Biochemistry. — *Complex systems of biocolloids.* I. *Survey and classification according to colloid-chemical and electrochemical points of view.* By H. G. BUNGENBERG DE JONG. (Communicated by Prof. J. VAN DER HOEVE).

(Communicated at the meeting of June 25, 1938.)

1. *Complex coacervation, Complex flocculation, Complex relations.*

Positive gelatin sol + negative gum arabic sol flocculate each other at a suitable mixing-ratio and P_H the "flocculation' having the nature of a liquid (*Coacervation*).

An elaborate research [1]) on the mechanism of this coacervation and the properties and analytic composition of these coacervates shows that we have to deal here with the electrostatic attraction of the oppositely charged colloid particles and the repulsion resulting from the tendency to hydration of the two hydrophilic biocolloids. This coacervation has been called *complex coacervation* [2]). Many examples of complex coacervation have been found, among which cases that are interesting from a biological point of view. But also in analogous cases, where the precipitate morphologically merely has the nature of flocculation, *complex flocculation,* the investigation showed that here also the same relations are found between the particles as have been mentioned above with complex coacervation. These relations were called *complex relations* and by this term is consequently indicated that simultaneously between the particles electric attraction and repulsion resulting from hydration are to be found.

2. *Auto-complex coacervation, Auto-complex flocculation* [2]).

Continued research revealed that there are also cases of coacervation and flocculation respectively with complex relations, where not two oppositely charged biocolloids are found but only one. They were, therefore, called auto-complex coacervation and auto-complex flocculation respectively. At the formation of the auto-complex colloid systems a well

---

[1]) H. G. BUNGENBERG DE JONG and W. A. L. DEKKEER, Kolloid Beihefte, **43**, 134 (1935), **43**, 213 (1936).

[2]) For summaries concerning complex and auto-complex coacervation compare:

H. G. BUNGENBERG DE JONG, Die Koazervation und ihre Bedeutung für die Biologie, Protoplasma, **15**, 110 (1932).

H. G. BUNGENBERG DE JONG, La Coacervation et son importance en Biologie. Tome I et II, Hermann et Cie; Paris 1936.

H. G. BUNGENBERG DE JONG, Koazervation, Kolloid Z., **79**, 223, 334 (1937), **80**, 221, 350 (1937).

chosen oppositely charged crystalloid ion already causes an opposition of charge between the particles of one biocolloid. According as this ion causes the opposition of charge either by means of chemical combination or by adsorption, 2 subdivisions may be distinguished:

1st Subdivision. To this belong the coacervation and flocculation respectively of isolabile proteins at the I.E.P. Starting from negative sols at a lowered P_H, combination of H-ions takes place, which bring about optimal complex relations at the I.E.P. by formation of positively charged groups ($NH_3^{\cdot}$) by the side of negative ones already present (COO'). If these complex relations attain a sufficient intensity, flocculation or coacervation occurs (Globulins), if however this is too small at the given tendency to hydration of the colloid, the protein sol remains stable at the I.E.P. (isostable proteins).

2nd Subdivision. Here a suitable (often polyvalent) oppositely charged ion by means of adsorption enters into an interrelation with the biocolloid. Consequently they are possible with negative biocolloids + crystalloid cations, e.g. gum arbic + Hexol nitrate, as well as with positive colloids with a crystalloid anion, e.g. positive gelatin + $K_4Fe(CN)_6$.

However, they are not limited only to polyvalent crystalloid ions since many cases are known of auto-complex flocculation or auto-complex coacervation occurring with monovalent organic ions, e.g. gum arabic + crystal violet; positive gelatin + sodium picrate.

3. *Complex and auto-complex systems and their general properties.*

The complex relations may occur not only in coacervate condition or in "amorphous" floccules but also in fibrillar systems and in gels. We examined, for example, complex gels, in which either one of the colloid components (gelatinized mixture of gelatin + gum arbic) or both colloid components (gelatinized mixture of gelatin and agar) are present in the gel condition. Auto-complex gels were found as well. We take together all the mentioned kinds of systems in which complex relations occur as *complex biocolloid systems* [1]) in a wider sense and distinguish them into complex and auto-complex biocolloid systems.

The complex relations may be found also in sols or sol mixtures without floccules or coacervates being separated. In these cases they may be recognized by finer methods of investigation, e.g. by means of viscosimetry.

