Chemistry. — *Changes in diameter of gelated coacervate drops of the complexcoacervate Gelatin-Gum arabic, resulting from a change in the pH, or from neutral salts added to the surrounding medium.* II.

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(Communicated at the meeting of November 29, 1947.)

1. *Introduction.*

In communication I the swelling-pH curve and the swelling at constant pH when salts are added, were studied [1]). They lead us to the conclusion that in gelating the complexcoacervate, the typical complex relations (electrostatic attraction versus water contents) have been retained. The *complexgel* which results after cooling down, distinguishes itself from the complexcoacervate by the coherence of the positively charged gelatin to a gel structure.

The changes in diameter studied in communication I, were reversible (or approximately so), and this was undoubtedly due to the relatively low saltconcentrations used.

Higher saltconcentrations, however, give irreversible diameter changes the cause of which will be studied in this second communication. In doing so we will restrict ourselves to study these phenomena for one salt only, viz. KCl, at room-temperature (which was during these experiments 20—23° C). The results obtained allow us to give a more complete picture of the nature of the complexgel, the negatively charged complexcomponent not partaking in the formation of the gelstructure, but remaining in principle mobile and bound to the positively charged gelatin gelstructure by electrostatical forces only.

2. *Deviations from a reversible behaviour at higher KCl concentrations.*

As stated in 1. the changes in diameter occurring in smaller saltconcentrations, are reversible, but in greater saltconcentrations deviations from reversibility are met with. The character of these deviations may be seen from fig. 1, which gives the diameters of a gelated coacervate drop, each time at the end of a 5 minutes' treatment with a very diluted acetate buffer of pH 3.7 (black dots) and at the end of 5 minutes' treatment with the same buffer containing 40, 60, 80, 100 and 120 m.aeq. p. L. KCl (white dots) [2]).

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[1]) BUNGENBERG DE JONG and J. M. F. LANDSMEER, Rec. trav. chim. Pays Bas **65**, 606 (1946).

[2]) For preparation of the gelated complexcoacervate drops and technique of the measurements see Communication I. The acetate buffer contains 2 m. aeg. p. L. Na acetate.

So the abscissa gives only the number of treatments, but since each treatment lasted 5 minutes, it indicates the time as well. The black and white dots are connected by lines, which show the successive order of the treatments. We see therefore, that the alternate treatments with buffer (B) and buffer containing salt (B + S)| was applied five times before we took a higher KCl concentration. The drop could not be followed further as in indicated in the figure, for it was washed away by the liquid streaming through the cuvette.

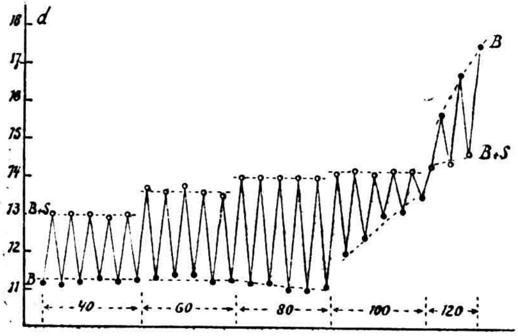

Fig. 1.

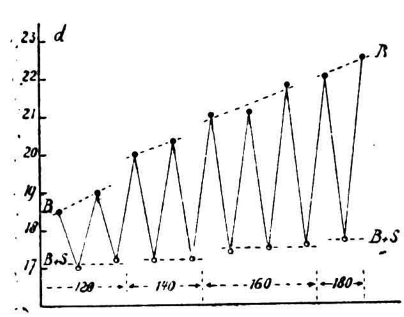

Fig. 2.

Fig. 1 shows the reversible character of the augmentation in diameters in 40 and 60 m.aeq.KCl, the white dots, apart from fluctuations (due to errors in the estimation) lying on a horizontal niveau (mean 11.16) the same being the case with the black dots at 40 and at 60 m.aeq.p.L. The first signs of deviation from strict reversibility are manifest during the treatment with 80 m.aeq.p.L. KCl, as the diameters after B treatment decrease a little.

Very conspiceous deviations, however, occur during the treatment with 100 m.aeq.p.L. KCl. After each 5 minutes' contact with the salt solution (B + S) the shrinking in buffer (B) becomes less.

