

the amount of copper lost due to surface oxidation and how to avoid it. The catalytic effect of the walls of the container has been demonstrated.

A procedure for the proposed method with comparison of results for the determination of the reduced copper has been described, including a discussion of the degree of accuracy of the new method.

The reducing action of sucrose on Fehling solution, alone and when mixed with different proportions of reducing sugars, has been investigated with the view of elimination of the use of empirical formulas and factors for calculation of errors involved.

The new method is designed for use in the analysis of saccharine materials containing sucrose, dextrose, levulose, invert sugar, lactose and maltose. It is not claimed that this method can be used for the accurate quantitative determination of 0.1% or less of invert sugar in approximately pure sucrose.

NEW YORK, N. Y.

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[CONTRIBUTION FROM THE RESEARCH LABORATORY OF THE NATIONAL BISCUIT COMPANY,  
No. 1.]

## VISCOSITY OF GELATIN SOLUTIONS.

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Owing to the divergence of results and conclusions arrived at regarding the various physical properties of gelatin solutions an investigation of the effects of different physical and chemical treatments of such solutions has been undertaken by this laboratory.

In this paper the investigation of the viscosity of gelatin solutions is described. Detailed results of the effects on viscosity of (1) method of effecting solution, (2) aging of solution, (3) concentration of hydrogen ion, (4) concentration of gelatin, (5) hydrolysis of gelatin are given for three different types of gelatins. Finally, a method is given whereby the viscosity of a gelatin may be determined with an accuracy well within 0.5%.

Previous work has shown that viscosity is affected by the age of the solution, concentration of the gelatin, the temperature employed for effecting solution and by the character of the gelatin. With the exception of the first two factors very few, if any, results of a definite nature have been published. Much work has been done on the effects of various salts, but Loeb<sup>1</sup> has shown the fallacy of the "Hofmeister Series" and has furnished adequate chemical data in explanation of such salt effects.

<sup>1</sup> Loeb, *J. Gen. Physiol.*, [III] 1, 85 (1920).

Gelatins are of at least three kinds, depending upon the method of manufacture. No. 1 is obtained by extracting ground bones with water, leaving the mineral matter behind. No. 2 is obtained as a residue from the same source by dissolving the mineral matter in dilute acid; No. 3 is obtained by extracting hides with water, concentrating, grinding and drying. Samples of all three kinds were used in this investigation. The various grades of these different kinds depend upon the temperature and length of time required for solution. The last fractions dissolved having been subjected to the water at a higher temperature for a greater length of time furnish the weaker jells and are probably more hydrolyzed as well as more affected by any bacterial action that may have been proceeding during the manufacture. Table I will show as nearly as is possible, at the present time, the characteristics of the three gelatins investigated.

TABLE I.

Gelatin	Moisture.	Ash.	$p_H$ in 1% Sol.	Jell strength.	Bacteria per g.
1	8.93	2.39	5.5	59	22,000
2	11.25	.84	4.1	430	200
3	5.25	1.03	4.0	800	100

The jell strengths of these gelatins were determined by a more or less arbitrary procedure. A solution of 30 g. of gelatin in 180 cc. of water was heated to 60° and placed in a standard glass of 250 cc. capacity. This was cooled to 21°  $\pm$  0.1° and maintained at this temperature for 16 hours. Then the number of grams required to force a plunger a certain distance into the surface of the gelatin was determined by a method which will be described in detail in a later paper.

The  $p_H$  values of the dry gelatin were determined by applying indicators directly. This gave an accuracy of 0.2 or 0.3 in the  $p_H$  value. In determining the hydrogen-ion concentration of solutions, comparison with the color of the indicators in buffer solutions of known hydrogen-ion concentration was necessary. By employing buffer solutions and indicators as described by Clark and Lubs<sup>2</sup> an accuracy of less than 0.2 was readily attained except at a  $p_H$  of 4 to 4.5 where the solution absorbs so much of the red rays as to render color comparison with buffer solutions difficult. 1% solutions of all gelatins gave the same hydrogen-ion concentration as did the dry gelatins.

