CXLIII. INVESTIGATIONS ON GELATIN. PART VIII. THE OSMOTIC PRESSURE OF GELATIN IN SOLUTIONS OF SODIUM SALICYLATE.

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THE present investigation was undertaken under the direction of Professor S. B. Schryver with a view to ascertaining the osmotic pressure of gelatin. Previous workers on this problem have not obtained their gelatin absolutely free from ash. Although the view is now commonly held that a low ash has no direct influence on the osmotic pressure it was thought desirable, since gelatin of exceptional purity has recently been prepared in this department [1923] to study the osmotic pressure of this preparation.

Since the osmotic pressure of gelatin is extremely low it is necessary to employ a fairly strong solution in order to obtain a pressure which can be easily and accurately measured with a very simple osmometer such as has been used in this investigation. If water be used as the solvent it is not possible to work at ordinary room temperature owing to the formation of a gel. It was therefore decided to employ as the solvent of the gelatin a solution of sodium salicylate, which, whilst having no chemical action upon the colloid, prevents gel formation. The liquefaction of gelatin by certain salts has been proved to be entirely reversible by Briggs and Hieben [1920] and the present investigator finds this to be the case with sodium salicylate. This reversibility proves that the salt in question has no chemical action upon the gelatin. The presence of this salt also serves to keep the solution sterile. A strong solution of sodium salicylate has therefore been used as the solvent of the gelatin in all the experiments herein described.

THE APPARATUS USED.

The osmometer is very simple in form. The membrane was prepared from a 10 % solution of a purified collodion ("Necolloidine") dissolved in equal parts of alcohol and ether. To prepare the membrane a test-tube is dipped into the solution and immediately inverted in a conical flask (to prevent evaporation of the solvent) and allowed to drain for a minute or so. It is then dried for about 15 seconds in air before plunging into water¹. The collodion sac is fitted with a straight rubber stopper through which is

¹ The writer is indebted to Dr Brown for his kind advice on the preparation of the osmometer.

passed a length of glass tubing. This acts as the manometer. The osmometer is filled through the manometer tube by attaching to it a small funnel containing the solution of gelatin, alternately expelling air from the sac and allowing some of the solution to take its place until it is filled to a convenient distance up the tube. It is supported in a vessel containing the solution used as solvent of the gelatin. A piece of sheet rubber, through which the manometer tube passes, is fixed (by means of a rubber band) over the top of the outer vessel to prevent concentration of the solution by evaporation. Three samples of gelatin prepared in this laboratory by Mr A. B. Manning have been studied. They are:

1. Coignet's Gold Label gelatin dialysed at the iso-electric point. Ash 0.054 %.
2. The same gelatin purified electrically [Knaggs, Manning and Schryver, 1923]. This sample was ash-free.
3. The same gelatin purified electrically and "recrystallised" [see Knaggs, Manning and Schryver, 1923]. Also free from ash.

The first series of experiments was carried out with a 5 % solution of the gelatin in *M* solution of sodium salicylate. Since this was found to be somewhat on the alkaline side of the iso-electric point—the p_H of the solution at the termination of the experiment being 6.3—two other series were prepared by adding salicylic acid to the solution of sodium salicylate until a solution with p_H 5.0 was obtained for one series and p_H 4.6 for a third series.

In series 1, when the osmometer was set up the p_H of the solution of gelatin was 5.4, that of the *M* sodium salicylate outside the membrane was 7.0. At the termination of the experiment the p_H was again determined in both the inner and outer solutions and was found to be in each case from 6.2 to 6.4. In series 2 and 3 where the initial p_H was the same on both sides of the membrane no subsequent change was observed. A Walpole electrode was used for the measurement of the hydrogen ion concentration, and, since it was found impossible to obtain a constant E.M.F. reading with the strong solution under investigation it was diluted with water to about 10 times its volume for the hydrogen ion determination in each case.

To prepare the solution about 5 g. of gelatin were added to 100 cc. of the solvent and warmed to 37° in an incubator, shaking occasionally until dissolved. Osmometers were filled in the manner described, the solution in the outer vessel being in each series the same as that used for dissolving the gelatin, and the height of the column in the manometer tube was noted. It was observed that in each case the pressure of the solution within the osmometer rose fairly rapidly, reaching a maximum in most cases within 6 or 7 days. It remains constant for a few days, then falls very gradually (see Fig. 1). This gradual fall of pressure continues even after six months' standing. Biltz, Bugge and Mehler [1916] observed a similar phenomenon working with a solution of gelatin in water, but they used a very dilute solution (0.1 to 0.25 %) and their observations extended over a relatively short period of time. The curve given

herein is representative of the change of pressure which occurs within the osmometer. A number of osmometers were dismantled when the pressure had remained constant for a few days, i.e. when it was at a maximum, the strength of the gelatin solution was determined by an estimation of the nitrogen content and the aggregate weight calculated by the usual method.