This change of behaviour continues during the treatment with 120 m.aeq. p.L. KCl. Though coming from B to B + S the first time a small swelling (13.6 → 14.3) takes place, from now on the character of the diameter changes has reversed.

Instead of shrinking, a swelling takes place when we return from B + S to B (14.3 → 15.8), and a shrinking when going from B + S towards B (the black dots are now above the white ones).

This reversal in the type of diameter changes is also present at still higher KCl concentrations. See fig. 2, in which the diameter changes of another complexcoacervate drop are given, which was exposed during 20 minutes to B + S containing 120 m.aeq.p.L. KCl.

The first black dot given in this figure, refers to the diameter at the end of this 20 minutes' period. The period of alternatively treating with B + S or with B was in this experiment 10 min., and in connection with this a fewer number of treatments (only 2) with each salt solution was taken. Comparied with figure 1, we see here a continuation of the changes, which began in fig. 1 at 100 and 120 m.aeq.p.L. KCl.

3. *Diameter changes in KCl solutions of pH 3.7, preceeded by and followed by a determination of the swelling pH curve.*

The method used in 1., in which gelated coacervate drops were successively exposed to different KCl concentrations, is not very suitable for studying the irreversible changes. In the following we take therefore for every KCl concentration a new object-slide covered with gelated coacervate drops from the stock, kept in the refrigerator.

Further we always stick to the following experimental procedure:

a. determination of the swelling pH curve, during which experiment the drops are successively exposed to diluted acetate buffers for 5 minutes (with 2 m.aeq.p.L. Na acetate constant) having the pH values 2.9; 3.1; 3.4; 3.6; 3.8; 4.0; 4.2; 4.4; 4.6;

b. then the salt treatment at pH 3.7 follows. To this end the drops are first exposed to buffer 5 min. (as above), then 5 min. to buffer + salt, and so on alternating B and B + S and ending with B, till a treatment with B + S has been given four times in all;

c. thirdly follows once more the determination of the swelling pH curve as described in a., in which if necessary the series of pH values is extended to other values.

In this experiment three gel-globules adhering to the object-slide, are measured which are so chosen that they have different diameters [3]) and the mean of these 3 values was approximately the same in every experiment with different salt concentrations. As an example the measurements for the case the KCl concentration was 110 m.aeq.p.L. are given in table I.

Inspecting this table, we recognise in the middle part (alternative treatment with B and B + S) the same points which were discussed in section 1. For we see that the values B + S hardly change, whereas the values B increase after each salttreatment, apart from a slight decrease in the very beginning.

For each of the four successive salttreatments the diameters B + S are given in the following table II expressed in per cent of the values B (1 → 2; 3 → 4; 5 → 6; 7 → 8). At the bottom of the table the average of these percentage values are given.

The increase (123 → 128) of the values 100 (B + S)/B, when passing from (1 → 2) to (3 → 4) is the result of the small decrease of the B values which takes place in the beginning. However, the strong decrease (128 → 113 → 109) of the values 100 (B + S)/B, when passing from (3 → 4) to (5 → 6) and to (7 → 8) is the result of the strong increase of the B values during the continued salttreatment.

[3]) Diameters in Table I and others are given in scale divisions of the objectmicrometer, 50 divisions = 670 μ.

TABLE I.

pH	Drop 1	Drop 2	Drop 3
2.9	31.3	17.0	13.2
3.1	27.0	14.5	11.2
3.4	25.3	13.5	10.8
3.6	24.8	13.6	10.8
3.8	24.9	13.8	10.8
4.0	25.0	14.0	10.9
4.2	26.0	14.4	11.1
4.4	28.5	15.8	12.3
4.6	32.4	17.8	13.9
B (1)	25.8	13.5	10.4
B+S (2)	31.0	16.9	13.0
B (3)	24.9	13.0	10.0
B+S (4)	31.0	16.9	13.0
B (5)	26.2	15.0	12.0
B+S (6)	30.9	17.0	13.0
B (7)	26.7	15.9	12.8
B+S (8)	31.0	17.1	13.1
B (9)	27.5	16.3	13.1
3.4	33.8	20.0	16.0
3.6	29.4	17.5	14.0
3.8	26.0	16.0	12.7
4.0	24.2	14.0	11.1
4.2	23.5	13.0	10.3
4.4	23.6	13.0	10.0
4.6	24.0	13.0	10.2
4.8	25.1	13.3	10.5
5.0	27.0	14.2	11.0
5.2	28.9	15.0	12.0
5.4	31.0	16.6	12.8

TABLE II.