The complex biocolloid systems have a number of properties in common, three of which we will mention here:

1. The neutralization of the complex relations by added neutral salts whose ions themselves cannot contribute to the formation of new complex relations. The rule is found then that this neutralizing action at an equal concentration is stronger according as either the valency of the cation or

---

[1]) H. G. BUNGENBERG DE JONG and collaborators, Rec. Trav. Chim., **53**, 163, 171, 607, 622, 737, 747 (1934); **54**, 1, 17 (1935).

the valency of the anion is higher. Consequently we find two parallel valency rules for the salts of the types n—1 and 1—n. The neutralizing action evidently decreases from left to right according to the series:

$4—1 > 3—1 > 2—1 > 1—1$ and $1—4 > 3—1 > 2—1 > 1—1$ resp.

2. The charge of the boundary plane complex colloid system/dispersion medium poor in colloids is according to the mixingratio of the components negative, uncharged or positive. At the iso-electric point or at any rate close to it the water content of the complex system is a minimum.

3. The charge of the macro boundary plane complex colloid systems/ dispersion medium poor in colloids is likewise influenced by neutral salts. Here, however, a so-called continuous valency rule is observed, indicating the positivization and negativization resp. with regard to the influence exerted by an equally concentrated salt of the type 1—1:

relative 4—1...3—1...2—1...1—1...1—2...1—3...1—4 relative
positivization negativization

4. *On new kinds of complex colloid systems whose classification according to the principle followed so far offers difficulties.*

The principle of classification followed so far, viz. according to the *number of colloid components* enables us to survey an extensive amount of data from one point of view.

A few years ago some new types of flocculation and coacervation were found, which, as appears from the presence of the three characteristic features mentioned in the preceding paragraph, are based on the formation of complex relations [1].

Striking examples are, for instance, the flocculations formed in mixtures which contain simultaneously:

$\underline{\text{egg lecithin}}$ — $\underline{\text{Ca}}\text{Cl}_2$ — $\text{K}_3\underline{\text{Fe(CN)}_6}$
$\underline{\text{I.E. gelatin}}$ — $\underline{\text{Ca}}\text{Cl}_2$ — $\text{K}_2\underline{\text{HgJ}_4}$
$\underline{\text{egg lecithin}}$ — $\underline{\text{La}}(\text{NO}_3)_3$ — $\text{Ca-}\underline{\text{arabinate}}$
$\underline{\text{I.E. gelatin}}$ — $\underline{\text{Ce}}(\text{NO}_3)_3$ — $\text{Ca-}\underline{\text{arabinate}}$

while in the 3 combinations which each time are possible of only 2 out of 3 solutions the flocculation is entirely wanting.

Although in the first two examples only one, in the last two on the other hand two biocolloids occur, yet they form a clearly connected group, in so far as it appeared that here is essential the presence of complex relations between *three* components simultaneously, viz. an amphoteric colloid, a well chosen cation and a well chosen anion or essentially negatively charged biocolloid. These three essential components have been underlined in the examples.

---

[1] H. G. BUNGENBERG DE JONG and G. G. P. SAUBERT, Biochem. Z., **288**, 1, 13 (1936).

The principle of classification used so far, according to the essentially required number of biocolloids, owing to which the first two examples would be based on the formation of auto-complex systems, the last two on the formation of complex systems in the strict sense of the word, cannot express this characteristic peculiarity.

5. *Can complex flocculation and complex coacervation be considered as salt formation? Crystalloid analogues of complex coacervation.*

The difficulties pointed out in the previous paragraph are based on the fact that we always assumed an essential difference between crystalloid ions and charged biocolloid particles. On continued examination of complex coacervation as well as of auto-complex coacervation, it became constantly more obvious that such an essential difference need not exist, but that there is only a difference in degree.

At the complex coacervation of positive gelatin and negative gum arabic in principle only the charged colloid particles, deprived of their opposite ions, enter into the coacervate, these oppositie ions themselves accumulating in the dispersion medium as a neutral salt [1].

Formally there can be no objection to consider complex coacervation as a double transmutation and the separating complex coacervate as a salt-like system, in which, besides water, gelatin colloid cations and arabinate anions take part.

However, it is necessary to point out that not too simple conceptions should be formed concerning these "colloid-colloid salts". In contrast with ordinary salts containing water of crystallization, these are liquid and as far as their analytic composition is concerned continuously variable with respect to the ratio of the two colloid components as well as the water content. Except the participation by hydrated ions, consequently they have nothing in common with ordinary salt hydrates.

