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.* III.

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

4. *Factors controling the rate of displacement of the swelling-pH curve.*

In the preceeding section *) the salt treatment was applied for a constant time and a displacement resulted which was the graeter the higher the chosen salt concentration.

This effect of the salt concentration might be due a. to a certain displacement characteristic of each salt concentration, and which will not further increase with time, b. to different velocities of displacement, depending on the salt concentration.

Though we met already in 2. indications in favour of explanation b., we have made experiments, which would ennable us to choose either a. or b. In this series of experiments the complexgel-globules are exposed for different spans of time (up to 5 houres) to KCl solutions of different concentrations (40, 100, 150, 250 and 500 m.aeq.p.L.).

As the procedure for measurements in order to enable us to study the 2 components a. and b. of the displacement (see the end of section 2) would be too cumbersome for this programme, we used a simplified procedure by which only component a. was determined.

We omitted to determine the swelling pH curve before the salt treatment, as the mean pH value of its minimum was already known from section 2. Further we also omitted to measure the diameters during the salt treatment and only determined the swelling pH curve after a well defined treatment, as regards concentration and time.

We therefore simply put a number of object-slides, covered with gelglobules (taken from the stock in the refrigerator) in a flat glass tray, filled with the desired KCl solution, and took one by one at stated intervals from the tray, which we then used to measure the swelling pH curve (on three geldrops). In order to obtain reproducible results, it was necessary to tilt the glasstrays gently during the salt treatment by means of a mechanical device. Table VI gives the pH values for the minimum for each of the three drops, while the diameters of these drops at their minimum is added in parentheses.

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*) See H. G. BUNGENBERG DE JONG and J. M. F. LANDSMEER, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, 51, 137 (1948).

TABLE VI.

KCl m, aeq. p. L.	2 min.	3 min.	5 min.	10 min.	15 min.	25 min.	30 min.	1 hour	5 hours
40 (20.4°)			3.74 (9.0) 3.71 (11.8) 3.76 (12.8) **3.74**				3.78 (8.8) 3.72 (11.9) 3.76 (12.8) **3.75**	3.80 (9.5) 3.79 (12.4) 3.76 (13.8) **3.78**	3.71 (11.8) 3.76 (13.2) 3.74 (14.2) **3.74**
100 (20°)			4.02 (11.3) 3.97 (13.8) 3.96)15.9) **4.01**		4.10 (10.3) 4.10 (13.8) 4.06 (14.8) **4.09**			4.36 (9.8) 4.34 (11.7) 4.29 (12.9) **4.32**	4.56 (8.2) 4.56 (11.4) 4.52 (13.9) **4.54**
150 (20.6°)		4.02 (11.1) 3.99 (13.0) 3.95 (14.9) **4.00**		4.28 (11.9) 4.18 (13.5) 4.22 (14.4) **4.28**				4.62 (10.2) 4.62 (11.3) 4.61 (13.3) **4.62**	4.80 (13.7) 4.80 (16.1) 4.78 (18.3) **4.81**
250 (20.8°)	4.20 (7.8) 4.16 (9.6) 4.10 (11.2) **4.10**			4.80 (7.1) 4.62 (9.6) 4.42 (14.1) **4.52**			4.92 (6.7) 4.74 (10.8) 4.72 (12.1) **4.71**		5.00 (9.9) 4.84 (12.5) 4.86 (16.6) **4.91**
500 (21°)	4.50 (6.8) 4.40 (7.6) 4.16 (11.5) **4.13**			4.80 (6.7) 4.70 (8.6) 4.68 (11.3) **4.67**		4.82 (6.0) 4.90 (7.7) 4.71 (10.4) **4.81**			5.02 (7.8) 5.00 (10.6) 4.84 (15.2) **4.93**

An inspection of the table reveals that the displacement is not determined by the KCl concentration only but by the time of contact with the KCl solution as well. So we see from this that of the above alternative case b. applies: the displacement with a given salt concentration is function of time, in which the rate of displacement is determined by the salt concentration. The velocity of displacement, however, is not only a function of the KCl concentration but of the sizes of the gelglobules as well. This is evident from the fact that we generally find a higher pH value for the minimum after the same salt treatment for small gelgobules (compare with each 3 values those in parenthesis).

