2. Colloid Systems

From the preceding section it has become clear that colloid chemistry is not a separate science, but that it should be seen as a part of physical chemistry. Perhaps it would be better to say two parts, as the hydrophobic sols and the colloid systems which represent equilibrium states (macromolecular and association colloids) are only very loosely bound together. The dimension of the kinetic units is the only important "theoretical" link. In practice, however, this weak link means some methodical agreement, which justifies the separation of colloid chemistry from physical chemistry to some extent.

The difference between a physical chemist and a colloid chemist would be—in theory at least—that a physical chemist is interested in all components of a poly-component system, while a colloid chemist is strongly biased in favour of the components having certain arbitrarily chosen dimensions (the colloid components). When trying to classify the various colloid systems, we must start from this predilection.

Let us take as an example a system of egg albumin and water. The colloid chemist calls it a *sol*, yet from a general physical chemical point of view it is simply a *solution*. We now mentally eliminate the kinetic units of all non-colloid components (thus all particles having a diameter below a certain value: "micro-units"). There remains a system of moving and colliding molecules, that on many points resembles a *gas*. Long ago Van 't Hoff discovered the "gas laws" in solutions of small molecules and colloid science recognised the validity of this principle in determining the molecular weight of colloids by osmotic methods, using these laws of dilute solutions.

Subsequently we take a solution of isoelectric gelatin (at 50° C.) and add alcohol (or Na_2SO_4). The mixture remains clear up to a certain concentration. On further addition turbidity is produced. This turbidity is caused by a large number of minute drops, which coalesce to a viscous liquid layer (coacervate). This layer contains relatively much gelatin; in the other layer—equilibrium liquid—the gelatin concentration is very low. At a sufficiently high concentration cohering masses of floccules are obtained. After a long time these masses of floccules are also transformed into a coherent coacervate layer. Applying the same elimination procedure which we used in the case of the egg albumin solution, we here have two phases (a) solution of gelatin and (b) coacervate of gelatin, which show an analogy to a *gas* and a *liquid* respectively. In phase *a* we have few free moving kinetic units, in phase *b* the kinetic units are closely packed, but they are still in movement and they show *no three dimensional regularity*.

In the third place we point to the crystals which may be obtained from solutions of globular proteins by addition of $(NH_4)_2SO_4$ or other means. These crystals are rather variable (1) as they may contain micro-units (water, ions, etc.) in varying amounts and (2) as these micro-units may be replaced by others without a radical change of the crystal structure. From

the colloid-chemical point of view they are homogenous; only the distance between the particles within the crystal may vary between certain limits. It is clear that these systems (which we will call *colloid crystals*) show an analogy to the normal crystalline solid. The corpuscular macromolecules are arranged in a three dimensional lattice.

Thus colloid science recognises three basic systems:

I Sol (solution),	analogy gas
II Coacervate,	analogy liquid
III Colloid crystal,	analogy solid (cristalline).

Just as in the cases of the normal gases, liquids and solids, a coacervate will be in equilibrium with a sol (equilibrium liquid), in which the concentration of the colloid particle may be high or low (compare the equilibrium between a liquid and its vapour). The same applies to the equilibria between the other colloid phases.

Already in the usual classification of gases, liquids and solids many instances are known of systems which are not easily classifiable. It is not surprising that in colloid science many systems are found which a first sight do not fall into one of the three classes given above, or appear to be intermediates.

In the first place we may mention the liquid crystals. Theoretically speaking two intermediate possibilities exist between the isotropic coacervate and the colloid crystal with three dimensional regularity.

These possibilities have indeed been found: "paracrystals" in the nematic state (an array of anisodiametric molecules with a common axial direction and a random distribution, mobile in two spatial directions of centres of gravity) and in the smectic state (the centres of gravity are mobile in one direction). There are instances known of a nematic colloidal phase (with relatively large amounts of water) in equilibrium with a—more concentrated—smectic phase. The well known myelin forms (originating from lecithin after the addition of water) fall into the category of paracrystals (smectic state). The X-ray diagrams show that these structures are built up from bimolecular layers (Fig. 5). It is interesting to note that the spacings characteristic for the bimolecular films increase continuously with increasing water content (from about 60 mμ to as much as 150 mμ).