As long as no analogous liquid systems consisting of exclusively crystalloid ions were known, the above-mentioned conception of complex coacervation as salt formation had only formal value.

Meanwhile these systems were indeed found [2] and some of them examined more in detail, e.g. unmixing in mixtures of aequeous solutions of Hexol nitrate and $\text{K}_3\text{Co(CN)}_6$, where in principle the Hexol-cobalticyanide is separated as a water-containing liquid [3]. All three characteristics of the complex biocolloid systems mentioned in § 3 are typically present here, in spite of the fact that we have only to deal with a system of crystalloid ions.

---

[1] At any rate at an optimal mixing-ratio and in sufficiently dilute systems.

[2] H. R. KRUYT and H. G. BUNGENBERG DE JONG, Proc. Kon. Akad. v. Wetensch., Amsterdam, **38**, 714 (1935); H. G. BUNGENBERG DE JONG and L. TEUNISSEN-VAN ZIJP, Proc. Kon. Akad. v. Wetensch., Amsterdam, **39**, 1103 (1936).

[3] H. G. BUNGENBERG DE JONG and K. C. WINKLER, Z. f. anorg. algem. Chem., **232**, 119 (1937).

With other examples we succeeded in realizing this unmixing already in the phase-theoretically binary system salt + water (e.g. Novocain perchlorate + H_2O) and stated that this unmixing in a certain range of temperature is stable [1].

Since we are indeed acquainted with analogous phenomena with crystalloid ions, there is no longer any objection to describe the charged colloid particles deprived of their opposite ions as ions.

It becomes apparent that the electrochemical conception has the advantage that not only the simple complex colloid systems but also the more complicated ones discussed in § 4 may be classified according to their nature.

6. *The connection of the complex systems in the limited sense and the 2nd subdivision of the auto-complex systems.*

As has been remarked in § 5, complex coacervation of positive gelatin and negative gum arabic may be considered as really being the unmixing of hydrated pairs of ions: gelatin cations + arabinate anions. In this example both ions are very large (colloid ions) and highly polyvalent. It is obvious that the auto-complex coacervation of gum arabic with Hexol nitrate from the electrochemical point of view has to be considered as exactly the same case. Here also unmixing of ions occurs, only the gelatin-colloid cation being replaced by a crystalloid cation. Similarly the auto-complex coacervation of positive gelatin with $K_4Fe(CN)_6$, where we find the unmixing of a colloid cation with a crystalloid anion.

Evidently from the electrochemical point of view the 2nd subdivision of the auto-complex coacervation forms merely a transitional case to the unmixing of 2 crystalloid ions discussed in § 5. From this point of view, consequently, they form a connected group with the characteristic feature that complex relations exist between a cation and an anion, the question whether both or one of the two are colloid ions or both crystalloid ions now being only of secondary importance. The same holds good as well when these complex systems are present, not as coacervates, but as flocculation, fibrils or gels.

7. *The isolated position of the first subdivision of the auto-complex systems.*

When in the interpretation and classification of the complex colloid systems we wish to stress the mutual relations of the ions, the first subdivision of the auto-complex systems appears as a separate group. The complex relations are present here between the zwitter ions or multipolar ions mutually.

This separation from the 2nd subdivision of the auto-complex systems, following from the electrochemical point of view, is besides in agreement with a long known distinction between the coacervates belonging to these two groups. Those of the 1st subdivision, namely, do not show disintegration phenomena in a direct current field, whereas those of the 2nd subdivision do. The connection discussed in § 6 of the latter with the complex systems in the limited sense, therefore, is manifested by the fact that also the complex coacervates show exactly the same disintegration phenomena.

8. *Nature of the complex systems discussed in 4.*

The complex systems discussed in § 4, which offer great difficulties with respect to their interpretation from a colloid-chemical point of view, may be easily explained from the electrochemical standpoint.

Whereas in the systems discussed above the complex relations occur either between a cation and an anion (§ 6) or between zwitter or multipolar ions mutually (§ 7), they are in the cases mentioned in § 4 present between these three genera of ions simultaneously: zwitter or multipolar ion + cation + anion. A more detailed discussion of the factors favourable to their formation will follow in the next communication.

9. *Nomenclature of the complex colloid systems according to electrochemical points of view.*

In §§ 6—8 already a classification of the complex colloid systems has been given and we found that here 3 types may be distinguished, according as the complex relations are present between:

A. Zwitter ions or multipolar ions mutually;
B. Cation and anion;
C. Zwitter ion or multipolar ion, cation and anion simultaneously.