### Method of Effecting Solutions.

Preliminary determinations of the variation of viscosity of solutions of a given gelatin with change in hydrogen-ion concentration gave erratic results. From these preliminary data we were convinced however of two things. First there is a distinct effect of hydrogen-ion concentration on viscosity and second the dissolving temperature has an effect on the nature of the solution. These first results plainly showed that the alkaline solutions were more difficult to effect than the acid ones. For concordant viscosity results on solutions of the same gelatin the dissolving temperature of the alkaline solutions must be greater than of the acid solutions. Upon a more complete investigation of the effect of the temperature employed for bringing about solution it was found that all solutions brought to a temperature of 75° gave concordant viscosity results. At temperatures somewhat lower than this the results on different portions of the same so-

<sup>2</sup> Clark and Lubs, *J. Wash. Acad. Sci.*, **6**, 483 (1916); *J. Biol. Chem.*, **25**, 479 (1916).

lution did not agree. At higher temperatures there was a rather rapid lowering in viscosity. At the boiling temperature this decrease in viscosity was extremely rapid as will be noted in the results given in Table II and plotted in Fig. 1. These results, which are quite in accord with the previous work of Von Schroeder,<sup>3</sup> show that a very few minutes' boiling reduced the viscosity of a high grade gelatin to that of a low grade one and entirely destroyed its tendency to increase in viscosity with aging of solution. Excessive temperatures should therefore be avoided in

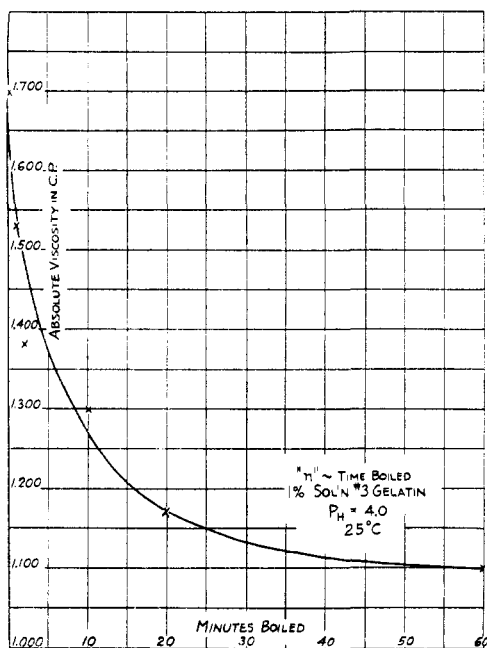


Fig. 1.

effecting solution. Since a temperature of 75° served to produce solutions whose viscosity could be accurately duplicated over the entire range of hydrogen-ion concentration and lower temperatures did not, this was selected as the standard. At this temperature the hydrolysis of the gelatin with its consequent lowering of viscosity was very slow except in certain cases detailed later.

From this preliminary work the following standard procedure was adopted. Ordinary distilled water ( $p_H$  value about 5.6) is made acid or basic by the addition of such quantity of 0.2 *M* hydrochloric acid or 0.2 *M* sodium hydroxide as will bring the resulting gelatin solutions to the required hydrogen-ion concentration. The gelatin is added to such

<sup>3</sup> Von Schroeder, *Z. physik. Chem.*, **45**, 75 (1903).

TABLE II.

Viscosity of Boiled Solutions of No. 3 Gelatin. Concentration 1% at 25°;  $p_H$  4.0.

Duration of boiling, min.	Age of sol. Min.	C.p. Absolute viscosity.
0	26	1.681
	39	1.769
	49	1.797
	60	1.816
	62	1.844
	91	1.960
	98	1.976
	114	2.056
	118	2.062
	1.0	65
76		1.503
91		1.518
2.0	84	1.371
10.0	72	1.304
	85	1.298
	97	1.306
	109	1.303
20.0	99	1.170
60.0	113	1.100

quantity of this solution as will give the required weight concentration. The age of the solution is reckoned from the time the gelatin is placed in contact with the water. As soon as the gelatin is added the flask is placed on the hot plate and brought to a temperature of 75° in 20 minutes, and stirred constantly. The solution is then filtered and placed in the thermostat at 25° ± 0.02°. When the solution registers this temperature 5 cc. is pipetted into an Ostwald viscometer and the whole immersed in the thermostat for at least 10 minutes. The solution is then drawn into the bulb of the viscometer and the run made while in the thermostat, timing the flow with a stopwatch. Three or four runs were made on each solution, using different viscometers of water equivalents of 73.8, 75.0, 78.4 and 75.8 seconds respectively. Specific gravity determinations were made by means of a pycnometer. The viscosity coefficient was calculated according to the customary formula,

$$\eta (\text{Gelatin}) = \eta (\text{Water}) \times \frac{(\text{Sec. gel. sol.}) \times (\text{Sp. gr. gel. sol.})}{(\text{Sec. water}) \times (\text{Sp. gr. water})}$$