Fig. 1

The results are given in the following table:

Series 1. p_H 6.3.

Sample 1. Ash 0.054 %				
Nitrogen in 5 cc. mg.	% gelatin in solution	Pressure in mm. solution	Temp. ° C.	Aggregate weight
30	3.33	451	14	16,620
26.8	2.98	402	14	16,640
Average				16,630 ± 1 %
Sample 2. Ash-free				
32.5	3.61	482	13	16,750
32.5	3.61	510	16	16,020
31.5	3.5	494	14	15,970
Average				16,246 ± 3 %
Sample 3. Ash-free				
25.15	2.79	382	13	16,380
27.8	3.09	420	17	16,700
Average				16,540 ± 1 %

Series 2. p_H 5.0.

Sample 3. Ash-free				
27.3	3.04	419	14	16,300
26.56	2.95	402	15	16,550
30.5	3.39	465	16	16,480
30.0	3.33	465	18	16,490
29.0	2.96	480	22	15,710
Average				16,300 ± 4 %

Series 3. p_H 4.6.

Sample 2. Ash-free				
30	3.33	405	15	18,540
32.8	3.63	472	15	17,380
29	3.22	394	17	18,570
30	3.33	436	19	17,480
Average				17,990 ± 4 %

Estimations of nitrogen were also made on the solution in the outer vessel at the conclusion of the experiment and the amount found was negligible, being in each case not more than 2 mg. nitrogen in 120 to 130 cc. solution.

For calculating the aggregate weight of the samples of gelatin used in this investigation it has been assumed that the maximum pressure obtained within the osmometer represents the osmotic pressure of the solution. The aggregate weights so calculated for the different samples of gelatin agree very closely, but from this investigation it appears that it is not possible to obtain a condition of equilibrium in a solution of gelatin, since it does not exhibit a constant osmotic pressure even after prolonged standing. The osmometer represented in the curve given herein was allowed to remain for 6 months undisturbed. It was found that the outer solution—which was 116 cc. in volume—contained only 2 mg. N. The solution removed from within the osmometer measured 19 cc. and contained 115 mg. N. The fall in pressure observed over this period of time may possibly be attributed to an increase in the average size of the molecular aggregate of the gelatin, the maximum pressure (which was attained after 6 days) being due to an average aggregate weight of 16,600 which has increased during the period of observation to 19,000. It might also be due to a gradual distension of the membrane, although Biltz and his co-workers [1916] obtained similar results using a rigid osmometer.

From the results recorded in the above tables it will be seen that there is no difference in the osmotic behaviour of the three samples of gelatin investigated, determinations made of each sample at the same hydrogen ion concentration (series 1, p_H 6.3) showing no difference in the aggregate weight as determined by the osmotic pressure. The presence of a very low ash in gelatin which has been dialysed at the iso-electric point therefore appears to have no influence on the osmotic pressure. The work of Loeb [1922] shows that the osmotic pressure is dependent on the p_H of the solution. The osmotic pressure is a minimum at the iso-electric point. It is a generally accepted theory that the increase in pressure which occurs with an increase in p_H is due to the formation of a salt of gelatin which ionises. The presence of another salt—sodium salicylate—increasing the concentration of the sodium ions will suppress such ionisation, so giving rise to a smaller increase in pressure as the p_H of the solution increases beyond the iso-electric point than would be the case in the absence of such a salt. The results of this investigation seem to confirm this. The increase in osmotic pressure is evidently small. Although in these results the pressure at p_H 5.0 appears to be the same as that at 6.3 whilst that at 4.6 is somewhat lower, it is probable that the pressures herein recorded at p_H 5.0 are all a little higher than the correct figure, whilst those observed at p_H 6.3 may be slightly lower than the correct figure, the possible experimental error due to the method employed being comparatively large, whilst the actual difference in pressure over the range of p_H studied is very small.

INFLUENCE OF THE CONCENTRATION OF THE GELATIN
ON THE OSMOTIC PRESSURE.

