

Biochemistry. — *Some instances of tricomplex flocculation. I. Soybean phosphatide + carraghene + crystalloid cation.* By H. G. BUNGENBERG DE JONG and C. H. RERING. (Communicated by Prof. H. R. KRUYT.)

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1. *Introduction.*

In the second part of a survey called: "Complex systems of biocolloids"¹⁾ we gave some details of the theory of the so-called "tricomplex systems", colloid systems for the formation of which three charged components:

Amphoion + anion + cation

are essential²⁾).

In this survey such a theoretical discussion was possible, as we had already experimental material at our disposal concerning some significant instances of tricomplex flocculation, namely:

soybean phosphatide	+ carraghene + crystalloid cation
egglecithin	+ carraghene + crystalloid cation
i.e. gelatine	+ carraghene + crystalloid cation.

These instances belong to the special case:

colloid amphoion + colloid anion + crystalloid cation.

They are biologically especially interesting, because here these tricomplex systems can be formed with Ca⁺⁺, whereas they are not possible with K⁺ (sometimes not with Na⁺). There is also pronounced antagonism between CaCl₂ and KCl, CaCl₂ and NaCl resp. Thus far, however, circumstances prevented the publication of this experimental material. In this and the following two Communications a survey will therefore be given in this place.

In this first Communication the first mentioned instance will be discussed.

As starting material for the preparation of the soybean phosphatide sol we used "Planctin alcohollöslich 90—95%" of J. D. RIEDEL, E. DE HAËN, A. G. BERLIN.

4.5 gr. of this preparation was dissolved in 30 cc petroleum aether and after filtration 28 cc of this solution was precipitated with 280 cc acetone. The precipitation was dissolved in 14 cc aether and precipitated with 140 cc acetone, the precipitation was dissolved in 35 cc ethyl alcohol and squirted in a thin jet into 170 cc dist. water, while the mixture was being stirred very vigorously. The transparent sol obtained was placed in a dialysator, a large funnel (diameter 36 cm) which was closed with a thin cellophane membrane³⁾ and was rocked continuously in a large dish of dist. water (volume 10 l, replaced after 4 hours). 8 hours dialysis practically freed the sol from alcohol. The very slight opalescence of the sol increases a little during the process, while owing to hydration the volume increases slightly (e.g. sol I 200 cc → 247.5 cc, sol II 200 cc → 245 cc).

The concentrations of the individual sols differed slightly e.g.:

sol I: 0.454 %, sol III: 0.451 %, sol V: 0.52 %.

¹⁾ H. G. BUNGENBERG DE JONG, Proc. Kon. Akad. v. Wetensch., Amsterdam, **41**, 776, 788 (1938).

²⁾ H. G. BUNGENBERG DE JONG and G. G. P. SAUBERT, Biochem. Z. **288**, 1, 13 (1936).

³⁾ In order to obtain a tightly stretched membrane it should be mounted wet and left to dry in the air. With the large diameter of the funnel (36 cm) however, the membrane was usually torn in drying. This inconvenience was prevented by soaking the membrane not in dist. water, but in a water alcohol mixture (1 : 1) before mounting it.

In the following prescriptions we shall only indicate the individual sols as "phosphatide sol 0.5%" with the addition of the roman numeral. For the preparation of the Na-carraghene see "Kolloid Beih." 47, 262 (1938).

From the air-dry preparation we obtained a ca. 0.1% sol by shaking (0.2025 gr. air dry preparation shaken with 200 cc dist. water, during the night).

2. *Specific differences of the cations as regards the tricomplex flocculation phosphatide + carraghene + crystalloid ion.*

The dialysed phosphatide sol, when mixed with a solution of CdCl_2 , ZnCl_2 , AgNO_3 , MgCl_2 , CaCl_2 , SrCl_2 , BaCl_2 , LiCl , NaCl , KCl resp., does not flocculate, there is not even any considerable increase of the slight opalescence.

The carraghene sol behaves in the same way, at most a slight increase of the opalescence occurring with KCl .

Neither is there flocculation or turbidity when the phosphatide sol is mixed with the carraghene sol.

