

Chemistry. — *Coacervate sols and their relation to the theory of lyophilic colloidal stability.* By H. G. BUNGENBERG DE JONG and P. V. D. LINDE. (Communicated by Prof. H. R. KRUYT).

(Communicated at the meeting of March 30, 1935.)

1. *Introduction and starting-material.*

In the extremely hydrophilic sols capillary electric charge and hydration play the part of more or less independent stability factors¹⁾. Thus dilute sols of Na-nucleinate, gum arabic, agar, and amyllum solubile (MERCK) (all of which are negatively charged) may be mixed with 2 or 3 volumes of alcohol (desolvation) without flocculation taking place. A trace of electrolyte, however, flocculates them.

It is always absolutely necessary that the sols are diluted and free from electrolyte impurities. Nevertheless, it appears that only the two first mentioned sols on mixing with alcohol remain almost perfectly clear but that, under otherwise comparable conditions, the sol-alcohol mixtures of the others become opalescent.

In the case of the agar sol it can be proved that the particles visible in these opalescent sols are not the original sol particles, but already aggregates of them¹⁾. The stability theory mentioned above consequently does not seem to be fully applicable here, since the dehydration, in spite of the negative charge, leads to a union of particles. However, it retains its validity in that the "flocculation" remains restricted to ultra-miscropic dimensions. On addition of alcohol to the $\frac{1}{7}$ per cent agar sol an opalescence suddenly sets in, but hardly increases in the course of time, or at least exceedingly slowly.

In sufficiently electrolyte-free sols of amyllum solubile the above-mentioned phenomenon occurs to a larger extent, and a slow increase of the opalescence in the course of time is clearly perceptible.

In the glycogen sol, which shows these phenomena to a still higher degree, we found a suitable object of investigation, where it was possible to explain the nature of these aggregations occurring with alcohol, and at the same time to understand why particularly in the order: nucleinate — gum arabic — agar — amyllum solubile — glycogen the above-mentioned phenomena highly increase.

As starting-material we made use of a glycogen preparation of SCHUCHARDT, practically free from N, but containing a trace of Ca. This impurity, however, appeared to be not the cause of the phenomena described below, which continue to exist after the practical removal of Ca.

For this removal first a large amount of Na Cl (e.g. 10 per cent) was dissolved in a 5—10 per cent glycogen solution, and the glycogen

¹⁾ H. R. KRUYT and H. G. BUNGENBERG DE JONG, *Kolloid chem. Beihefte* **29**, 454 (1929).

precipitated by addition of a sufficient quantity of alcohol (while care is taken that the NaCl itself is not yet deposited). This treatment, which removes the Ca, is 2 or 3 times repeated. Subsequently the glycogen solution is repeatedly precipitated exclusively with a sufficient amount of alcohol, in order to liberate NaCl. Contrary to the behaviour of gum arabic (in the preparation of Na-arabinate), here addition of alcohol keeps depositing the colloid during continued removal of NaCl, in which case, however, soon fairly high concentrations are required (e.g. 1 vol. sol + 5 to 7 vol. alcohol).

2. Behaviour of the glycogen sols with regard to rising alcohol concentrations.

Starting from 3 sols, we made mixtures, keeping the final glycogen concentration constant but varying the relation water-alcohol:

- I) x cc alcohol 96% + $(48 - x)$ cc dist. water 2 cc $\frac{1}{2}$ % sol of glycogen
 II) x " " + $(35 - x)$ " " " 5 " " " "
 III) x " " + $(35 - x)$ " " " 5 " 1.7% sol of purified glycogen.

After a certain interval of time (I 20 hours, II 5 hours, III 16 hours)

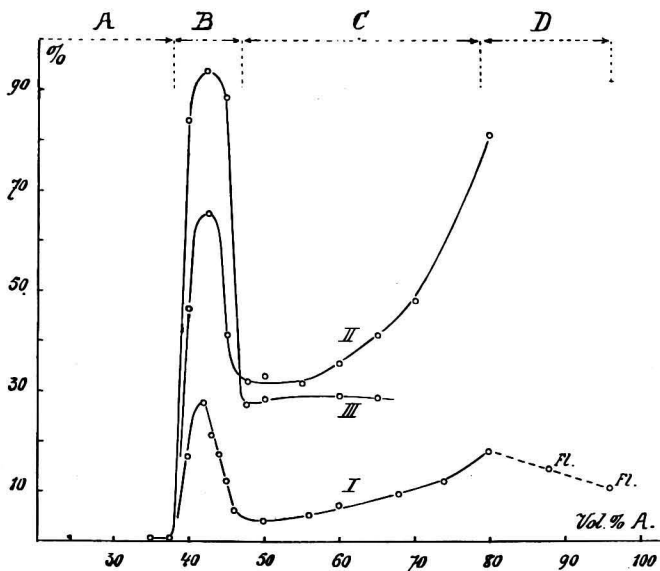


Fig. 1

the turbidity was measured in a layer of 10 mm with the aid of MOLL'S extincometer. These turbidities have been graphically represented in fig. 1 as function of the alcohol concentration. Although I, II and III have very different final glycogen concentrations (I=0.02%, II=0.06%, III=0.21%), further in I and II the Ca-containing glycogen is used, in III the purified preparation, yet all three curves display the same

character. Without being able to indicate distinct boundaries, we can distinguish 4 ranges of the alcohol concentrations:

