

**Biochemistry.** — *Som2 instances of tricomplex flocculation. III. The flocculation of gelatine + carraghene with alkali and earth alkali cations.* By H. G. BUNGENBERG DE JONG and C. H. RERING. (Communicated by Prof. H. R. KRUYT.)

(Communicated at the meeting of May 30, 1942.)

For the following experiments we started from a 3% sol of purified gelatine<sup>1)</sup> and a 0.1% sol of Na-carraghene<sup>2)</sup>.

When we mixed at 40° 11 cc dist. water with 4 cc 0.1% carraghene sol and 10 cc 3% gelatine sol, we obtained a mixture which, measured with the extinc-tometer of MOL in a cuvette 10 mm deep, gave a turbidity of 4% (non-transmitted light). The same turbidity is obtained by mixing 15 cc dist. water with 10 cc 3% gelatine sol. So it is not caused by the interaction of gelatine and carraghene.

Neither does extra turbidity arise when CaCl<sub>2</sub> is added to the carraghene sol, to the gelatine sol resp. but CaCl<sub>2</sub> (and other salts see below) cause strong flocculation when gelatine as well as carraghene are present.

With the following experiments we left the mixtures 30 min. after preparation at 40°; then they were well shaken, so that any deposited floccules were divided equally over the liquid and then we measured the turbidity as quickly as possible in the extinc-tometer.

We always kept a final volume of the mixtures of 25 cc, in which there was for the experimental series pictured in Fig. 1, 4 cc carraghene 0.1% and 10 cc gelatine 3%, but the salt concentration was greatly varied. In Fig. 1 the turbidity has been set out against

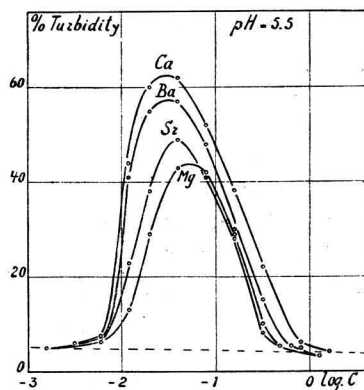


Fig. 1.

the logarithms of the salt final concentrations. We see here that on the turbidity level discussed above of ca. 4% (owing to the turbidity of the gelatine sol itself) a turbidity curve with a maximum is obtained with all four salts examined.

There are great differences between the salts (which are here of the same type, viz. 2—1) themselves, namely that the flocculation decreases from left to right in the following series:



1) Gelatine F00 extra of the "Lijm- en Gelatinefabriek 'Delft'" at Delft. Preparation of the isoelectric gelatine see Koll. Beihefte 43, 256 (1936).

2) Na-carraghene preparation see Koll. Beihefte 47, 262 (1938).

When with the same mixing proportion of gelatine and carraghene we make analogous experiments with LiCl, NaCl resp. KCl, only very slight turbidity is produced. So we can already state that the intensity of the flocculation with salts of type 1—1 is much less than with the salts mentioned of type 2—1. The turbidity obtained with salts 1—1 is too slight to conclude from it with any certainty the sequence of the monovalent cations.

We found that a different mixing proportion of the two colloids gives a better result, namely when in the final mixtures of 25 cc there is 12 cc 0.1% carraghene and 5 cc 3% gelatine. This experiment (Fig. 2) shows that also with alkali cations there are great

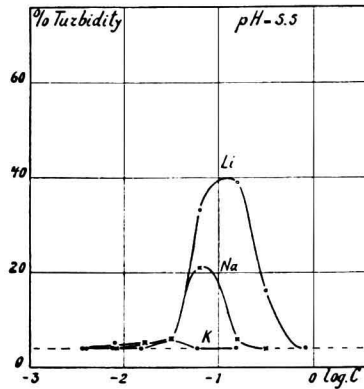


Fig. 2.

specific differences. The flocculation decreases from left to right (being even absent in K) in the order:



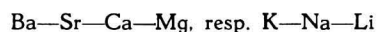
when we combine the results of the two series we get the order:



We also made electrophoretic measurements (microscopic cuvette, measurements at  $1/5$  height) of the carraghene sol separately and of the gelatine sol separately ( $40^\circ$ ). The results obtained are given in the following table, where we give the logarithms of the reversal of charge concentrations (concentrations in equivalents p. L) in columns 2 and 3.