With the aid of graphs we have therefore computed the pH value which a gelglobule of 12 scale divisions would show. These pH values given in the table in fat print, have been used in fig. 5, which gives an idea of the rate of displacement of the swelling pH curve, while the latter is represented in the figure by the pH values of their minimum points only (the component a of the displacement, see above).

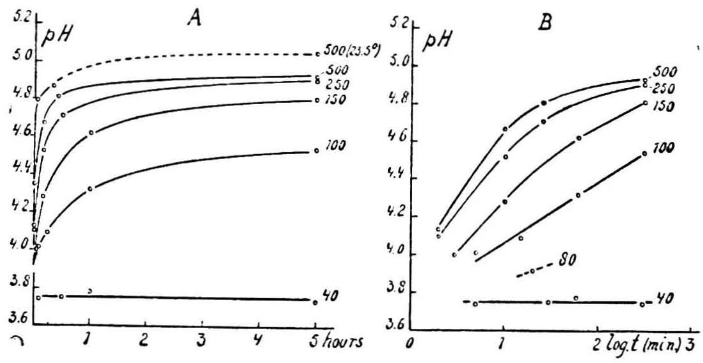

Fig. 5.

We see from fig. 5 A, that after a rapid change the process slows down very considerably. A representation, which allows us to follow also the courses of the curves after brief treatments is given in fig. 5 B, in which we have used the logarithms of the time in minutes as abscissa.

The figure has two curves for treatment with 500 m.aeq.p.L., the lower one corresponding with the data of table VI at 21° C and the higher (dotted) one corresponds which with measurements performed a few days later at 23.5° C. We see that the rate of displacement is probably also dependent on the temperature. We have therefore made some experiments at different temperatures, using a specially constructed cuvette, which could be cooled or heated by streaming water of the temperature desired.

In these experiments which were of the type described in section 1., we used a new preparation of complexgel globules [4]).

[4]) At this place we thank Mr. A. M. VAN LEEUWEN for his valuable help in the experiments and also in some others reported in this communication.

Fig. 6 gives a comparison of the treatments at 18.8 and at 22.9° C for 10 minutes alternately with diluted acetate buffer (pH 3.7, always containing 2 m.aeq.p.L. Na acetate) and with this buffer to which 500 m.aeq. p.L. KCl was added.

We see the strong influence of the temperature. At 18.8° C a reversal of the swelling character tends to occur only after several salt treatments,

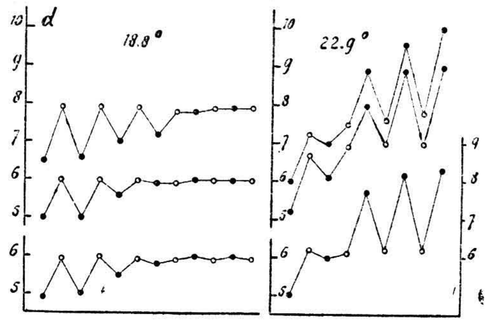

Fig. 6.

at 22.9°, however, this reversal occurs very soon already. The three gelglobules, taken for the experiment at 22.9°, did not differ much in diameter, for which reasen the influence of the diameter on the rate of the changes was not clearly seen. This influence is obvious in the experiment at 18.8° C, where the differences in sizes of the drops was a little greater. We clearly see, that the largest drop had to be given at least

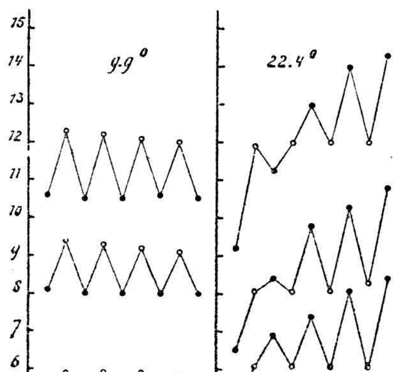

Fig. 7.