It seems desirable to be able to indicate these 3 types by short terms. This is particularly the case with type C which, as continued research makes us expect, may be of biological importance and which then each time would have to be indicated by long descriptions. A short terminology is possible when we express in it the *number of principal types of ions essentially required* for each type of system, A, B, C. Of the latter we know three: multipolar or zwitter ion, cation and anion. For the formation of complex relations an opposition of charge is required. This is already possible in case of participation of only *one* principal type of ions: zwitter ion or multipolar ion.

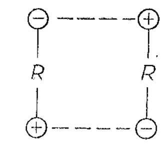
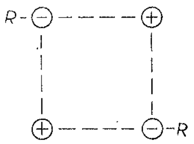
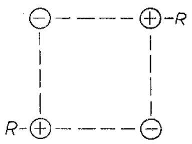
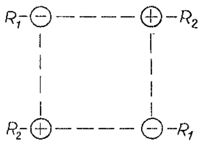
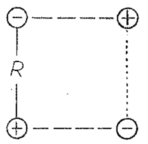
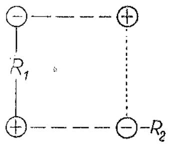
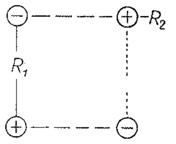
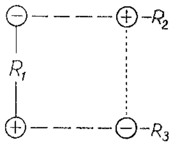
Type A of the complex colloid systems corresponding to this may consequently be indicated by the term: *Unicomplex colloid systems.*

Complex relations may exist as well between 2 principal types of ions: cation and anion. Type B of the complex colloid systems may then be defined by *dicomplex colloid systems,* type C, where complex relations between the 3 principal types of ions are present: zwitter or multipolar ion, cation and anion, then being indicated by *tricomplex colloid systems.* Analogous terms may then be used for the flocculation and coacervation

---

[1] H. G. BUNGENBERG DE JONG and L. W. J. HOLLEMAN, Proc. Kon. Akad. v. Wetensch., Amsterdam, 40, 2 (1937).

TABLE

Examples	Classification according to the number of biocolloids	Ion diagrams	Classification according to the number of principal types of ions
Globulin at the I.E.P.	Auto-complex systems 1st subdivision		Unicomplex colloid systems
Hexol nitrate + gum arabic (neg.)	2nd subdivision		Dicomplex colloid systems
Gelatin (pos.) + Na picrate	" "		
Gelatin (pos) + gum arabic (neg.)	Complex systems (in a limited sense)		
I. E. gelatin + $K_2HgJ_4 + CaCl_2$			Tricomplex colloid systems
I. E. gelatin + carragheen (neg.) + $CaCl_2$			
Examples not yet known			
" "			

respectively by which these systems are formed, e.g. tricomplex flocculation.

In the following table we give examples of each of the discussed kinds of complex colloid systems, their connection according to the old principle of classification by the number of participating biocolloids and according to the one proposed here by the number of essentially participating principal types of ions. Since the latter classification seems to concern more the nature of the complex colloid systems, it has not been tried to classify the tricomplex colloid systems according to the old principle. In fig. 1 diagrams have been added to illustrate the new classification, cations and anions being taken monovalent and eventual multipolar ions simplified to zwitter ions. The complex relations present between them have been indicated by dashes.

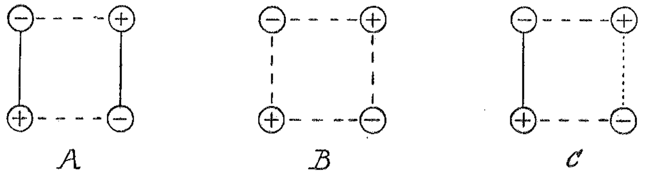


Fig. 1.

Although the dicomplex colloid systems (B) may be symbolized by a figure in which only one cation and one anion are reproduced, their number has been doubled in order to point out the mutual connection with the two other diagrams.

The complex relations observed in the diagram of the unicomplex colloid systems (A) are equal, in the same way as those existing between the two pairs of ions in the diagram of the dicomplex colloid systems. However, this is not the case with the tricomplex colloid systems (C), where the three given complex relations are of a different intensity. In this diagram the complex relations between cation and anion have been indicated by dotted lines, those between each of them and the charges of the zwitter ion by dashes. It is tried to express in this way that the dotted complex relations as a rule have to be weaker here than the two others for the formation of a tricomplex system. This rule, which can be applied only provided no other cohesive forces ("Symplex relations", e.g. V. D. WAALS forces) really contribute to the binding of the components mutually, will be illustrated more in detail in the following communication.