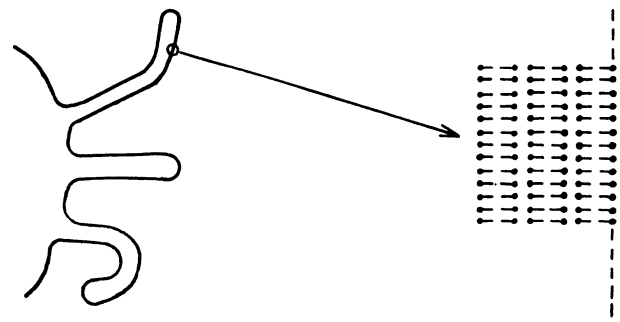

Fig. 5. Molecular structure of myelin tubes.

In many cases it is far from easy to distinguish to which class a colloid-rich phase belongs. Everyone knows that in trying to separate a colloid from a solution one often gets a mass of floccules. Only exceptionally the colloid will separate out in the form of a clear coacervate layer or a colloid crystal. It frequently costs a great deal of trouble to determine the nature of the "floccules" microscopically. They may be formed of minute highly viscous coacervate drops. Then gentle heating will sometimes produce larger drops which may be easily recognized. On the other hand they may be masses of cohering minute colloid crystals. Then identification with simple means is practically impossible.

The phases discussed up till now (sol, coacervate, colloid crystal and paracrystals) might be characterised as systems being macroscopically, microscopically as well as submicroscopically homogeneous. On the other hand many instances are known of colloid systems which are microscopically and macroscopically homogeneous, but submicroscopically inhomogeneous. We will give some examples:

a) *Inhomogeneous sols.* The whole system is liquid and has the character of a sol. It contains, however, submicroscopical aggregates which causes deviations of the normal properties of liquids (structural viscosity, sometimes even distinct elasticity).

b) *Inhomogeneous coacervates.* The coacervate shows the same deviations from the normal properties as were seen in the first case. Here too submicroscopical aggregates are the cause of the deviations.

c) *Gels.* The whole system is solid. When the solvent is added the gel may swell and in some cases it even dissolves. Then the gel has become a sol.

The term gel has been applied to several systems with rather different properties. One might try to distinguish two classes: one-phase gels and two-phase gels.

The first type is characterized by the fact that the single macromolecules [2], though for the greater part of their length freely dispersed in the surrounding liquid, are bound together at certain points by cohesion forces or stronger chemical bonds. Though their character of independent kinetic units is thus lost, the free chain elements of the macromolecules still execute kinetic movements.

In its extreme form, this type of gel consists of macromolecules forming a coherent network throughout the whole system. One and the same macromolecule takes part in regions of the gel which might be called crystalline and in other regions, that resemble macromolecular solutions (Fig. 6). Thus there is formed a continuous lacunary system throughout the whole gel. It seems presumable that the one-phase concept is here more preferable than the two-phase concept. Still it is difficult to characterise the nature of this phase. The gel might for instance be considered as a crystalline phase with very extensive lattice disturbances. In a 2%

[2] or micelles of association colloids.

gelatin gel, the lattice disturbances would even amount to more than 98% of the total volume.

When we now suppose that the cohesion forces between the macromolecules are weak (and the time of contact short) we will get systems which show properties of solids as well as of liquids. We might then speak of easily deformable "solids" or of "liquids with structural viscosity and clearly visible elasticity". There is no sharp boundary between this type of gel and the inhomogeneous sols and coacervates.

Other gels, however, consist of a cohering mass of highly dispersed flocculation aggregates (the agar gel presumably belongs to this group). Here one is inclined to consider these gels as two-phase systems. For these two-phase gels special phase boundary considerations may be of use in explaining part of their properties. We approach the hydrophobic colloids. Of course, this type of gel is not as important for biology as the one-phase gel.

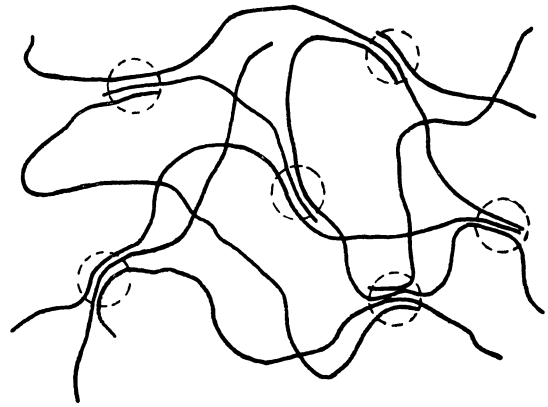

Fig. 6. Structure of a gel.

d) *Apparently single colloid systems.* We have seen that under certain circumstances insoluble inorganic substances like gold, sulfur, etc. may be subdivided to submicroscopical dimensions. The particles may form a more or less stable sol by virtue of the charged surfaces. It should be possible that in a macromolecular solution separation of a coacervate or a crystalline phase sets in, while this new colloid-rich phase remains subdivided in the range of colloidal dimensions. This system does not represent an equilibrium state, though it might at first sight give that impression, just like the "real" hydrophobic sols.