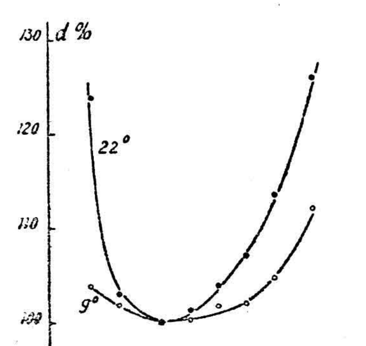

Fig. 8.

one salt treatment more than the other two before the black dots came on the same level with the white ones.

In a further experiment we compared the treatment with 500 m.aeq.p.L. at 9.9° C and at 22.4°. See fig. 7, which needs no further comment as it clearly shows the very strong influence of temperature (and also the influences of the sizes of the gelglobules).

In this experiment we followed the procedure described in section 2. by which we also determined the swelling pH curves before and after the salt treatment. The minimum before KCl treatment was found at pH 3.51 after the treatment at the mean values pH = 4.13 (9.9° C) and pH = 4.69 (22.4°). The shifting of the minimum towards higher pH values was therefore 0.62 at 9.9° C and 1.18 at 22.4° C, which once more demonstrates the accellerating influence of higher temperatures.

In the individual pH values for the globules the influence of their sizes was also clearly visible. In the series at 9.9°, we found 4.06 (10.6); 4.13 (8.1); 4.20 (5.0), in the series at 22.4° the values were 4.63 (9.2); 4.70 (6.5) and 4.73 (4.9), while the values, added between brackets, represent their sizes in scale divisions at pH 3.7 before KCl treatment.

It may be added that another influence of temperature was visible during these experiments, viz. that the steepness of the branches of the swelling pH curve is much smaller at lower temperature. Compare fig. 8, which gives two such curves (before the salt treatment), in which the ordinate values are expressed in percentages of the ordinate value at the minimum.

5. *Discussion.*

All experiments described in the preceeding sections can be simply explained by assuming that the negatively charged component of the complexgel, the gum arabic, which is attached by electrostatical forces to the positively charged gelatin, does not share in the formation of the gel structure.

So long the electrical attraction is sufficient, the gum arabic remains attached to the positively charged frame work of the gel structure formed by the gelatin. If, however, this attraction is diminished by salts added, the gum arabic, which is mobile in principle, really gets a chance of diffusing out of the gel globules, into the liquid streaming along the globules. This hardly occurs in a short time with smaller salt concentrations up to 40 m.aeq.p.L. KCl, but at higher concentrations the conditions for this loss of gum arabic becomes more and more favourable.

As the liquid, surrounding the gel globules, is constantly streaming through the cuvette, the gum arabic, which leaves the gel globules, is lost for good and all. Hence, the irreversible character of the change in properties brought about by the treatment which such higher KCl concentrations.

This change in character of the properties, manifests itself in a reversal of the swelling behaviour by adding KCl to the dilute buffer. Whereas originally KCl causes a swelling of the gel globule, after a sufficient KCl treatment the gel globules shrink, if KCl is added to it (see figures 1, 2, 6 and 7).

This reversal of the swelling behaviour is exactly what should be expected in consquence of a rigorous loss of gum arabic from the gel globule, as it will then closely approach the nature of a gel globule, which only consists of swollen gelatin. But we know that a positively charged gelatin gel shrinks when salts are added [5]).

All other experimental results are in accordance with the above postulated loss of gum arabic from the complexgel globules, such as, for instance, the displacement of the swelling pH curve towards higher pH values (see the figures 4 and 5). Accordingly it must be expected that this displacement will come to an end, when all gum arabic has diffused out, leaving gel globules, which contain gelatin only. The pH at the minimum of the swelling-pH curve of a gel globules which have so rigorously been treated with salt should then be found at the I.E.P. of the gelatin used.

We have, therefore, prepared gel globules existing of gelatin only according to a method described previously [6]) and have measured quite in the same way as in the sections 2. and 3. the swelling-pH curves.

By the same graphical method we found values between pH $= 5.04$ and 5.09 for the pH at the minimum.

