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).

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

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 amylum 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, desolvatated 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.

From a certain alcohol concentration onwards these mixtures are slightly opalescent. Regarding the change of this opalescence in the course of time there is a certain analogy with the behaviour of glycogen[1]). Whereas at higher alcohol concentrations the opalescence after 1 day has only slightly increased, this is the case to a larger extent in the concentration range (at 50 volume % alcohol) on the boundary of the clear and opalescent mixtures, although microscopically visible coacervate drops are not obtained here.

If 3 vol. alcohol are added quickly to 2 vol. 2 % cold Huron starch sol, the result is a feebly opalescent mixture, which is stable at room-temperature. Upon heating this mixture in a water-bath during about 15 minutes at 70°, very striking changes occur. Gradually a "streamer" effect sets in: at the slightest convection currents silky shiny streamers are formed in the liquid. On subsequent cooling to room-temperature the streamer effect remains. Ultramicroscopic examination of the liquid shows that it is completely filled with small, extremely thin plates.

If we bring about this "platelet sol", avoiding liquid currents, we may succeed in obtaining plates with a diameter of 5—6 μ. It is then possible to state with certainty that the plates are more or less regular hexagons.

Besides these plates very small granules are always found as well in the liquid. When putting a series of tubes, all containing an equal amount of Huron sol and filled up to the same final volume with varying quantities of alcohol and water, in the water-bath at 70°, we are struck by the fact that in a certain range of the ratio alcohol-water the streamer effect is optimal and the largest plates are formed. This lies about at the ratio indicated above. At very high alcohol concentrations the formation of the plates becomes minimal.

Some observations may follow here regarding the influence of small amounts (to 10 cc of the sol mixture 1 drop or a small crystal) of added substances on the formation of the platelet sol at 70° (judged by the extent of the streamer effect obtained).

No influence: resorcin, tannin, menthol, tributyrin.

Slight decrease: triolein. No platelet sol: oleic acid (if 1 drop of oleic acid is added to an already formed, the latter continues to exist). Electrolytes have a strongly decreasing or impeding influence, even in concentrations which do not flocculate the cold sol mixture but only intensify the opalescence.

3. *Properties of the platelet sols.*

Similarly to the opalescent sol, from which the platelet sol originates on heating, the platelet sol also flocculate with small amounts of neutral salt.

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[1]) See the preceding paper.

Electrophoretically it may be ascertained that the plates carry a negative charge. In their relative motion with respect to the liquid they show the tendency to place themselves in such a position that the direction of the relative motion coincides with the planes of their hexagons. (The streamer effect, on shaking, is also based on this common orientation). The amount of light transmitted through a certain layer also changes when perpendicularly to it an electric field is placed. Thus we found a light-scattering of 52 per cent for a plate-suspension formed according to the method described above, with the aid of Moll's extinctometer in a cuvette 10 mm thick. On application of an alternating current field (220 V. at a distance between the electrodes of circa 16 cm) this scattering diminishes in 70 seconds to 36 per cent, and after switching off the field the scattering returns in 140 seconds to its original value (52 per cent).

Although the plates consequently carry a capillary electric charge, the platelet sols are stable only to a small extent. Shaking for some time effects complete flocculation of the plates. The cooled platelet sol does not directly lose its streamer effect on dilution with water, though it becomes weaker in the course of time, and more rapidly as the dilution increases. However, on heating of the platelet sol diluted with water, the streamer effect disappears at once.

For the behaviour of the plates with respect to iodine and ptyalin see below.

4. *Fractionation of the Huron sol.*

After the mechanical coagulation the plate-substance may be centrifugated off, washed out with an alcohol-water mixture (3 : 2), and finally washed out repeatedly with alcohol and ether and subsequently dried. In this way we ascertained that only 18—19 per cent of the Huron starch had separated out in the form of plates.

The colloid fraction which did not separate from the alcohol-water surroundings may be easily flocculated with a little KCl.

This fraction is soluble in water and, at a well chosen alcohol concentration with a little neutral salt, may separate out in distinctly liquid coacervate drops.

The centrifugate, (damp platelets) changes into an opalescent sol, if water is poured on and shaken (outward appearance of a glycogen sol). On heating the opalescence disappears. It is remarkable that, if we try with alcohol and heating once more to obtain a platelet sol out of this, we generally succeed very badly: if plates are formed at all, they are far less beautiful and smaller than in the original sol.