$\eta$  for water being taken as 0.008937 absolute units or 0.8937 c. p. as given by Bingham.<sup>4</sup> Curiously enough no appreciable difference could be detected in the density of the solutions of the different grades of gelatin from the poorest to the best. The variation of the densities with different

<sup>4</sup> Bingham. Bur. Standards. *Bull.* 298, 74 (1917).

values of the concentration of the hydrogen ion was negligible in its effect on viscosity results.

In all our computations the density of the 1% gelatin solutions at 25° was taken as 1.0000. Actual variations were encountered from 1.00008 to 0.99988. The densities of gelatin solutions will be discussed in detail in a later paper.

The instruments were cleaned after each run by sucking through hot tap water and then distilled water by means of the filter pump. They were then placed in an air-oven and heated and air drawn through them while they were still warm. It is extremely important that the viscometer be absolutely dry when the gelatin solution is drawn into the side tube, as otherwise bubbles will be formed and correct results cannot be obtained. The  $p_H$  values between 2 and 9 were determined colorimetrically with the indicators and buffers of Clark and Lubs.<sup>2</sup> Other values were determined with the hydrogen electrode, using the condenser method described by Beans and Oakes.<sup>5</sup> The hydrogen electrode also served to check colorimetric determinations.

This procedure was found to give such satisfactory results that it was followed for all the viscosity determinations. It was found that under identical conditions the viscosity of solutions of any gelatin could be duplicated at will within the accuracy of the stopwatch. This gave an accuracy in the absolute viscosity values of  $\pm 5$  in the fifth decimal place, or in the third decimal place where viscosity is given in c. p. or an error of about  $\pm 0.5\%$  at the most.

#### Effect of Age of Solution on Viscosity.

Attention has been directed to the fact that gelatin solutions increase in viscosity for a period of time, reach a maximum and then diminish in viscosity. This phenomenon has been investigated for all three kinds of gelatin. It has been found that the maximum viscosity is attained in about 24 hours. Solutions of the higher grade gelatins may then still continue to increase slowly in viscosity. The low grade gelatins show a fairly rapid decrease especially where the  $p_H$  value of the solution is between about 5 and 8. It was noted that in every case where the viscosity began to decrease the solution smelled putrid; it was the gelatin of the highest bacterial content that decreased most rapidly; the decrease was accelerated by conditions favoring bacterial growth.

Table III shows the effect of age on the lowest grade of gelatin which also contains the greatest number of bacteria per gram. In Table IV will be found data covering the effect of age on solutions of the highest grade gelatin over periods of 1 and 2 hours.

<sup>5</sup> Beans and Oakes, *THIS JOURNAL*, **42**, 2116 (1920).

TABLE III.

Effect of Age of Solution on the Viscosity of 1% Solutions of Gelatin No. 1 at  $p_H$  5.0.

Age of solution.		C. p. Absolute viscosity.
Hrs.	Min.	
0	48	1.062
0	95	1.058
0	108	1.060
25	48	1.126
66	35	1.096
90	48	1.075

The viscosities of solutions of various concentrations of all three gelatins were investigated over a range of  $p_H$  value 1.5 to 12.9. In order to show in detail the method of procedure the complete results on 1% solutions of Gelatin No. 3 are given in Table IV. The last column gives the increases in viscosity during the second hour multiplied by 100 to avoid the use of small fractions. Similarly 0.5% and 0.25% solutions of this same gelatin were investigated.

TABLE IV.

Viscosities of 1% Solutions of No. 3 Gelatin at 25°.