Fig. I curve „blank” shows the effect of CaCl_2 on a mixture of the phosphatide sol + the carraghene sol. The other curves of Fig. I can be compared direct with the blank

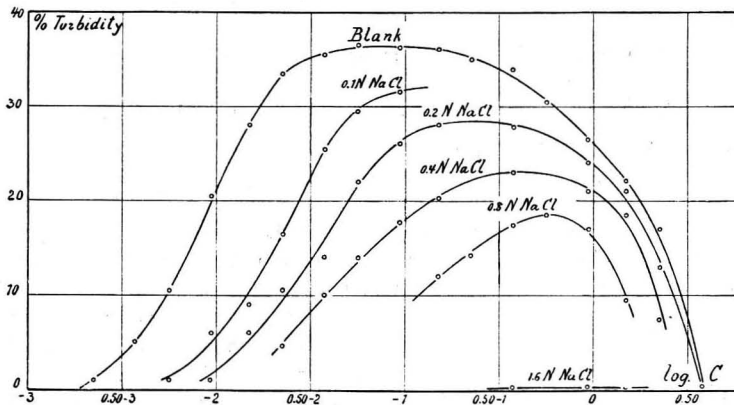


Fig. 1

curve, but now there is NaCl present, of which the concentration increases but is kept constant for each curve.

In preparing the mixtures we proceeded as follows:
first we prepared a mixed sol by mixing 2 vol. phosphatide sol 0.5% I with 1 vol. 0.1% Na carraghene sol, pipetting 2 cc of it to 23 cc mixtures prepared beforehand:

a cc CaCl_2 solution + (23 — a) cc dist. water (blank)
a cc CaCl_2 solution + bcc NaCl solution + (23 — a — b) cc dist. water (remaining curves).

Addition b was chosen so that the final NaCl concentrations were 0.1; 0.2; 0.4; 0.8; 1.6 N resp. ¹⁾

In the 6 series measured thus we varied a and the strength of the CaCl_2 solution in such a way that the CaCl_2 final concentrations varied over a very large concentration section (from ca. 0.001 N to ca. 4 N). Therefore the logarithms of the concentrations have been set out along the absciss in Fig. 1 and in the following figures (so in Fig. 1 $\log C_{\text{CaCl}_2}$).

¹⁾ The measuring was done 30 min. after the addition of the sol mixture, after each tube had been well shaken just before measuring, so that any sediment floccules were divided equally over the liquid. Immediately after shaking the turbidity was measured as quickly as possible, before sedimentation had again set in.

Fig. 1 curve blank shows that already at small CaCl_2 concentrations ($\log C = 0.50-3$, i.e. $C = \text{ca. } 3 \text{ m.aeq. pL}$) the sol mixture begins to flocculate, that the flocculation is about maximal in the section of ca. 30–300 m.aeq. pL ($\log C = 0.50-2$ to $0.50-1$), decreasing to 0 with higher concentrations, ca. 300–4000 m.aeq. pL ($\log C = 0.50-1$ to 0.60). Fig. 1 also shows that NaCl counteracts flocculation with CaCl_2 , even entirely neutralising it with 1.6 N.

Fig. 2 gives the results of a comparison of the flocculation effect of MgCl_2 , CaCl_2 , SrCl_2 , BaCl_2 , LiCl, NaCl and KCl.

The preparation of the mixtures was the same as indicated above (2 cc mixed sol, from

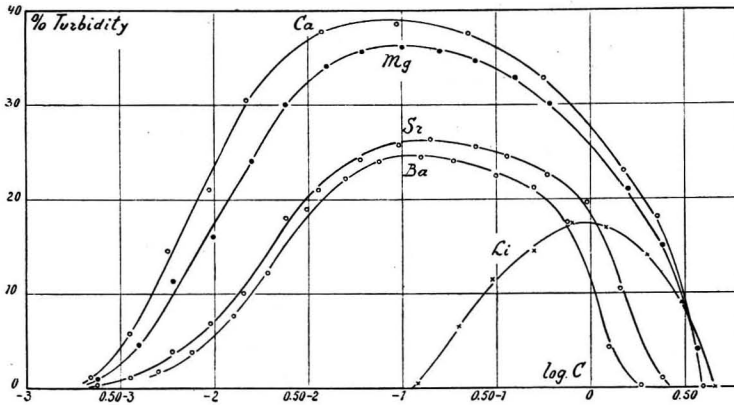


Fig. 2

2 vol. phosphatide sol 0.5% + 1 vol. carraghene sol 0.1% + a cc salt solution + (23-a) cc dist. water, measurement 30 min. after sol was added).