These systems do indeed exist in the field of macromolecular and association colloids. They behave like hydrophobic sols, they are flocculated [3] by neutral salts in small concentrations.

It will be clear that the "apparently single colloid systems" are closely related to the two-phase gels.

In the following chapters a survey of experiments on coacervates will take the greater part of the space available. Two reasons might be given for this preference. The first reason is that we have a wealth of experimental methods for studying coacervates in contra-distinction to the other colloid systems. The simplest method consists of measuring the coacervate

[3] Finally a single colloid-rich layer may sometimes be formed.

volume in relation to the total volume. Moreover one may measure the density, viscosity, refraction and similar properties. With other systems, e. g. colloid crystals or floccules, the number of experimental methods is far more limited.

The second reason is that of all colloid systems the coacervates resemble protoplasm most. This—perhaps superficial, perhaps important—resemblance has led to the study of *colloid morphology.* The important question whether or not protoplasm should be called a coacervate will be treated in one of the following chapters.

We will close this chapter with some general remarks on coacervates. As we have already seen a sol (one-phase system) may separate into two phases under the influence of various factors (change in temperature or pH, addition of a substance) which cause a reduction of the solubility of the colloid. The separated colloid phase may appear in a low dispersed state (either liquid—*coacervate*—or solid—*colloid crystals)* or in higher dispersed states (*floccules* or even *apparently single colloid systems*).

In former times—when the colloid crystals were not yet known—the existence of liquid colloid-rich layers in a two-phase system caused some surprise. This phenomenon led Wo. Ostwald to his well known classification of the sols into "suspensoids" and "emulsoids." He believed that the striking differences were based on respectively the solid and the liquid nature of the dispersed phase. In the gelatin-sol for instance, the protein particles would be present as ultramicroscopic liquid drops, as they would be united with a relatively large quantum of water. In this line of thought a visible separation into two liquid layers is only a change in the degree of dispersion of the second liquid phase already present in the emulsoid sol. Bungenberg de Jong and Kruyt, starting from much the same point of view, introduced the term *coacervation* for the phenomenon described.

Subsequently it became clear that coacervation and flocculation are very closely related phenomena and thus it was thought that coacervation too, might be explained by their stability theory (compare Fig. 1). The original sol particle was considered to be surrounded by a hydration coating of considerable size, in which the water was bound less and less tightly towards the periphery. Such a sol particle would owe its stability just to this diffuse character of the solvate coating, since the latter is not sharply defined at its periphery. Consequently it possesses no free surface energy. Transformation of the diffuse solvate coating into a sufficiently concrete outer boundary (consequently with free surface energy) will result in a union of the sol particles through their solvate coatings. As the actual particle nuclei are still displaceable with respect to each other the coacervate has the nature of a liquid (Fig. 7). This theory has served as a useful guide in the further experimental work, but its fundamental assumptions became more and more doubtful. One of the reasons to forsake this theory was the fact that the measurements of the amount of hydration of macromolecular biocolloids—though giving widely diverging

results—never gave the enormous quantity of "bound water" required by this theory.

With the development of the modern theory of macromolecules came the conclusion that coacervation is a true partial miscibility in the sense of the phase theory. In this respect the phenomenon resembles the partial miscibility which occurs in systems consisting exclusively of micro-units (e. g. phenol/water, alcohol/water/$(NH_4)_2SO_4$, etc.). Nevertheless it is an extreme case of partial miscibility in so far as the two phases (coacervate and equilibrium liquid) differ practically only in colloid content (the con-

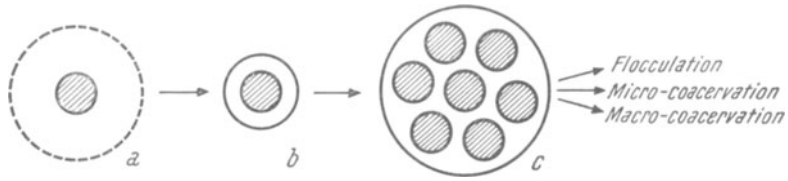

Fig. 7. Original scheme for the mechanism of coacervation.

centration of the colloid is very high in the coacervate and very low in the other phase). The concentration of the micro-units is more or less the same in both phases.

