as with the simpler phenols, we also obtained coacervation in iso-electric gelatin with the following "crystalline tanning materials": d. catechin, chebulinic acid and Digalloyl glucose and further also with tannin. Fig. III shows a microphoto of this last combination, the same preparation showing a beginning of drops melting together, after it had stood for four hours at room temperature (fig. IV), a proof that at this temperature we have still to do with a liquid, though with an exceedingly viscous one.

4. *Flocculation with a (poly) phenol + little electrolyte.* Well purified agar does not flocculate with well purified tannin, a little electrolyte added brings about flocculation at once. On application of high temperature and slow cooling the micro-coacervation of the agar sol with this tannin solution succeeds easily.

5. *The salting out.* At higher temperature the micro-coacervation of the gelatin sol succeeds very easily with the following salts: Na_2SO_4 [1]), $(NH_4)_2SO_4$, $MgSO_4$, $ZnSO_4$, $HgCl_2$, Na-tartrate, Na-citrate, Na-lactate, Na-succinate, Na-formiate, $NaNO_3$, KNO_3, $NaNO_2$, $MnSO_4$, $FeSO_4$, $NiSO_4$, $Na_2S_2O_3$, K_2CO_3, NaCl, KCl, NH_4Cl. To this category belong probably also the salting out phenomena, which may be observed in not too greatly diluted soap solutions, as sodium oleate with NaCl or K_2CO_3; the "coagulate" may then also appear in the form of drops.

6. *Flocculation of iso-unstable proteins at the iso-electric point.* Addition of diluted NaOH to a caseinsol peptized in diluted HCl to near the iso-electric point brings about typical micro-coacervation at higher temperature (fig. V).

7. *Flocculation of hydrophile sols with small electrolyte concentrations.* The iso-unstable proteins are flocculated from their sols by some electrolytes in small concentrations. At 50° we obtained micro-coacervation in an alkalic casein sol by addition of a small quantity of $CaCl_2$, $SrCl_2$ or $BaCl_2$. To this category belongs also the flocculation shown by a number of carbohydrate sols (gum arabic, Carraghean- and Semen Lini mucilage) with small concentrations of electrolytes with multivalent kations. It is easy to convince oneself that hexol salt brings about micro-coacervation of the gum arabic already at ordinary temperature.

8. *Flocculation of the globulins after removal of the neutral salt.* Already OSBORNE and STRAUSS [2]) observed that in dialysis of an

[1]) WO. OSTWALD. Kleines Praktikum der Kolloidchemie, TH. STEINKOPFF. Dresden und Leipzig, 1922 p. 147.

[2]) TH. B. OSBORNE and E. STRAUSS in Handbuch der biolog. Arbeitsmethoden herausgeg. von E. ABDERHALDEN, Abt. I Teil 8, p. 413 (1922).

amandine sol in NaCl solution this globulin separates out in the form of small globules. We have followed OSBORNE and STRAUSS's prescription to the first dialysis (inclusive), and made the micro-photograph reproduced in fig. VI of the "precipitated" globulin obtained.

9. *Temperature influence in protamin sols.* KOSSEL [1]) describes that at higher temperature a "clupein sulphate"-solution is clear, but that on cooling part of it separates from it as an "oil". Similar indications for coacervation in consequence of decrease of temperature are also found for other protamins, e.g. salmin.

10. *Flocculation of proteins with some special reagents.* WO. OSTWALD (l.c.) already published an extensive investigation of the macro-coacervation of gelatin sols with sulfo-salicylic acid.

Micro-coacervation of the gelatine sol succeeded further with picric acid (by slow cooling of the sol mixture which was clear at higher temperature), with $K_4Fe(CN)_6$ + a little HCl (fig. VII), and finally with trichlor acetic acid.

11. *Mutual flocculation of a positively and a negatively charged hydrophile colloid.* The typical example of this flocculation is the combination gelatin + gum arabic + a trace of acid, first described by TIEBACKX [2]), and later studied by REINDERS [3]), who observed that at somewhat higher temperature macro-coacervation easily sets in. We found micro-coacervation with the combination of gelatin with four vegetable mucilages: Carrhagean, Semen Lini, Cetraria Islandica, Semen Psyllii. A very beautiful coacervation was presented by the combination gelatin + "Na-nucleinate".

Instead of gelatin also another protein may be taken, e.g. serum albumin, egg albumin or an aqueous extract of "Ichtyocolla". The condition for the appearance of flocculation, resp. micro- or macro-unmixing is always that the P_H is such that one component the (protein) is positively charged, and the other component has not yet lost too much of its negative charge [4]).

A micro-photograph for the combination gum arabic + serum albumin (prepared according to the THE SVEDBERG method [5]) is represented in fig. VIII. To this (for biology highly important) group we can probably take also the flocculation of "basic" albumens with serum albumin gelatin, Na-nucleinate, etc.

12. *Flocculation of the prolamine-sols by water.* In contrast with other proteins the prolamines are peptized well by strong alcohol solutions, while

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[1]) A. KOSSEL, Z. f. physiol. Chemie **22**, 178 (1896).