10. *The complex colloid systems as special cases of the complex systems. Limit cases of the latter.*

In column 3 of the table ion diagrams have been inserted for each of the examples mentioned in column 1, the colloid ions being distinguished from the crystalloid ions by addition of —R. This distinction makes us foresee some more cases for the tricomplex colloid systems, examples of which are not yet known. In addition of the four diagrams given in the table three other cases may be found, where the zwitter ion is a crystalloid ion. However, since from the electrochemical point of view the distinction of colloid ions and crystalloid ions is not essential but merely a matter of degree, we have to consider for the principal classification only the three principle-diagrams reproduced in fig. 1, where now we admit also the possibility that none of the participating ions are colloid ions.

If we drop the distinction between colloid and crystalloid ions, we may consequently speak still more generally of "complex systems". In the dicomplex systems we know indeed cases (cf. § 5) where the participating ions are exclusively crystalloid ions. Finally it may be remarked that actually also the crystalline salt hydrates are special cases of the complex systems, with this difference that here the ions are arranged in regular patterns and the water content is only discontinuously variable. The three principal types of the complex systems finally reach their limit when the content of the water of crystallization is reduced to zero (examples: amino-acid crystals; NaCl crystals; crystalline "molecular combination" amino acid + NaCl).

11. *Remarks concerning the proposed classification.*

The 3 principle-diagrams given above (fig. 1) only represent the nature of the complex relations concerned. Consequently they are not meant at all as a stereometrical reproduction, e.g. of the complex colloid systems. However, it is conceivable that in some cases indeed ordered patterns occur. Besides, in these diagrams the ions or ionized groups are represented only as monovalent. In many cases the crystalloid ions and as a rule the colloid ions are polyvalent. It may also occur here that the colloid ions carry positive or negative ionized groups, whose chemical composition differs, e.g. with protein cations guanidine groups by the side of $NH_3^{\bullet}$, with protein anions COO' groups by the side of phosphate groups. This may occur likewise in essentially negative biocolloids, e.g. SO_4'' groups by the side of COO' groups in chondroitin sulphate.

All these distinctions, however important for judging the intensity of the complex relations in each case to be considered separately, do not give rise to an extension of the proposed general classification, since the number of varieties would be practically endless.

Finally a few words about the question whether it is advisable in a general classification to include as separate cases those in which one of the biocolloids is partly replaced by another, which however belongs to the same principal type of ions.

The usefulness of such a distinction, although of importance in cases to be considered separately, in our opinion should be denied for a general classification, for then we should introduce again the old principle of classification, according to the number of biocolloids, which appeared to be not real.

In speaking of unicomplex systems, we merely mean systems in which the complex relations are present between zwitter ions or multipolar ions respectively. The most simple case is, of course, that only one chemically well defined individual takes part in it. However, in cases where a mixture of chemical individuals has part in it, we still will speak of a unicomplex system.

Similarly with the dicomplex systems: In mixtures of gum arabic (negative) + gelatin (positive) + ichthyocoll (positive) a complex coacervate is formed containing all three biocolloids. Nevertheless, the coacervate has to be classified as a *dicomplex system*, since we are concerned here only with complex relations between cations and anions. The cations, however, are not only gelatin cations but the latter are partly replaced by ichthyocoll cations. In mixtures of gelatin (positive) + gum arabic (negative) + yeast nucleinate (negative) at a certain P_H and mixing-ratio analogously a dicomplex coacervate is formed, in which all three colloids take part. At other P_H's and mixing-ratios two coexisting dicomplex coacervates are formed, again containing all three colloid components but one being richer in nucleinate than arabinate, the other just the reverse.

12. *Discussion of the influence of the P_H, the mixing-ratio and concentration of the colloid components from the electrochemical point of view.*

The great variety of influences from the surroundings displayed by the complex colloid systems can, of course, not be expressed in the classification diagrams which have been kept as simple as possible, e.g. that of the P_H influence determining the charge of the protein ions, the influence of the mixing-ratio of the components, of added electrolytes, etc. The influence of the P_H is connected with the continuously variable valency, in particular of the protein ions. The other mentioned influences are the result of the fact that in describing the complex colloid systems as "salt-like" systems we have to remember that, if they are "amorphous", they are continuously variable in composition.