Fig. 2 shows that there are great specific differences between the bivalent cations. This is also true of the monovalent cations, as LiCl only causes flocculation, the sol mixtures remaining perfectly clear with NaCl and KCl.

So we find the following series for flocculation decreasing from left to right



As from a theoretical point of view it is desirable also to know the order of Na and K we also compared LiCl, NaCl and KCl in the presence of 25 vol % aethyl alcohol.

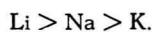
In order to obtain greater turbidity we did not use 2 but 4 cc sol mixture to 25 cc final volume (4 cc sol mixture from 2 vol. phosphatide sol 0.5% V with 1 vol. carraghene sol 0.1 cc + 6 cc alcohol + a cc salt solution + (15-a) cc dist. water).

The result of these measurements is shown in Fig. 3. It appears that LiCl and NaCl both produce considerable flocculation, but the turbidity is slight with KCl.

Control experiments with only phosphatide sol and with only carraghene sol both with 25 vol % alcohol convinced us that no turbidity arises in the phosphatide sol with LiCl NaCl and KCl, neither in the carraghene sol with LiCl and NaCl. But there is slight turbidity of the carraghene sol with KCl, which is about as strong as the one which was produced with the sol mixture in Fig. 3.

So we conclude that the flocculations produced with LiCl and NaCl are typical tricomplex flocculations, but that KCl has not this power (the slight flocculation which occurs is owing to the flocculation of carraghene with K in a medium containing 25% alcohol).

We may therefore conclude that the order of a tendency to tricomplex flocculation decreasing from left to right is



In a watery medium we also found in separate experimental series (with phosphatide sol VI)

$\text{Ag} > \text{Li}$ (Fig. 4a) and $\text{Cd} > \text{Ca}$ (Fig. 4b).

By comparing the curves with each other (degree of the max. turbidity) we arrive at

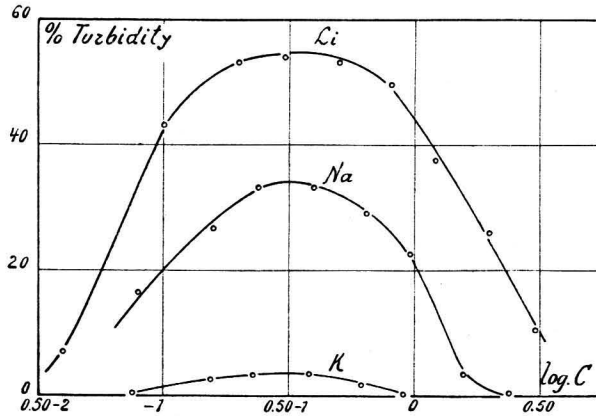


Fig. 3

the following series of a tendency to tricomplex flocculation, decreasing from left to right:

$\text{Cd} > \text{Ca} > \text{Mg} > \text{Ag} > \text{Sr} > \text{Ba} > \text{Li} > \text{Na} > \text{K}$.

There was no indication that tricomplex flocculation can be realized with K ions.

3. *Measurement of the reversal of charge concentrations and explanation of the cation order found in 2.*

With the aid of the technique described in detail elsewhere¹⁾, (microscopic cuvette, measurements at $\frac{1}{5}$ height, SiO_2 powder as electrophoretic indicator) we determined the reversal of charge concentrations of the salts used in 2. with the phosphatid sol (5 cc $\frac{1}{2}$ % to 50 cc final volume) and with carraghene sol (5 cc 0.1 % to 50 cc final volume), separately for each sol.