[2]) TIEBACKX. Kolloid Z. **8** (1911); **9** (1911); **31** (1922).

[3]) REINDERS. Chem. Weekblad **10** (1913).

[4]) H. G. BUNGENBERC DE JONG en W. A. L. DEKKER. Bioch. Z. **212**, 318 (1929).

[5]) THE SVEDBERG and BERTIT SJÖGREN. J. Am. Chem. Soc. **50**, 3318 (1928).

they flocculate on dilution with water. According to OSBORNE and STRAUSS's prescription [1]) we prepared zein and slowly added water to a 1 % sol in 70 % alcohol at 40°, till a slight turbidity arose. After the substance had been left for some time at 40°, it was slowly cooled down to room temperature. Then a micro-photograph was made (fig. IX), which again beautifully reveals micro-coacervation.

Theoretical Considerations.

Coacervation and flocculation are, accordingly, very closely allied phenomena, both require elimination of the stability factors, charge and hydration. This can, of course, best be verified by studying how the relative viscosity of the system changes, when gradually the conditions are created that lead to coacervation. We have actually examined this in all the cases we have just described, and we shall soon publish the results in the "Koll. Zeitschr.". In these investigations we have indirectly succeeded in ascertaining that an amicro- resp. ultramicro-coacervation frequently precedes the perceptible coacervation.

Hence we have found that removal (resp. great decrease) of charge and hydration are conditions both for the coacervation and the flocculation ; under these circumstances partial or entire separation of dispersion medium and dispersed phase sets, therefore, in ; in flocculation the dispersed phase presents itself as solid aggregate, in coacervation as a viscid but yet "tropfbare Flüssigkeit", a liquid, which must therefore contain the solid particles, but also liquid (i.e. water in a hydrophilic colloid). Accordingly water in the coacervate is in a condition in which it is not miscible with the large mass of the other water.

What image must we form to ourselves of the structure of the coacervate, so that this image is in harmony with that for the state of sol and flocculation ? The image must account for the fact that the particles *in the sol* continue in permanent division in their surroundings, also e.g. when water is added, but that in the *coacervate* they remain bound to the smaller quantity of water in the coacervate. Besides their condition must be thought so, that reversibly the sol-condition can be restored, as soon as charge and (or) hydration are restored. These considerations at once suggest the following image : in the coacervated phase are the particles with a limited quantity of water of hydration [2]), but they maintain themselves as primary particles ; this limited quantity of water must, therefore, necessarily be somewhat differently bound than the water of hydration in the sol-condition.

Two things are to be considered here : first the name. As we saw the coacervate is a drop or a layer, in which the particles have flocked together as bees in a swarm. Unfortunately it is hardly possible to form a word from

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[1]) TH. B. OSBORNE and E. STRAUSS, loc. cit. p. 443.

[2]) For the sake of simplicity we confine ourselves for the moment to water; one may of course, read every time equally well solvation — and intermicellar liquid.

the Latin word : *examen* that does not lead to comical associations ; the Greek word *smenos* is unsuitable, and the German word Schwarmbilding has already been taken possession of in the theory of the mesomorphous phases. We, therefore, chose the Latin *acervus* = aggregation, heap, and wish to express the previous combining in the prefix *co*.

In the second place we should try to make it clear to ourselves what these two kinds of water bonds are ; in this we may make use both of classical conceptions of VAN BEMMELEN and of modern analogies in the theory of the electrical double layer (GOUY).

For certain reasons the solvation of the lyophilic colloids suggests the word "solvation mantle" to the investigator. Not that it would a priori be certain that all the water, like a mantle, would quite envelop a solid nucleus ; on the contrary, it is possible to advance objections to such a rigid image. But part of the water *must* surround the particle like a mantle, if we wish in any way to account for the fact that the solvation acts as a stabilizing factor ; for on discharge of lyophilic colloid particles it often prevents the coalescense, which might be expected on the ground of the free surface energy. Hence the solvation must change something in this surface, and this so, that this free surface energy does not manifest itself. It is for this reason that the conception according to which the hydration mantle would surround the particle as a sharply defined phase, does not satisfy us ; for in a collision these sharply defined mantles would come in contact, and it is not easy to see why they should not unite.

We should be more inclined to suppose the original surface energy between particle and dispersion medium to be practially annihilated by the solvation. A concrete boundary can, therefore, not exist ; we must rather assume with VAN BEMMELEN [1]) that the solvation consists of liquid bound more and more loosely towards the outside, and finally passes imperceptibly into the perfectly free liquid of the dispersion medium. A real boundary can scarcely be indicated, we, therefore, propose to denote such a solvate mantle by the term of *diffuse solvation mantle*.

When, therefore, by the aid of EINSTEIN's formula we calculate from the viscosity of a sol, how many cc. of solvate liquid are bound by 1 gr. of colloid substance, we obtain a number that is of course quantitatively wrong, for the application of EINSTEIN's formula assumes a rigid particle, i.e. in our case, therefore, a particle with rigid solvate mantle with a concrete limitation with regard to the dispersion medium.