The glycogen-like sol of the plate-substance only shows a slight violet iodine reaction; however, after boiling for a short time and cooling the blue iodine reaction is obtained. Before heating ptyalin affects this substance only slightly (to be ascertained by subsequent boiling and after

cooling application of the iodine reaction), after heating this takes place very rapidly.

In this respect there is analogy with the flocculation of Merck's amylum sol, which is formed after digestion with ptyalin. There also, after boiling, we obtain a system which again shows the iodine reaction and can be further affected by ptyalin [1].

5. *Structure and properties of the plates; change into coacervates.*

On ultramicroscopic examination of the platelet sol very small granules are always observed together with of the plates. The plates themselves also have a fine granular appearance and frequently show clearly concentrically situated hexagonal zones with a slightly differently reflecting capacity. This seems to indicate that the plates originate by concentric apposition.

If a platelet sol is left for a day in a beaker on the bottom of which lies a cover-glass, a layer of sedimented plates is formed on it. Under crossed nicols an exceedingly feeble double refraction is then observed.

We do not consider it likely that the plate should be a crystal in the ordinary sense of the word, though it is very likely indeed that the amylum micells themselves are crystalline.

The nature of the plate is rather closely related to that of a coacervate. After rubbing the cover-glass under strong pressure to and fro on the object-glass, we observe that the plates have coalesced into liquid rolls. Only at the edges of these rolls accumulations of granules are still observed, but for the rest they are optically empty and not to be distinguished from a coacervate [2].

6. *The plates as oriented coacervates; irreversible and reversible formation of the plates.*

We consider it very likely that the opalescent hydrophobe sols, serving as starting-material for the preparation of the platelet sol, are coacervate sols. Contrary to the coacervate sols of glycogen, however, the micells, in consequence of a pronounced unequal distribution of the charged spots on their surface, must have coalesced here under observance of a certain prefered orientation.

If we assume, for want of further data, that these oriented coacervate

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[1] J. R. BROEZE. Bioch. Z. **204**, 286 (1929).

[2] In solvate-poor coacervates, separating out in the form of granular flakes, the liquid nature may be demonstrated in the same way, e.g. in the complex coacervate lecithin + gelatin at P 3.5. Cf. H. G. BUNGENBERG DE JONG and R. F. WESTERKAMP, Bioch. Z. **234**, 367 (1931).

units are globular, the charged spots must be situated chiefly at two opposite poles, while in a zone round the equator these stabilizing factors are wanting.

These coacervate units will now in principle be able to unite and fuse along their equators. Round one unit six others may be arranged, etc., and in this manner we can imagine the hexagonal, exceedingly thin coacervate plate to have originated by apposition in one plane. In this way the charged spots of the coacervate units remain free and are then present on the upper and lower surface of the plate.

This formation of the plates, however, does not take place (or exceedingly slowly) at room-temperature. Why exactly is rise of temperature needed for the formation of the platelet sol? The answer to this question is already included in principle in the discussion of an analogous phenomenon in the coacervate sol of glycogen. Although this latter coacervate sol is more or less stable at 50 vol. per cent alcohol, addition of so much water that the alcohol concentration is decreased to 42 vol. per cent causes progressive coalescence of the ultramicroscopic coacervate droplets into larger ones.

It has earlier been shown that the desolvating action of alcohol is clearly a function of the temperature: it decreases, at a constant alcohol concentration, if the temperature is raised.[1])

Heating at a constant alcohol concentration will consequently from the view-point of stability have the same result as decrease of alcohol concentration at a constant temperature.

In both cases, therefore, it will be possible for the ultra-microscopic units to coalesce to larger ones, as has already been discussed in the preceding paper for the coacervate sol of glycogen.

However, since in glycogen no pronounced prefered orientation of the micells are present, whereas they occur in amylum sol, in the first case coalescence into larger coacervate drops will take place on all sides, in the Huron sol on the other hand the coalescence into plates.

This transition of coacervate units into larger coacervate drops (glycogen) and plates (Huron-amylum) respectively is, of course, irreversible on reversal of the factors enabling the coalescence (i.e. increase of the alcohol concentration with glycogen and cooling with the Huron-amylum respectively).

The explanation given above leads to the conclusion that the plates must be able to originate also reversibly, viz. by coalescence of the sol particles. At certain alcohol concentrations, lower than those indicated above as optimal (2 vol. 2 per cent sol and 3 vol. alcohol) we observe the streamer effect setting in exactly by means of *cooling* of the mixture of the sol and alcohol, which is clear at 90°.

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[1]) L. W. J. HOLLEMAN, H. G. BUNGENBERG DE JONG and R. S. TJADEN MODDERMAN, Kolloid Beihefte **39**, 334 (1934), cf. p. 349—350.