Figures 5 and 6 give some of the interpolation graphs obtained²⁾. From it we have taken the logarithms read of the reversal of charge concentrations, mentioned in columns 2 and 3 of Table I (the extrapolated values are placed in brackets).

We shall not further discuss here the location with regard to each other of the reversal of charge concentrations with the carraghene sol, resp. the phosphatide sol separately. It is sufficient to state here that the sequences are in agreement with previous measurements, and that they were discussed in detail elsewhere³⁾.

It was seen that the sequences may be explained from the difference in polarizability

¹⁾ H. G. BUNGENBERG DE JONG and P. H. TEUNISSEN, *Kolloid Beihefte* **47**, 254 (1938) compare p. 265—274.

²⁾ Here we used a cuvette with auxiliary electrodes in order to measure the potential gradient during the measurements. The electrophoretic velocities (U) in Figures 5 and 6 have been corrected for the same potential gradient per cm. This causes the U-log C curves to be nearly straight lines, which is necessary for the interpolation and especially for the extrapolation unavoidable with some salts (on account of their insufficient solubility). The extrapolated parts of the curves on the right top of the curves, indicated by a dotted line, are thus extrapolated linearly. The dotted parts at the left lower end of some curves probably represent a yet incomplete covering of the SiO_2 particles with a colloid film.

³⁾ H. G. BUNGENBERG DE JONG and P. H. TEUNISSEN, *Kolloid Beihefte*, **48**, 33 (1938).

TABLE I.

Salt	log of the reversal conc.		$\log \frac{C_{\text{Carraghene}}}{C_{\text{Phosphatide}}}$	Maximal turbidity ¹⁾
	Carraghene	Phosphatide		
KCl	(0.81)	(0.70)	0.11	— (3.5)
NaCl	(0.88)	0.47	0.41	— (34)
LiCl	(1.00)	0.00	1.00	18 (54)
BaCl ₂	(0.60)	0.13—1	1.47	25
SrCl ₂	0.70	0.06—1	1.64	27
AgNO ₃	(0.86)	0.95—2	1.91	34.5
MgCl ₂	0.86	0.70—2	2.16	37.5
CaCl ₂	0.84	0.59—2	2.25	40
CdCl ₂	0.70	0.13—2	2.57	43

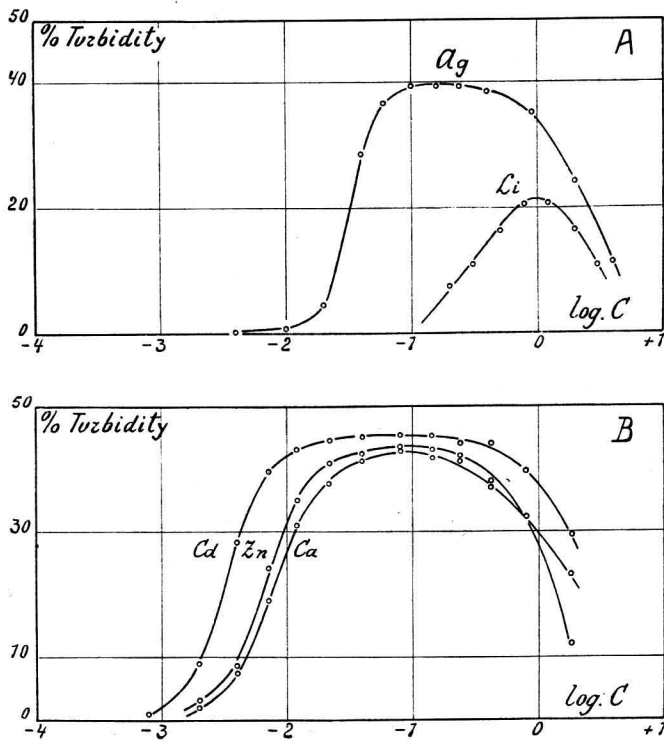


Fig. 4

¹⁾ The values placed in brackets refer to a medium of 25 vol % alcohol (Fig. 3) the others to a watery medium. As for the latter experiments different sol individuals were used, the maximal turbidities have been calculated from each other, with as basis a maximal turbidity of the Ca flocculation of 40 % so the turbidities of Mg, Sr, Ba and Li mentioned in the table were obtained from Fig. 2 by multiplying the values read there by 40/38.5 (38.5 is the max. turbidity of CaCl₂ in Fig. 1). The value for Ag in Fig. 4a was obtained by taking 18/22.5 times the max. value read (22.5 is here the max. turbidity of Li) in the same way we obtained the Cd value by multiplying the value read in Fig. 4b, 45.5 by 40/42.5 (the max. value of CaCl₂ is here 42.5).