Salt	Log of the reversal of charge conc.		$\Delta \log C$
	Carraghene	Gelatine pH 5.5	
CaCl <sub>2</sub>	0.84	0.60—2	2.24
BaCl <sub>2</sub>	0.60	0.63—2	1.97
SrCl <sub>2</sub>	0.70	0.18—1	1.52
MgCl <sub>2</sub>	0.86	0.52—1	1.34
LiCl	1.00	0.32	0.68
NaCl	(0.88)	0.51	0.37
KCl	(0.81)	(>0.8)	negative

The reversal of charge concentrations united to so-called ion spectra are set out in Fig. 3 for earth alkali-chlorides (top) and for the alkali chlorides (bottom). The sequence obtained for the carraghene:



together with the slight valence effect, which appears from the little distance horizontally from the centre of gravity of the former (2—1) and of the latter (1—1) group, are in agreement with previous measurements. They are characteristic of a negative colloid with ionized groups (ester-sulphate) which are less polarizable than the watermolecule.

The sequences obtained for the (weakly negative) gelatine, Ca—Ba—Sr—Mg (a "transition" series) and Li—Na—K, besides the very pronounced valence influence (2—1 ..... 1—1), on the other hand, are indicative that the negative ionized groups of the protein (COO') are considerably more polarizable than the watermolecule<sup>1)</sup>.

The theory of the flocculations advanced here of gelatine + carraghene with inorganic cations as *tricomplex flocculation* is in agreement with what has been discussed in the previous pages.

For a more detailed discussion of the nature of the tricomplex systems we must refer to a previous Communication<sup>2)</sup> in which we have pointed out that it is favourable for the formation of these systems when the negative group of the amphiion (here gelatine near its I.E.P.) is better polarizable than the negative group of the anion (here the colloid anion: carraghene with ester sulphate groups). In the Communication mentioned we also discussed in how far the ion spectra of the two colloids can be of importance for specific differences which are so striking among the inorganic cations with regard to the tricomplex flocculation (Fig. 1 and 2).

It is there namely seen that these ion spectra are not themselves determining, but the distances between the place of an ion in the two ion spectra.

This is made clear in Fig. 3, where we must compare the lines drawn under the ion spectra, with regard to their lengths.

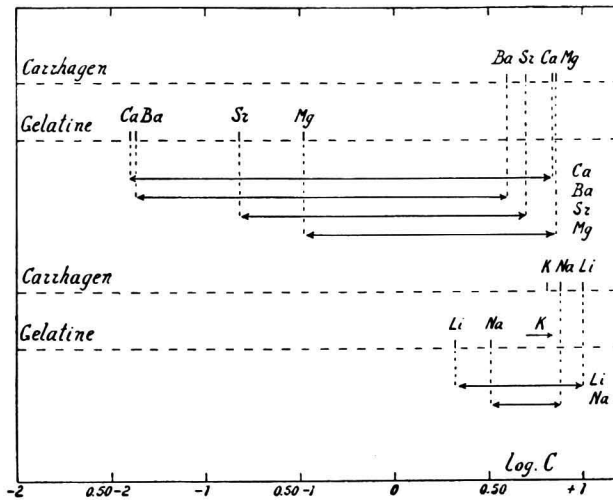


Fig. 3.

These lengths (difference of the logarithms of the reversal of charge concentrations) are given in the last column of Table 1. In this Table we have purposely arranged the salts

<sup>1)</sup> As regards their polarizability the COO' group of the proteins is apparently between that of the carbon hydrate colloids with carboxyl group (pectate, arabinat etc.) and those of the soaps, which is seen from a comparison of the ion spectra. These spectra have been discussed in H. G. BUNGENBERG DE JONG and P. H. TEUNISSEN, Koll. Beihefte, **48**, 33 (1938).

<sup>2)</sup> H. G. BUNGENBERG DE JONG, Proc. Ned. Akad. v. Wetensch., Amsterdam, **41**, 788 (1938).

below each other in such a way as agrees with the sequence following from Fig. 1 and 2 for the decreasing tendency to flocculation. It is then to be expected that the logarithmic distances ( $\Delta \log C$ ) will decrease from top to bottom in the Table, which is indeed the case.

Finally we mention the fact that the experiments discussed thus far were made with  $\text{pH} = 5.5$ , that is a little higher only than the I.E.P. of the gelatine used (ca. 5.1). With this  $\text{pH}$  the gelatine behaves still very markedly as an amphoteric. But we have been able to state that even until ca.  $\text{pH} = 10$  the amphoteric character is noticeable. With  $\text{pH} = 8$  we have still made turbidity measurements analogous to those of Fig. 1, with which we still found considerable flocculation with the earth alkali-chloride and with it likewise the order  $\text{Ca} > \text{Ba} > \text{Sr} > \text{Mg}$ .