- A. 0—37 volume % alcohol, in which the mixtures remained clear.
- B. 37—47 volume % alcohol, between which, with a maximum at about 42—43 volume %, a strong turbidity gradually developed.
- C. 47- circa 80 volume % alcohol, where a strong opalescence is present (in a thin layer the mixtures are more or less transparent at 50—60 vol. %), gradually giving way to a strong turbidity upon increase of the alcohol concentration.
- D. 80 volume % and higher, where the mixture is flocculated (the boundary between C and D cannot be distinctly marked).

3. Coacervation of the glycogen sol with alcohol.

On microscopic investigation the cause of the strong turbidity in *B* appears to be a large amount of small coacervate droplets. Compare fig. 2, representing the coacervation of the glycogen sol at 40 volume % alcohol (in order to accelerate the coacervation we chose the final sol concentration higher here: 10 cc 0.56 % sol of purified glycogen + 7.2 cc 96 % alcohol).

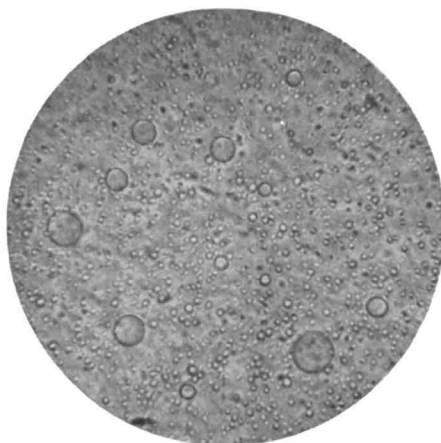


Fig. 2
Glycogen + 40 % alcohol (300 X).

It is characteristic of range *B* that the turbidity here develops only slowly to the high value. The gradual increase of turbidity in range *B* is due to a continued fusion of the first submicroscopic coacervate droplets into larger ones. The average size of these droplets after an equally long interval of time consequently increases strongly with the sol concentration.

The fact, however, that the negatively charged glycogen sol already coacervates with alcohol seems at first sight contradictory to the stability

theory mentioned in the introduction. However, it should be remembered that this theory would also apply to this case, if namely the electric charge after desolvation is not sufficient to ensure the sol condition any longer as the only stability factor.

Determinations of the reciprocal hexol number supply a possibility to characterize negative colloids with regard to their charge numerically.

They showed indeed that glycogen possesses a very small charge (of the order of $100 \times$ so small as gum arabic), so that the coacervation of the glycogen sol by means of alcohol in the absence of electrolyte is not in contradiction with the stability theory mentioned above.

4. *Influence of the increasing desolvation on the coacervation process.*

The rapidity of formation of microscopically visible coacervate droplets depends on *a.* the rapidity with which the electrically insufficiently protected sol particles unite to smallest submicroscopic coacervate droplets, *b.* the rapidity of fusion of these droplets into larger microscopic drops.

It is characteristic of the desolvation of negative hydrophilic sols with alcohol that it highly increases in an often relatively narrow range of the alcohol concentrations (e.g. in case of the agar sol from 40—60 per cent alcohol).

The result of this will be that the rapidity *a.* will be practically zero below a certain alcohol concentration, above, it will become finite and soon strongly increase. The rapidity *b.*, closely connected with the richness in solvate of the coacervate drops, will on the contrary be maximal where the rapidity *a.* is still very small, but it will diminish very strongly in a comparatively small concentration range (owing to the small amount of solvate these coacervate drops become more or less rigid, which greatly impedes their fusion).

All this entirely agrees with the occurrence of a considerable turbidity top in a comparatively narrow range of the alcohol concentrations in fig. 1 (range *B*) and with the manner in which it develops in the course of time.

The mixture with 40 volume per cent alcohol practically does not even show any opalescence directly after mixing, but opalescence occurs gradually, followed by considerable turbidity. Here, consequently, the rapidity *a.* is small with regard to *b.* The mixture of 50 vol. per cent, on the other hand, is opalescent directly after mixing, but this opalescence increases only slightly in the course of time. In this condition of desolvation, therefore, it is the rapidity *b.* that is small with regard to *a.*

However, since the process of formation of primary aggregates from the sol particles at any reasonable magnitude of the rapidity *a.* will cease very soon, it is to be expected that the top of the curve in range *B*, upon gradual development of the considerable turbidity, in the course of time

will shift to the lower alcohol concentrations. Fig. 3 shows what may be expected here and indeed has been found experimentally.