But they are the very values which were obtained under the most rigorous conditions in section 3 (e.g. in table VI the very small globules after a 5 hours' treatment with 500 m.aeq.p.L. KCl, and in fig. 5 A also larger globules with the same KCl concentration at 23.5° C, which value was no further changed after 24 hours' treatment).

The influence of the size on the rate of displacement (section 3) is quite in accordance with the above explanation, (diffusing out of the gum arabic) for the time necessary for a same procentual loss of gum arabic is shortened if the globules are smaller.

There are, however, two points in section 3 which seem not to be in accordance with this diffusion: a. the very considerable slowing down of the rate of the displacement in fig. 5 A, and b. the very great influence of temperature.

As to a., a certain amount of slowing down of the rate of displacement on itself does not form the main difficulty, as the concentration gradient must necessarily decrease in the beginning as diffusion proceeds. But as the globules are spherical, the diffusion will continu at a greater rate at the end, whereas in reality the displacement continues very slowly. We think the very considerable slowing down could be explained by considering, that the gum arabic is present as kinked macromolecules and that a certain fraction of the latter is in a favourable position to diffuse out, the remaining fraction being more or less enmeshed in the gelatin gel

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[5]) H. G. BUNGENBERG DE JONG and J. PH. HENNEMANN, Kolloid Beih. **36**, 123 (1932), see p. 137—138 and fig. 4.

[6]) H. G. BUNGENBERG DE JONG, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **41**, 646 (1938). Because of the poor visibility of these globules, when swollen at either side of the minimum, we used globules slightly stained with a dye not interfering the experiment. To this end we added some collargol to the gelatin solution before adding the $(NH_4)_2SO_4$ solution. The coacervate drops, which after cooling down and washing out furnished our globules, are then stained a slightly brown.

structures, so that its diffusing-out is considerably hindered, though not completely.

A factor which favours the diffusing-out will, no doubt, be the swelling of the gel globules, which accompanies the decrease of the effective attraction of negative gum arabic and positive gelatin.

And this effect, being the graeter, the higher the salt concentration, explains why the loss of gum arabic is considerably increased by increasing the salt concentration (see fig. 5).

The great influence of temperature may be explained on similar lines, the gelatin gel frame work being evidently more deformable at higher than at lower temperatures (compare the swelling-pH curves in fig. 8). This influence will be very great at the higher temperatures, e.g. 23.5° C, which lays already near to the temperature at which the complexgel globules melt down to complexcoacervate drops (near to 27° C).

For the explanation of the irreversible change in the swelling behaviour, described in section 1, fig. 4 has a central importance.

Though this figure gives the positions of the swelling-pH curves after a treatment with different KCl concentrations for a constant time, it also represents, as is clear from the results obtained in section 3, the successive positions of the swelling-pH curve at constant KCl concentration and increasing times of treatment.

This whole series of the changes in the positions of the curve takes place with decreasing velocity (this follows from the slowing down discussed above) and the "mean" rate of this passage depends on the height of the KCl concentration, and is the faster the higher the salt concentration.

These different "mean rates of passage" explain a further detail of the experiments in section 1. In that section we worked at constant pH (= 3.7). When we examine fig. 4, we see that the points at pH 3.7 have different ordinate values on the successive curves. As the successive curves also indicate (as discussed above) the successive positions of the swelling pH curve for one constant salt concentration and increasing time, we notice that the gel globule must first shrink a little, pass through a minimum diameter and afterwards must increase [7]) as the loss of gum arabic proceeds.

Indeed, if now the "mean rate of passing" is sufficiently slow (as for the experiment with 100 m.aeq.p.L. KCl in section 1. we get a change of observing this initial small decrease of the globules before the large increase sets in (see the trend of the black dots in fig. 1).

If, however, the "mean rate of passing" is much greater, it may be that with the chosen experimental procedure the first detail (the decrease of the ,,B" values) cannot be observed, but only the rapid increase (see black dots in fig. 6 and fig. 7).

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[7]) This change in diameter as time goes on, may be expected, not only for pH 3.7, but, as can be seen from the relative positions of the curves in fig. 4, for any other point on the orriginal swelling pH curve as well.