of the negative ionized groups. This difference in polarizability is of the greatest significance, however, for the formation of the tricomplex systems. It appears from theoretical considerations (see the publication mentioned in the introduction), that for the formation of these systems it is conducive when the negative group of the amphotoion (here the phosphatide ¹⁾) is more polarizable than the negative group of the anion

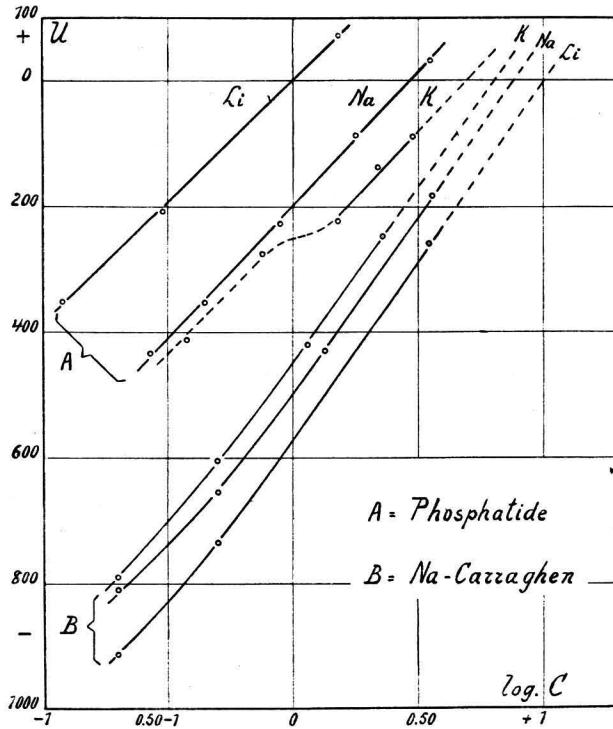


Fig. 5

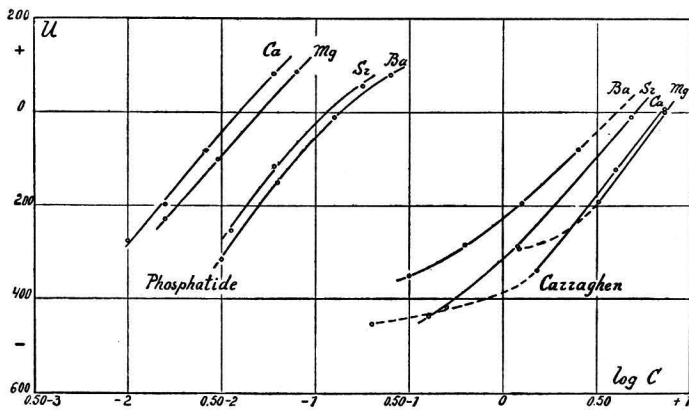


Fig. 6

¹⁾ Although the phosphatide used here is negatively charged (owing to phosphatidic acid) the greater part yet consists of phosphatide amphotoions.

(here the colloid anion of carraghene with ester sulphate groups). In the publication mentioned it has also been discussed in how far the "ionspectra" of the two colloids are of importance for the specific differences, which are so predominant among the inorganic cations with regard to the tricomplex flocculation.

It is seen there that these ionspectra are not themselves determinant but that the value of the quotient of the reversal of charge concentrations of both colloids with the same cation determines the tendency to tricomplex flocculation. As a matter of fact this is reflected in Table 1, column 1 where we have arranged the salts in such a way beneath each other as agrees with the increasing tendency to tricomplex flocculation (see the cation series found in 2).