Let us take e.g. the complex coacervation gelatin (positive) + gum arabic (negative). In practical use the dicomplex system arabinate anion + gelatin cation is obtained by mixing sols of gum arabic and positive gelatin, or expressed electrochemically: by double transmutation of gelatin chloride + Ca arabinate.

The coacervation is optimal when equivalent quantities of gelatin chloride and Ca arabinate are mixed at one given P_H. This mixing-ratio is, of course, dependent on the P_H, since the charge particularly of the gelatin is a function of the P_H. But at one P_H the coacervate has only at a single mixing-ratio, viz. the optimal one, really the composition of the formally dicomplex system:

$$\text{gelatin cation} + \text{arabinate anion} + nH_2O.$$

It is then uncharged at the boundary plane coacervate/dispersion medium, poor in colloids. At other mixing-ratios the coacervate partly absorbs the complex component, which each time is present in excess, and weakens the complex relations, which is accompanied by increased solubility of the coacervate and charging of the boundary plane coacervate/dispersion medium poor in colloids:

e.g. [gelatin cation + arabinate anion
Ca-ion + arabinate anion] negatively charged

[gelatin cation + arabinate anion
gelatin cation + chlorine ion] positively charged

These formulations are besides only approximately true when the concentrations of the two sols which are mixed are very low. At concentrations which are usually taken, e.g. with sols of a few percentages, it should be borne in mind that if the double transmutation were absolutely completed a concentration of $CaCl_2$ would be formed which has already a perceptibly neutralizing effect (cf. § 3) on the dicomplex system. In these cases the dicomplex system is separated, not all opposite ions having left the coacervate and consequently the complex relations have a smaller intensity. This is manifested by a larger water content than when the coacervate is formed out of highly dilute sols. Thus it is also conceivable that, if the concentration of the sols is taken sufficiently high (e.g. 6 %), the complex coacervation does not take place. The $CaCl_2$ concentration, which might be regarded as formed by double transmutation, then reaches a value which also has a neutralizing effect on the complex coacervation with highly dilute sols.

From a phase-theoretical point of view, however, this dependence of the unmixing on the concentration in these systems which apparently consist of three components (gelatin, gum arabic, H_2O) is quite conceivable, if we accept the electro-chemical conception.

Phase-theoretically, namely, we are not concerned with a ternary system but with (at least) a quaternary one, since we have to deal with a system from H_2O + two salts without a common ion:

gelatin chloride + Ca arabinate + H_2O

Similarly in the auto-complex coacervation of negative gum arabic with Hexol nitrate we have to deal with a system consisting of 4 components: Ca arabinate + Hexol nitrate + H_2O, the $Ca(NO_3)_2$ formed by the double transmutation representing a counteracting factor.

To the tricomplex flocculation, as it is observed in practical use, e.g. in mixing of egg lecithin sol with gum arabic sol and $La(NO_3)_3$ solution, etc., applies also that we have always more components than would be essentially necessary for the formation of the tricomplex system.

Summary.

1. A survey is given of the colloid systems known so far, in which complex relations occur (simultaneous electrical attraction and repulsion as a result of solvation).

2. It became apparent that the classification followed before according to the number of essentially participating biocolloids does not concern so much the essence, since exclusively analogous systems containing crystalloid ions were found. Moreover, in the previous classification into complex and auto-complex colloid systems it is difficult to insert a number of newly found cases, of which it is characteristic that complex relations between 3 complex components are simultaneously present.

3. From electrochemical points of view it is possible to obtain a more satisfactory classification, the question whether an ion is either a colloid ion or a crystalloid ion being left in the background. We are then able to distinguish three principal types, according as the complex relations are essentially present between:

a. zwitter ions or multipolar ions mutually
b. cations and anions
c. simultaneously between zwitter ions, cations and anions.

4. A short terminology is obtained by taking the number of minimally required principal types of ions as a criterion: The types mentioned in 3. are then called: *a.* unicomplex, *b.* dicomplex, *c.* tricomplex colloid systems.

5. The connection is discussed with analogous systems consisting only of crystalloid ions and their limit cases (animo-acid crystal, NaCl crystal, crystalline double compound of amino acid with NaCl).

Laboratory for Medical Chemistry at Leiden.

May 1938.