Cc. of 0.2M HCl and NaOH contained in 200 cc. of solution.		$p_H$	Age of solution in min.	C. p. absolute viscosity.	$10^2 \frac{\Delta\eta}{\Delta T}$ For 2nd hour.
HCl.	NaOH.				
6.0	0	3.0	65	1.990	10.5
			86	2.020	
			114	2.067	
0	0	4.0	26	1.681	24.9
			39	1.769	
			49	1.797	
			60	1.816	
			62	1.845	
			91	1.960	
			98	1.976	
			114	2.056	
1.0	4.6	4.6	68	1.788	29.6
			91	1.927	
			110	2.005	
			127	2.081	
			127	1.923	
2.0	4.8	4.8	70	1.960	21.6
			87	1.960	
			108	2.074	
			127	2.126	
3.0	4.9	4.9	74	1.724	39.0
			90	1.793	
			107	1.924	
			120	2.032	

TABLE IV.—(Continued).

Cc. of 0.2 M HCl and NaOH contained in 200 cc. of solution.		$p_H$ .	Age of solution in min.	C. p. absolute viscosity.	$10^3 \frac{\Delta \eta}{\Delta T}$ For 2nd hour.
HCl.	NaOH.				
4.0	4.0	5.2	67	1.756	45.6
			89	1.931	
			101	1.985	
			117	2.143	
5.0	5.0	6.3	59	1.634	73.5
			72	1.813	
			161	2.840	
			177	3.023	
5.5	5.5	7.1	63	1.592	74.2
			93	1.987	
			111	2.250	
			136	2.497	
6.5	6.5	8.4	67	1.831	87.1
			90	2.151	
			124	2.701	
			170	3.460	
10.0	10.0	10.0	66	1.443	16.3
			91	1.489	
			127	1.609	
			157	1.648	

In Fig. 2 the viscosity values for all three concentrations are plotted for  $p_H$  4.0, while in Fig. 3 corresponding values for  $p_H$  6.5 are plotted. Reference to these curves (Figs. 2 and 3) shows that the rate of increase

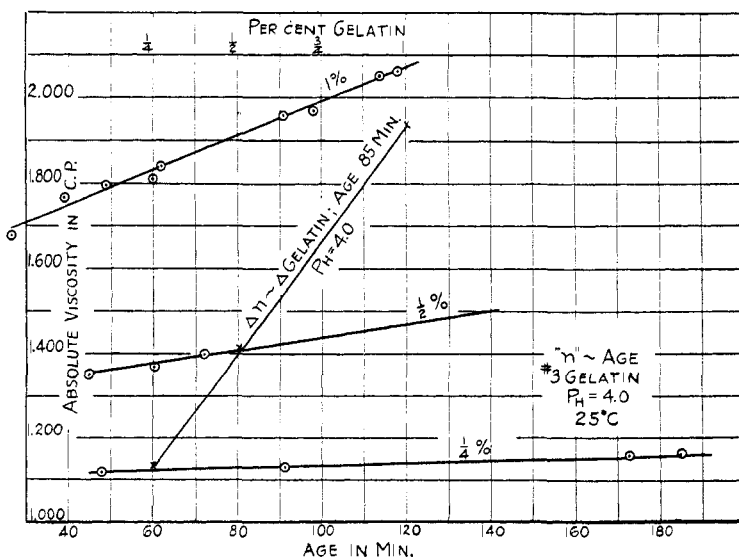


Fig. 2.

in viscosity with aging of solution differs at different  $p_H$  values. In Fig. 4 the viscosity-age of solution results are plotted for several different  $p_H$  values for 1% solutions of No. 3 gelatin. From this figure it is seen that the slopes of the curves increase with concentration of hydroxyl ion until a maximum is reached when the slope drops rapidly to a new low level. In order to represent this more clearly the values of  $10^3 \frac{\Delta\eta}{\Delta T}$  for the second hour of age of solution have been plotted in Fig. 5. In this figure the corresponding values of No. 2 gelatin are also given, No. 1 gelatin gave such small values as could not be plotted on the same scale with No. 2 and No. 3. From Fig. 5 it is seen that the viscosity is increasing most rapidly around a  $p_H$  value of 8. At a concentration of hydroxyl ion greater than  $p_H = 9$  there may even be a decrease in viscosity. This effect is taken up later under hydrolysis of gelatin.