When each time we subtract the values of column 3 from those placed by the side of them in column 2, we obtain column 4, the logarithms of the quotients of the reversal of charge concentrations mentioned above. As a matter of fact we see that these values in col. 4 also increase from top to bottom. The connection is also expressed in Fig. 7, in which we have set out the maximal turbidity in a watery medium against the values of column 4.

Apparently the value of the quotient is too slight for K and Na to cause tricomplex flocculation in a watery medium. In 25% alcohol tricomplex flocculation may still be produced with Na, but not with K (see the turbidity figures placed in brackets in the last column of the table).

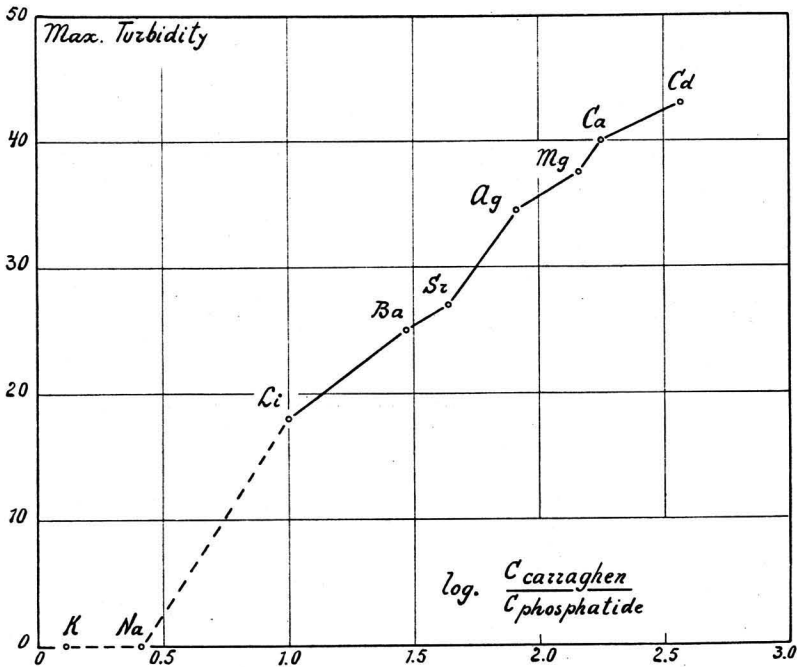


Fig. 7

4. Neutralization of the Ca, Li tricomplex flocculation respectively with salts of type 1—1.

With other complex systems tricomplex flocculation has this characteristic in common, that neutral salts in general have a neutralizing effect. This is already indicated by the curves of Figures 1, 2 and 3, where the same salts which smaller concentration cause tricomplex flocculation, neutralize it in greater concentrations. But this also happens on addition of another salt (e.g. NaCl in Fig. 1). The neutralization of the approximately

maximal CaCl_2 , resp. LiCl flocculation by salts of type 1—1 has been investigated systematically.

We shall however delay the discussion and the explanation of the specific sequences found of cations and anions to a subsequent communication, in which we shall find the same results for the cations and more evident spreading of the anions with the tricomplex flocculation egglecithin + carraghene + crystalloid cations, to be discussed there.

Summary.

1. In mixtures of soybean phosphatide sol, soluble in alcohol (negative, but consisting mainly of phosphatide amphiions) and carraghene sols (negative) a number of salts of types 1—1 and 2—1 cause flocculation.

2. The tendency to flocculation of the sol mixture decreases in the following series from left to right



In a watery medium Na and K cause no flocculation, the order placed in brackets appeared from an investigation in 25 vol. % alcohol.

3. Electrophoretic measurements show that the same sequence as in 2 is obtained for the value of the logarithms of the quotient of the reserval of charge concentrations of the two sols separately with the salts mentioned.

4. The results obtained in 2 and 3 are in agreement with the opinion that the flocculation is tricomplex, so that as real components of the flocculation we can consider: the phosphatide amphiion, the carraghene colloid anion and the crystalloid cation used for het flocculation.

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