In the experiments just recorded a solution containing about 5 g. gelatin per 100 cc. was used throughout the investigation.

In order to discover whether the osmotic pressure is proportional to the concentration of gelatin in the solution a number of experiments have been made using gelatin solutions of different concentrations, viz. 1 %, 1.25 %, 2.5 % and 3.5 %. The gelatin was dissolved in a molar solution of sodium salicylate (as in the previous experiments).

It is found that the osmotic pressure of gelatin in these solutions is not proportional to the concentration of the colloid but increases very considerably with increased concentration of gelatin in the solution.

Fig. 2. Pressure per unit concentration of gelatin in sodium salicylate solution
 ○ 2M sodium salicylate × 1M sodium salicylate

The osmotic pressure of a 1 % solution of gelatin corresponds with an aggregate weight of 45,500 (if the usual formula be applied), whilst that of a 3 % solution corresponds with an aggregate weight of 16,500, the other concentrations employed giving intermediate figures as shown in the accompanying tables and curves (Fig. 2) (the curve showing the relation between the "pressure per unit concentration" and the concentration of the solution

indicates that the *increase* in pressure is proportional to the concentration of the solution).

The pressure within the osmometer (if started below the maximum obtainable for the solution under investigation) rises to a maximum, then falls gradually—as observed in the previous experiments. The results here given have been obtained by estimating the nitrogen in the solution at the conclusion of the experiment and assuming that the maximum pressure attained within the osmometer represents the osmotic pressure.

Some of the osmometers were filled to a greater height than the maximum obtainable when the initial pressure is low and in these cases the pressure fell rapidly until it agreed with the maximum pressure obtained by the first method of filling, then continued to decrease gradually as in the previous examples. As the point corresponding with the maximum obtained in the first type of osmometer cannot be readily estimated, no calculations of osmotic pressure (and corresponding aggregate weight) have been made in these cases.

Similar results were obtained when the gelatin was dissolved in $2M$ and $M/2$ salicylate solutions. In the latter case, owing to the tendency to gel formation, the readings are not very reliable. The osmotic pressures are somewhat higher in M than in $2M$ solutions (see Fig. 2).

Osmotic Pressure of Gelatin in Sodium Salicylate solution.

Nitrogen in 10 cc. solution mg.	Concentration of gelatin solution % (N = 18 %)	Pressure in mm. solution	Pressure per unit concen- tration of gelatin mm.	Temperature ° C.	Aggregate weight calculated from pressure
<i>M sodium salicylate</i>					
15	0.83	41	49.4	14	45,500
15.25	0.85	41	48.2	11	45,750
17.2	0.95	52	54.7	13	41,140
28.6	1.59	116	73	14	30,770
30.7	1.71	126	73.9	13	30,310
31.4	1.74	130	74.7	15	30,420
39.4	2.19	194	88.6	11.5	25,110
40.7	2.26	207	91.6	13	24,470
55.4	3.09	420	135	17	16,700
60	3.33	451	135	14	16,620
65	3.61	482	134	13	16,750
65	3.61	510	141	16	16,020
<i>M/2 sodium salicylate</i>					
33.1	1.84	116	63	14.5	35,690
34.1	1.91	139	72.8	14	30,890
35.2	1.95	136	69.7	15	32,420
13.07	0.726	32	44	14	51,100
14.43	0.8	40	50	14	45,130
<i>2M sodium salicylate</i>					
14.48	0.80	33	41.2	14	60,980
12.2	0.68	25	36.8	14	53,730
28.8	1.60	101	63.1	14	35,550
33.1	1.84	128	69.6	14.5	32,330
35.48	1.97	141	71.6	13	31,330
50.83	2.82	280	99.3	13	22,580
56	3.11	337	108.3	13	20,660

It is not possible with our present knowledge to explain the results obtained in this investigation, as so many factors such as solvation, salt-formation, and others which are perhaps not yet known, may have to be taken into account before any satisfactory generalisations can be made.

SUMMARY.

(1) The osmotic pressure of gelatin in sodium salicylate solutions has been determined.

(2) This depends on the concentration of the gelatin in the salicylate solution. The lower the concentration, the lower the osmotic pressure and the higher the apparent aggregate (or molecular) weight; thus in a 3 % solution in *M* sodium salicylate the apparent aggregate weight is about 16,000; this rises to about 40,000 in 0.9 % solutions.

(3) The osmotic pressure is not apparently influenced by small amounts of inorganic material in the gelatin.

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