At 90° the surroundings are still too feebly desolvating, i.e. the sol micells can not yet coalesce. On cooling, however, the desolvatation increases and, if the temperature is sufficiently low, the sol particles themselves can coalesce to oriented coacervates, i.e. to plates. On heating this platelet sol to 90°, of course we again obtain a perfectly clear sol.

Here also a parallel may be drawn with the glycogen sol, in which case, at a constant temperature increase of the alcohol concentration of 35 to 42 vol. per cent, the coacervation becomes possible, but disappears reversibly on addition of water.

The above may be represented in the following scheme:

System	Decreasing solvatation		
Glycogen	sol particles $\underset{\text{(water)}}{\overset{\text{(alcohol)}}{\rightleftarrows}}$	coacervate drops $\overset{\text{(water)}}{\longleftarrow}$	ultramicroscopic coacervate units
Amylym solubile (Huron)	sol particles $\underset{\text{(heating)}}{\overset{\text{(cooling)}}{\rightleftarrows}}$	plates (oriented coacervates) $\overset{\text{(heating)}}{\longleftarrow}$	ultramicroscopic oriented coacervate units

In comparison with the corresponding system in glycogen (see scheme) the platelet sols exhibit an increased stability, owing to the slightly charge on upper and lower surface of the plates. After sedimentation part of the plates may on shaking again be brought in free suspension: Coalescence of the plates, accumulated in layers in the sediment, is impeded by the presence of the charged centres. Vigorous shaking, however, enforces this contact: the plates coalesce (mechanical coagulation). It is similarly understandable that a little electrolyte at once occasions flocculation of the platelet sol.

7. *Formation of platelet sols with other desolvating agents and in other preparations of Amylum solubile.*

Starting from sols of the Huron preparation, we can also obtain platelet sols with methyl- and isoproply alcohol, although the plates do not become so large as in case of aethyl alcohol. Streamer effects were further obtained with ethyl urethane in a high concentration. With acetone (5 cc 2 per cent Huron sol and 5 cc acetone) turbidity sets in slowly on heating, but ultramicroscopically it appears that plates are not formed but trachyte bundles.

Heating with tannin does not result in the formation of plates. This is

not wholly unexpected, since tannin, at the high temperature needed for the formation of the platelet sol, has no or hardly any desolvating effect on amylum solubile.

Finally, we also succeeded in obtaining the streamer effect with other preparations of amylum solubile. The 2 per cent sol of Amylum solubile MERCK apparently contains a sufficient amount of electrolyte impurities for the mixture of 2 vol. sol and 3 vol. alcohol to flocculate at once. If this precipitate is centrifugated and once more dissolved in distilled water, the streamer effect is obtained on heating the mixture of 2 vol. sol with 3 vol. alcohol. The platelet sol may likewise be flocculated again by vigorous shaking.

Even without previous removal of electrolyte impurities the streamer effect may be obtained in MERCK's preparation as well as in that of KAHLBAUM, but only at coordinated values of alcohol concentration and temperature. For example: in the series of mixtures of the composition 10 cc 2 per cent sol (MERCK) + x cc alcohol at 90° the mixtures with $x = 1$ to 7 inclusive are clear, with $x = 8$ on the other hand the mixture reveals the streamer effect.

This series being slowly cooled to room-temperature, the streamer phenomenon is first observed at $x = 7$, then at $x = 6$, and finally at room-temperature at $x = 5$, while $x = 3$ and lower remain clear.

Simultaneously with the cooling, however, the mixture with $x = 8$ shows increasing turbidity and at room-temperature it is flocculated. The same changes occur at $x = 7$ and $x = 6$ after passing the typical stage of the platelet sol, and every time to a smaller extent.

The order of the phenomena may be understood from the above discussion of the Huron sol. The plates originate here (reversibly) from the sol particles themselves, whenever at sufficient desolvation (sufficient cooling) the oriented coacervation becomes just possible. The electrolyte impurities present, however, flocculate the platelet sol at a slightly stronger desolvation (further cooling).

Finally we mention the behaviour of the sol-alcohol mixtures at room-temperature:

Amylum solubile Kahlbaum. In mixtures of the composition 10 cc 2 per cent sol-x cc alcohol 96 per cent-(10-x) cc distilled water maximal instability occurs at $x = 6$ and $x = 7$. Although these mixtures immediately after preparation are only opalescent, they are maximally flocculated after 16 hours. On microscopic examination the sediment at $x = 6$ appears, besides many small granules, also to contain fairly large globular coacervate drops.