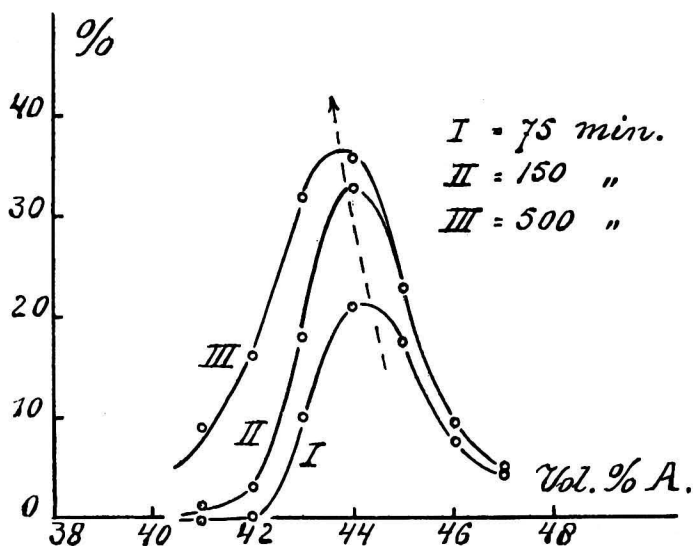


Fig. 3

5. Coacervate sols.

The opalescent mixtures, formed in the left hand part of range C in fig. 1, on microscopic examination display non- or hardly visible particles. From the above it is clear that the glycogen has separated out here in the form of solvate-poor submicroscopic coacervate droplets. It is due to the small amount of solvate that the fusion into larger coacervate drops is highly impeded (a much longer contact is needed here than on an average takes place at the meeting of the droplets, as the result of their Brownian movement).

Their small negative charge (in the maximum of B no change of polarity takes place, and the flakes in D also are still negatively charged) now acts rather protectively against such a superficial adhesion, which as an introduction to the gradual fusion has to take place; at any rate during a certain minimum interval of time.

A sufficient discharge (CaCl_2 1 m. aeq. to 50 volume per cent alcohol, the flakes still having a very feeble negative charge) however leads at once to flocculation. The submicroscopic droplets flocculate superficially into flakes. The contact of the droplets now brought about still permits the very gradual fusion into larger ones. The microscopic image of the flake is in agreement with this: the flake consists of an aggregate of connected, rounded little lumps, showing various stages of fusion.

As is shown in fig. 1, however, the turbidity gradually increases with the rise of the alcohol concentration, and, without a distinct boundary, passes into range D, where the glycogen is flocculated. Microscopically

these flakes appear to have a different character: they consist rather of granular masses, i.e. of very solvate-poor coacervates, which no longer fuse perceptibly in the course of time.

Consequently, as might be expected, the rapidity *b*. diminishes more and more upon increase of the desolvation and finally becomes practically zero. The gradual increase of turbidity in the right hand part of range C and the flocculation in range D can, therefore, be based only upon a decrease of the electric protection of the submicroscopic coacervate droplets, owing to the rising alcohol concentration.

A similar secondary action of the desolvation medium, alcohol, on the double layer (attaching the opposite ions to the inner coating of the double layer) was known to us from previous investigations.

The mixtures in the left hand part of range C may be regarded as colloid systems of higher order. They behave as negatively charged hydrophobe sols; their kinetic units, however, are already colloid systems themselves (solvate-deficient ultramicroscopic coacervate droplets).

We suggest for them the term *coacervate sols*.

They owe their (relative) stability to the capillary electric charge of their kinetic units (capacity to be flocculated by means of very low electrolyte concentrations).

6. *Unequal distribution of the charge on the surface of the sol particle as condition for the formation of coacervate sols.*

The following question still remains to be answered: How is it that the capillary electric charge of the submicroscopic coacervate drops is indeed sufficiently large to ensure relative stability for the coacervate sols? Their kinetic units have namely originated as the result of the very fact that the electric protection of the original sol particles is insufficient in the given condition of desolvation.

If, however, it is taken into consideration that the reciprocal hexol number of glycogen is very high (i.e. the charge per gram glycogen is very small), there is every chance of an unequal distribution of the charge on the surface of the sol particle. It is also very likely, then, that not all particles carry an equally high charge. At coacervation a certain fractionation will then take place. The less charged particles unite first; however, as more highly charged particles follow, the rate of coacervation decreases. Moreover, the particles still joining will be taken up in such a manner that they turn their charged spots as much as possible outward¹). The surface of the submicroscopic coacervate droplets then consists of the charged spots of the united sol particles, which causes the formation of a relatively stable coacervate sol.