	$100\frac{B+S}{B}$ (1 → 2)	$100\frac{B+S}{B}$ (3 → 4)	$100\frac{B+S}{B}$ (5 → 6)	$100\frac{B+S}{B}$ (7 → 8)
Drop 1	120	125	118	116
Drop 2	125	130	113	108
Drop 3	125	130	108	102
Average	123	128	113	109

When we now direct our attention to the upper and lower section of table I, we see that the minima in the swelling pH curve (in the upper section laying somewhere between 3.6 and 3.8) have been considerably displaced by the salt treatment (in the lower section the minima lay somewhere between pH 4.2 and 4.4). With the aid of the graphical method described in communication I (construction of bisector lines), we find for these minima:

prior to KCl treatment pH = 3.70; 3.66; 3.68 mean pH = *3.68*
after KCl treatment pH = 4.30; 4.48; 4.48 mean pH = *4.39*.

However, for the interpretation of the phenomena described in 1. it will not suffice only to state that a salt treatment results in a shifting of the swelling pH curve in horizontal direction, i.e. towards higher pH values, but we must also take into consideration the simultaneously occurring shifting of this curve into the vertical direction.

To this end all ordinate values were expressed in percents of the ordinate value at the minimum of the swelling pH curve before the salt treatment.

For drop 1 (See table I) the ordinate values are therefore expressed in per cent of 24.8, for drop 2 in per cent of 13.5 and for drop 3 in per cent of 10.8. This has been done in the next table III.

TABLE III.

Ordinate values of the swelling pH curves before and after the treatment with 110 m. aeq. p. L. KCl, expressed in per cent of this value at the minimum of the curve before the salt treatment.

pH	Before KCl treatment			Mean	After KCl treatment			Mean
	Drop 1	Drop 2	Drop 3		Drop 1	Drop 2	Drop 3	
2.9	126	126	122	125				
3.1	109	107	104	107				
3.4	102	100	100	101	136	148	148	144
3.6	100	100	100	100	119	130	130	126
3.8	100	102	100	101	105	119	118	114
4.0	101	104	101	102	98	104	103	102
4.2	105	107	103	105	95	96	95	95
4.4	115	117	114	115	95	96	93	95
4.6	131	132	129	131	97	96	94	96
4.8					101	99	97	99
5.0					109	105	102	105
5.2					117	111	111	113
5.4					125	123	119	122

We see that after the salt treatment the minimum of the swelling pH value has not only shifted towards anoher value on the abscissa axis (pH 3.68 → 4.39) but to another value of the ordinate (100 → 95) as well.

In the same way as has been described above for 110 m.aeq.p.L. KCl, other series were measured, the KCl concentrations being then 40, 70, 100 and 140 m.aeq.p.L. KCl. We further added a series with zero KCl concentration, i.e. a series in which between the 2 swelling-pH curves, only B was lead through the cuvette, and the diameters of the three drops chosen were measured nine times with 5 minutes' intervalls

The results (already averaged for the three drops) of these series are given in table IV.

TABLE IV.

KCl m. aeq. l. L.	pH of the minimum be-before KCl treatment	$100\frac{B+S}{B}$				pH of the minimum after KCl treatment
		1 → 2	3 → 4	5 → 6	7 → 8	
0	3.65	99	100	100	100	3.69
40	3.62	115	115	115	115	3.67
70	3.63	119	121	123	122	3.74
100	3.65	125	126	126	122	4.15
110	3.68	123	128	113	109	4.42
140	3.57	129	97	89	85	4.81
Mean	3.63					

Fig. 3 gives the values $100\frac{B+S}{B}$ as function of the salt concentrations after the first (1 → 2), second (3 → 4), third (5 → 6) and fourth (7 → 8) salt treatment.