The concept of the statistically kinked macromolecule can be of service in explaining the facts on which the old coacervation theory was based. An example will make this clear.

1. When adding certain micromolecular substances (e. g. Na_2SO_4) to a dilute sol of a linear macromolecule (e. g. isoelectric gelatin) the viscosity $\left(\frac{\eta_s - \eta_0}{\eta_0}\right)$ decreases sharply in a certain concentration range of the added substance, previous to the coacervation.

2. On just exceeding the "coacervation limit" (the minimal concentration of Na_2SO_4 required to get coacervation), the coacervate still contains a relatively large amount of water and other micro-units. This amount decreases on further addition of the micromolecular substance.

The macromolecule is present in the form of a more or less dense coil. Addition of the micromolecular substance will decrease the solubility of the macromolecule, in other words, the affinity of various groups along the macromolecule for the solvent (water) decreases. Eventually it reaches the same value as the mutual affinity of the groups, and finally it decreases to lower values. Consequently the macromolecular coils grow much denser (lowering of viscosity) and some points of contact of more or less long duration are formed between the loops of different macromolecules. If this inter-molecular association is sufficiently great, coacervation takes place. Thus the fall of viscosity and coacervation are not the result of dehydration, but of the large reduction of the amount of occlusion liquid inside the macromolecule (see for a simple scheme Fig. 8).

Further the coacervate is to be regarded as an association of macro-

molecules (or micelles of association colloids) in which the points of contact are of a dynamic nature, since it is still a typical, though viscous, liquid.

Coacervation may be brought about in very different ways but we can give a classification into two large groups. These groups can be illustrated by two examples of micromolecular systems (it should always be remembered that coacervation results from a decrease in solubility).

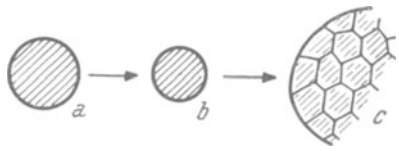

Fig. 8. Modified scheme of coacervation. In the coacervate there are mutually associated macromolecules, which penetrate each other, at any rate with their peripheral loops. At the circumscribed volume of a loosely built macromolecular coil in the original sol.

1. The solubility of phenol in water is lowered as a result of a decrease in temperature (a 1 : 1 mixture is stable at 70° C., but it separates into two phases at 60° C.).

2. We get the formation of an insoluble salt when mixing solutions of $BaCl_2$ and Na_2SO_4.

Thus we distinguish:

1. Simple coacervation; concerned with the non-ionised groups.

2. Complex coacervation; salt-bond formation, the charges on the macromolecules play the important part.

From a biological point of view the simple coacervation (e. g. gelatin with alcohol, resorcinol or Na_2SO_4) is not very interesting, so we will turn our attention to the second type of coacervation. Before doing so we must discuss the experiments on the charge of macromolecules.

References

Bungenberg de Jong, H. G., 1949: Chapter VII in Kruyt's Colloid Science II. Amsterdam.

3. Colloids with Electrolytic Nature

The difference between the electric charges of hydrophobic colloids and macromolecular colloids is that the former derive their charge from ions adsorbed to the particles, in the latter it is due to the dissociation of groups firmly attached to the macromolecule. Thus the macromolecular and association colloids with electrolyte character can be divided into:

a) Colloids with acid character, which carry only anionic groups such as $-COO^-$, $-OSO_3^-$ and $-OPO_3H^-$.

b) Colloids with basic character, which carry only cationic groups, such as $-NH_3^+$, $-NHC(NH_2)_2^+$ and $-N^+(CH_3)_3$.

c) Colloids with amphoteric character, which carry both types of groups.

When looking for a quantitative explanation of the way in which the composition of the medium determines the charge of the macromolecular electrolytes, one should use the theories of electrolytic dissociation rather than the theory of the double layer. Thus gum arabic will loose its negative charge at pH = 2 as the —COOH group acts like a rather weak acid