The same order ( $\text{Ca} > \text{Ba} > \text{Sr} > \text{Mg}$ ) proved to be present with  $\text{pH} = 9.7$ , although there the flocculation was already fairly weak. With this  $\text{pH}$  we still found very weak flocculation with  $\text{Li}$ , but it was absent with  $\text{Na}$ .

All this is of biological importance, as from it is seen that it is possible that acid proteins (which are of so frequent occurrence in the organism) with  $\text{pH}$  values of 6–8 can sufficiently react as amphoteric to make it possible for tricomplex systems to occur with natural medium conditions.

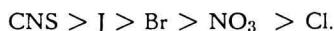
We finish here with a few remarks:

a. We must assume that the carraghenate colloid anion attaches itself to positive ionized groups of the protein (for which the arginine and lysine groups for instance come into consideration). Although in diluted sols without the addition of salts there is nothing striking in this respect, this is different when the 3 % gelatine sol and the 0.1 % carraghenate sol mentioned are mixed undiluted. It can then clearly be seen macroscopically that the viscosity of the sol mixture increases considerably.

b. The tricomplex flocculation has the characteristics of the complex systems, that neutral salts in general have a neutralizing effect. This is already seen from the curves of Fig. 1 and 2, where the same salts which in smaller concentrations cause tricomplex flocculation, neutralize it in greater concentrations. But we also find this when a second salt is added. The tricomplex flocculation with  $\text{CaCl}_2$  disappears, for instance, when some solid  $\text{KCl}$  is added. With mixing proportions of gelatine + carraghenate with which  $\text{LiCl}$  and  $\text{NaCl}$  themselves cause practically no tricomplex flocculation (the conditions of Fig. 1) the same may be said of these salts.

We have measured a few more series with the extincometer, with which with constant  $\text{Ca}$  concentration the concentration of another salt increases. We then found again specific differences between the monovalent cations themselves and the monovalent anions, namely on comparison of the chlorides the neutralization is more difficult (i.e. with higher concentrations) from left to right with:  $\text{K} > \text{Na} > \text{Li}$ .

Likewise on comparison of a number of  $\text{K}$  salts the neutralization is more difficult from left to right with



This anion sequence is the same as O. BANK and E. G. HOSKAM<sup>1)</sup> found for the neutralization of complex systems with positive proteins. It is namely the sequence of affinity decreasing from left to right of the positive ionized groups of a protein<sup>2)</sup>.

From the order  $\text{K} > \text{Na} > \text{Li}$ , which is the one of decreasing affinity of carraghenate we think we may conclude that on neutralization of the tricomplex system — as far as the cation action is concerned, the screening of the carraghenate colloid anion is the determining factor in the final result. (Compare § 4 of Communication II)

<sup>1)</sup> O. BANK and E. G. HOSKAM, *Protoplasma* **34**, 188 (1940).

<sup>2)</sup> L. TEUNISSEN VAN ZIJP, *Diss. Leiden* 1939.

*Summary.*

1. In mixtures of gelatine and carraghene sols flocculations are formed in the pH section between the I.E.P. of the gelatine and pH ca. 10 with a number of inorganic salts, which do not occur in the presence of only gelatine or only carraghene.
2. The tendency to flocculation of the sol mixture decreases in the following series from left to right:  $\text{Ca} > \text{Ba} > \text{Sr} > \text{Mg} > \text{Li} > \text{Na} > \text{K}$ .
3. Electrophoretic measurements show that the same order is obtained for the difference decreasing from left to right — of the logarithms of the reversal of charge concentrations of the two colloids.
4. The results obtained in 2 and 3 are agreement with the theory that this flocculation is "tricomplex", so that as real components of the flocculation we may consider: the gelatine amphoteric, the carraghene colloid anion and the crystalloid cation used for the flocculation.
5. In a pH section considerably above its I.E.P. the gelatine has still sufficiently the character of an amphoteric to make tricomplex flocculation possible.
6. The neutralizing effect of neutral salts, expressed a.o. in an „antagonism"  $\text{Ca}-\text{K}$  and  $\text{Ca}-\text{Na}$  is discussed briefly.

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