Rigid is, in our opinion, possibly only the very first molecule layer round the particle, but the further we get towards the outside, the greater becomes the mobility of the solvate molecules, so that finally the solvate mantle imperceptibly passes into the free dispersion medium. We can now readily see that the preparative process that eventually renders possible the combination of the particles to a coacervate, can only consist of the transition of

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[1]) J. M. VAN BEMMELEN, Z. anorgan. Chem. **13**, 234 (1896).

the diffuse solvate mantle into a solvate mantle with a concrete boundary at its periphery. We shall denote such a solvate mantle by the term of *concrete solvation mantle*. To make the matter clearer we may point out that such a condition of the solvate mantle differs from a diffuse solvation mantle only at its periphery. In its inner layers we still suppose it to consist of concentric shells of decreasing degree of binding, i.e. increasing mobility of the solvate molecules, hence still far from rigid.

The mechanism of the process is in outline represented in fig. X. Symbol A represents a particle with a diffuse solvate mantle (dotted periphery).

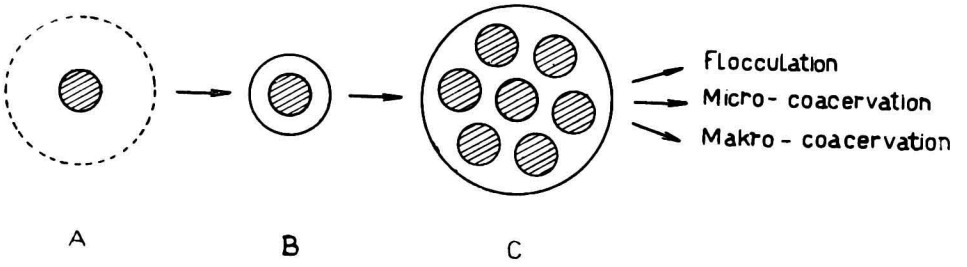

Fig. X.

According as desolvation sets in, we must assume that at the same time with the shrinking of the solvate mantle, this becomes more and more concrete at its periphery. Let symbol B now represent such a particle, in which, to indicate the concrete boundary, we have no longer dotted, but traced the periphery of the solvate mantle. In the case of sufficient free surface energy of the mantle boundaries such particles can now combine to a coacervate with their solvate mantles.

According as the process of combining has advanced in a given time, we get typical flocculation, micro- or macro-coacervation.

We see accordingly that according to our view the original particles in the coacervation process have only combined with their solvation mantles. This accounts for the fact that coacervations have the nature of a liquid, because the solvate liquid should not be considered as rigid, but consists of liquid which in a more or less degree has lost its free mobility. We have seen that the most obvious preparation for the coacervation process is desolvation. It can be brought about by change of the material constitution of the dispersion medium (categories 1, 2, 3, 4, 5, 8, 10, 12).

In other cases it can be brought about by decrease of charge (categories 6, 7). It is not at all strange that also in the latter case a diffuse solvate mantle could pass into a concrete one, considering the well known influence of charge on the surface tension. It is not quite so easy to see that if two opposite charges are balanced, the mantles can also become concrete (category 11).

So we see that the finer mechanism through which a diffuse mantle can pass into a concrete one, can be very different, and it will be reserved for

later researches, to study this mechanism more thoroughly. The conception of the nature of the coacervation process set forth here, now enables us to study the properties of the solvate liquid more closely also experimentally. For the equilibria between the originally free particles and the dispersion medium have their continuation in the equilibria between the coacervate and the equilibrium liquid.

Both liquid layers are now accessible to chemical analysis (i.e. material constitution) as well as to physicochemical investigation. Researches in this direction have already been started.

Dr. H. L. BUNGENBERG DE JONG and Miss W. KLAAR have, as we heard from them, studied analogous phenomena simultaneously with us but quite independently. They investigated especially the gliadin sol and came to conclusions similar to ours.

Biological Significance of the Coacervates.

When we pose the question whether the coacervates have biological significance, we must for the present observe all possible reservation.

On closer inspection of the ground mass of the protoplasm, it strikes one that this has some properties in common with the coacervates, so that there is a possibility that this ground mass may be considered as a coacervate or as a system of coacervates. For the protoplasm is often considered as an isotropic, liquid, concentrated colloid water system limiting itself [1]), and often presenting a tendency to vacuolisation [2]) (compare fig. II).

Coacervate drops further exhibit a tendency to absorb solid particles e.g. carbon and indigo carmine particles. They often assume a very intense colour with several pigments, (e.g. coacervate drops of gum arabic + gelatin, with lithium carmine, Eosine, Nigrosine, and they also strongly absorb Collargol).

Whether the points of resemblance summed up here are only a coincidence, we cannot yet decide.

It certainly seems justifiable to assume that for the structure of living matter, and also for its outer limitation, not only sols and structure elements (gels, fibrils etc.) have significance, but that by the side of them, likewise coacervates play a part.

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[1]) W. LEPESCHKIN, Kolloidchemie des Protoplasmas, JULIUS SPRINGER, Berlin. 1924, p. 142.

[2]) L. V. HEILBRUNN, The Colloid Chemistry of Protoplasma, Gebr. BORNTRAEGER, Berlin 1928, p. 233—255.