The explanation given here accounts at the same time for the fact that in the order Na-nucleinate — gum arabic — agar — amyllum solubile

¹) H. R. KRUYT. Proc. Acad. Sci. Amsterdam 32, 849 (1929).

(MERCK) — glycogen, the tendency to form coacervate sols in comparable concentration and absence of electrolyte impurities) is absent in the first two, present in agar, but only occurs conspicuously in the two last substances.

The charge per gram colloid substance indeed diminishes very strongly in the order indicated above¹), in other words, the chance of the occurrence of an unequal distribution of the charge on the surface of the sol particle strongly increases. Supposing that the charge per gram for the Na-nucleinate = 1, we find from the determinations of the reciprocal Hexol numbers for the others, approximately:

Na-nucleinate	gum arabic	agar	amylum solubile MERCK	glycogen
1	0.3	0.15	0.01	0.004

We have now to go back to the fact that range C of the coacervate sols is preceded by a range of macro-coacervation, in which the irregular distribution of charge does not impede the continued fusion of the submicroscopic coacervate droplets into larger ones (range B).

Contrary to the charge, the solvation will be equally distributed on the surface of the particle. In conditions of slight desolvation, such that a very slow union of the sol particles into submicroscopic coacervate droplets is possible, the unequal distribution of the charge will not yet greatly influence the manner of union of the particles. Owing to this fact the surface of these droplets will differ only slightly from that of the sol particles, so that mutual contact (followed by fusion into larger drops) remains possible.

In comparison with the solvate-poor coacervates in range C, the coacervates here are much richer in solvate liquid, which moreover causes the charged spots on the surface of the drops to lie farther apart.

Summary.

1. The behaviour of glycogen sols with regard to alcohol was investigated. In a narrow range of concentration of the alcohol slow coacervation occurs, in slightly higher concentrations, however, strongly opalescent mixtures are formed, and in very high alcohol concentrations the glycogen is flocculated.

2. The very low charge (very high reciprocal hexol number) of glycogen explains the observed phenomena.

3. The opalescent mixtures may be characterized by the term *coacervate sols*. They behave as hydrophobe sols carrying a small negative charge. Their kinetic units, however, consist of submicroscopic solvate-poor coacervate droplets.

4. The relation of the distribution of the charged spots on the surface

¹) H. G. BUNGENBERG DE JONG, P. VAN DER LINDE and A. DE HAAN. Recueil trav. chim. 54, 17 (1935).

of the primary particles to the relative stability of these coacervate sols has been discussed.

5. As the charge per gram colloid becomes larger (the reciprocal hexol number smaller), the chance of an unequal distribution of the charged spots on the surface of the particle decreases together with the tendency in suitable alcohol concentrations to spontaneous coacervation and to formation of coacervate sols.

Chemistry. — *Oriented coacervates and their bearing upon the formation of colloid-crystals.* By H. G. BUNGENBERG DE JONG. (Communicated by Prof. H. R. KRUYT).

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1. Introduction.

Without special precautions being taken, completely liquid coacervates of Amylum solubile MERCK or KAHLBAUM are as a rule hard to realize. A high temperature is always needed. For example, 100°, with propyl alcohol and a little neutral salt. Upon cooling the drops soon become rigid and assume irregular shapes. Similarly with aethyl lactate as a desolvating agent coacervation may be obtained at a high temperature. However, coacervates are also possible at a lower temperature, but then one is confined to a very narrow range of comparatively low alcohol concentrations, in which case phenomena occur as have been described in the preceding article regarding the glycogen sol. The behaviour of the amyllum sols, however, is more complex.

Together with a colloid fraction, corresponding in its colloid chemical behaviour with glycogen, they also contain a fraction differing considerably from it. Under conditions that coacervation might be expected, this fraction does not separate in droplets but in a form strongly deviating from the globular shape.

For a close examination of this form of separation the preparations of KAHLBAUM and MERCK are not very suitable. From their sols the two fractions separate together too easily. With *Sizing Starch "Hercules"* of the *Huron Milling Company*, on the other hand, it is possible to effect the separation of the corpuscles mentioned above without coacervate drops being formed simultaneously. In the following, therefore, we restrict ourselves mainly to the sols of this preparation (indicated as "Huron sols") and at the end of this paper refer briefly to the behaviour of the sols of the preparations of MERCK and KAHLBAUM.

2. Behaviour of the sols of Huron starch, desolvated with alcohol, upon heating; formation of "platelet sols".

The 2 per cent sols of the Huron preparation are sufficiently electrolyte-free to be capable of being mixed with alcohol without flocculation.