We see that all concentrations of KCl used, produce a swelling in the beginning (1 → 2), but this swelling is only reversible with sufficiently

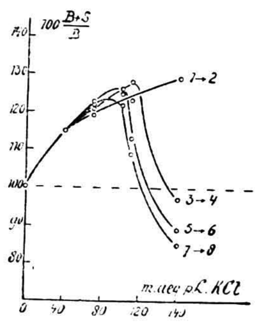

Fig. 3.

low KCl concentrations (0 and 40 m.aeq.p.L.). Indications of deviations from strict reversibily occur already with 70 m.aeq.p.L. KCl, and with sufficiently high concentrations (e.g. 140 m.aeq.p.L.) the second salt treatment gives already a shrinking instead of a swelling.

From the displacement of the steep branches of the curves (3 → 4), (5 → 6), (7 → 8) to the left, we are lead to the conclusion that this reversal of the swelling behaviour is not only a function of the height of the KCl concentration, but also of the time during which the complexgel globules are exposed to a given KCl concentration. (This will be confirmed later in section 4).

Next we direct our attention to the displacement of the swelling pH curve. Quite in the same way as was discussed above for the series with 110 m.aeq.p.L. KCl, all ordinate values are expressed in table V in per cent of this value at the minimum of this curve before KCl treatment.

Table V is therefore quite analogous to table III, only with this difference that the numbers in the columns 3—8 are already the mean values of the three drops measured. The values in column 2 are the means of all blanc series before the KCl treatment and relate to $6 \times 3 = 18$ drops, consequently.

TABLE V.

Mean values of the ordinates of the swelling-pH curve before and after treatment with KCl solutions, expressed in per cent of the ordinate value of the minimum before KCl treatment.

pH	Before KCl treatment	After treatment with KCl of the indicated concentrations in m. aeq. p. L.					
		0	40	70	100	110	140
2.9	124	130	137	137	150		
3.1	106	109	108	116	134		
3.4	101	102	100	98	112	144	
3.6	100	100	98	96	101	126	
3.8	101	100	99	96	96	114	
4.0	102	102	100	98	93	102	145
4.2	106	104	104	99	95	95	136
4.4	115	113	116	105	99	95	111
4.6	127	125	130	117	106	96	103
4.8				127	114	99	102
5.0					125	105	103
5.2					132	113	108
5.4					141	122	117
5.6							123
5.9							130

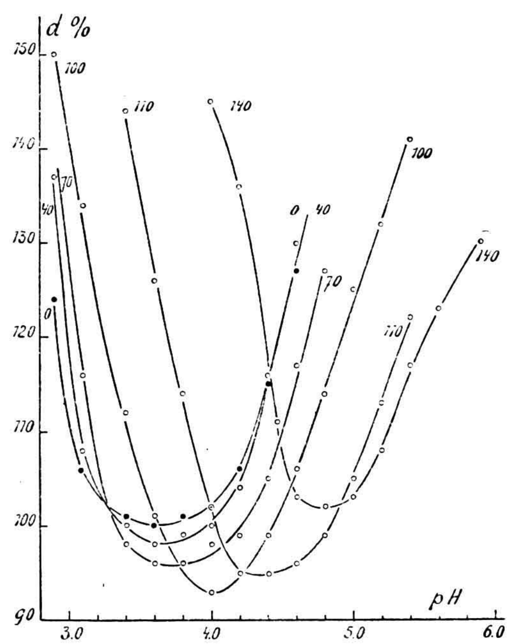

Fig. 4.

The percentage values of table V are given in fig. 4 as functions of pH. The curve corresponding with column 3 has not been embodied in this figure as its minimum tract practically coincides with the one of the blanc curve of column 2, and we are especially interested in the relative position of the curves in the neighbourhood of their minima.

So we see in this figure that with a treatment during a constant time with KCl solutions of increasing concentration, the swelling pH curve is displaced from its original position, the more so, as the KCl concentration used is higher.

This displacement contains two components:

a. a displacement in horizontal direction, as a result of which its minimum is displaced towards higher pH values,

b. a displacement in vertical direction, which takes place at first in a downward direction, and afterwards in an upward one.

(To be continued.)