Together with the plate fraction which leads to oriented coacervation another colloid fraction is thus present, corresponding in its behaviour with glycogen, — maximally large coacervate drops in a narrow range of alcohol concentrations.

In *Amylum solubile Merck* the phenomenon is even more striking. With

mixtures composed in the same way as in KAHLBAUM's preparation, the mixture with x = 10 is after 16 hours more or less transparent in a thin layer (coacervate sol), the mixtures with x = 5 and 6 on the other hand are maximally flocculated.

In the sediment with x = 5, besides a finely granular precipitate, also very peculiarly formed coacervate drops are found, making the impression of being centrally pierced. In all probability the coacervate has been deposited on the periphery of a centrally situated, extremely thin plate, owing to which fact these bodies have obtained the appearance of lifebelts.

The two preparations of Amylum solubile mentioned above consequently show a more complex behaviour than that of the Huron Milling Company. In this connection an observation may be mentioned, pointing to irreversible changes of the desolvated sols in the course of time:

On addition, during vigorous stirring, of 1 vol. alcohol to 1 vol. 2 per cent Amylum solubile Merck, the result is a milk-white hydrophobe sol, transparent in a thin layer. If this sol is left standing for about 40 hours and subsequently flocculated with little electrolyte (to 250 cc sol is added 0.4 cc 3n KCl = 5m. aeq. final concentration), the precipitate obtained cannot again be brought in sol condition, not even by boiling for hours with distilled water.

In the liquid are found only suspended, swollen lumps of gel.

In the preparation of the Huron Milling Company this insolubility does not take place (10 cc 2 per cent sol + 12 cc water + 13 cc alcohol).

Significance of oriented coacervation for the nature of protein crystals.

On consideration of the various directions for the preparation of protein crystals, it appears to be always essential that the protein sol is brought to the very verge of instability, i.e. the particles are in a condition which is exceedingly favourable to oriented coacervation.

It appeared indeed that nicely shaped trachyte bundles of egg albumin, lying in the medium in which they had originated, coalesce by means of a slight shifting of the cover-glass into a small liquid pool, bij the eye not to be distinguished from a coacervate.

Summary.

1. The coacervation of Amylum solubile sols at very definite alcohol concentrations and the formation of coacervate sols at higher alcohol concentrations is discussed.

2. In comparison with glycogen the behaviour is here more complex, owing to the presence of various colloid fractions with different properties.

3. Besides fractions which may give rise to ordinary coacervates, these sols also contain fractions, the micells of which can coalesce under observance of prefered orientations.

4. A more elaborate investigation was made of the oriented coacervates from sols of Sizing Starch of the Huron Milling Company, which separate out as hexagonal, very thin plates to a platelet sol upon heating of the coacervate sol.

5. The properties of these plates are described with respect to the electric field common orientation), to mechanical influences (transition to coacervates, flocculation by means of shaking), to coloration with iodine, and to the action of ptyalin.

6. The explanation given for the origin of the plates raises the expectation that, besides by the irreversible way indicated under 4), they must also be able to originate reversibly from the sol particles. This has indeed been observed.

7. The formation of oriented coacervates with other desolvating agents and in other preparations of Amylum solubile is briefly described.

8. The principle of oriented coacervation is of importance for the "crystallization" of proteins from their sols.

Chemistry. — *Researches on adsorption-electrodes* II. *Mineral-electrodes.* By H. J. C. TENDELOO. (Communicated by Prof. H. R. KRUYT).

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

In the first communication [1]) researches were described on the application of glass-electrodes as calcium-electrode. These researches were continued; a number of 50 glass-electrodes, all of the glass with 72 % SiO_2, 6 % CaO, 22 % Na_2O, were examined and, by replacing the exchangeable cations by others, we occasionally succeeded in employing glass-electrodes as Ca-, Na-, or K-electrode. It appeared, however, that the reproducibility of the electrodes was very poor and that the results were totally unreliable, owing to the presence of already very low concentrations of hydrogen-ions. Moreover, the fragility of these electrodes is a very great inconvenience. Consequently the investigation was not continued in this direction.

Instead of glass other silicates were then taken, as they are found in nature in the form of minerals. Many of these minerals possess a strong exchange-capacity for other ions. The crystallagraphic structure and the chemical composition are factors largely influencing the exchangeability of ions. Mica (muscovite) appeared to answer the purpose; from this mineral thin plates may be easily split off. However, we also examined other minerals.

[1]) Kon. Akad. Wet. Amsterdam, Proceedings 37. 212. 1934.