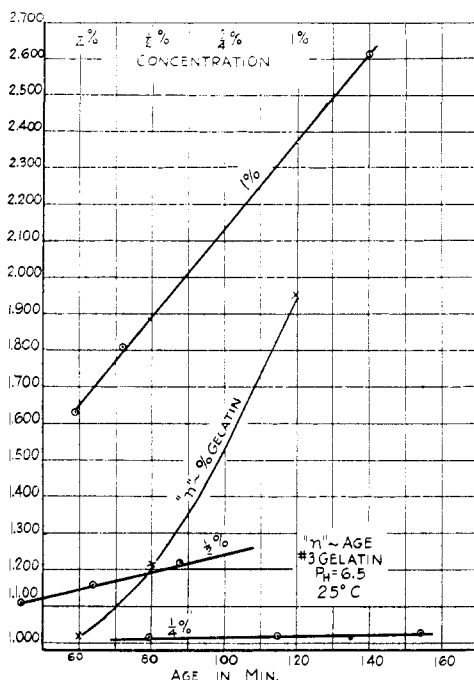


Fig. 3.

We may then conclude from the results described above that a solution of gelatin of  $p_H$  value less than 9 increases in viscosity with increase in age, reaching a maximum in about 24 hours. A decrease in viscosity after the maximum is reached indicates bacterial decomposition. The increase in viscosity with age varies directly with the hydroxyl-ion concentration until a maximum is reached at  $p_H$  8 to 8.5. At greater hydroxyl-ion concentration the in-

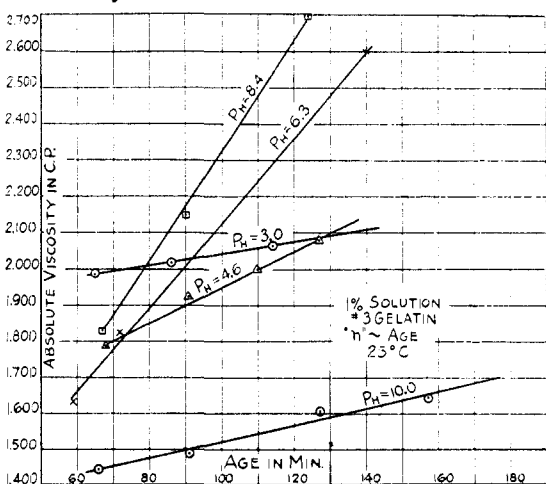


Fig. 4.



crease diminishes rapidly and actually becomes a minus quantity at  $p_H$  values somewhat greater than 9. In the case of pure, strong, slightly hydrolyzed gelatin the increase is very rapid. Very low grade gelatins show but slight increase in viscosity with age of solution.

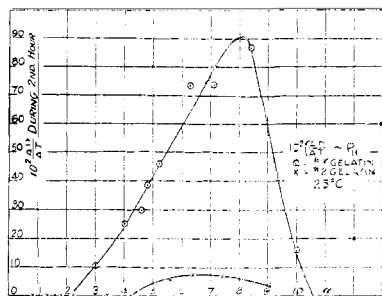


Fig. 5.

#### Effect of Concentration of Gelatin on Viscosity.

In Figs. 2 and 3 the viscosity-concentration values of No. 3 gelatin are plotted for  $p_H$  4.0 and 6.5 respectively. In Fig. 8 the corresponding values for No. 1 gelatin are plotted for  $p_H$  3.6 and 5.0. In the case of No. 1 the concentrations were carried to 3%. With No. 3, solutions of greater concentration than 1% jellied too rapidly to give reliable results. For low concentrations of fairly acid solutions the viscosity is approximately a linear function of the concentration.

On the alkaline side of the iso-electric point ( $p_H$  4.7) the viscosity increases so rapidly with increasing concentration that it does not even approximate a linear function of the concentration. It is quite evident from the above that neither Einstein's<sup>6</sup> formula for the viscosity of a colloid solution nor any modification so far proposed for it can be used to compute the viscosity of gelatin solutions at different concentrations. Viscosity-concentration formulas for gelatin solutions would require an entirely different set of constants for each gelatin, each value of the hydrogen-ion concentration, each age of solution, and in addition the method of effecting solution would have to be defined.

#### Effect of Concentration of Hydrogen Ion.

Since at a temperature of 25° gelatin solutions increase in viscosity with age it is obvious that viscosity values are worthless unless the age of solution is stated. Accordingly in all our work a number of determinations were made on each solution. From these values a graph was constructed from which the viscosity of the solution at any particular age

<sup>6</sup> Einstein, *Ann. physik.*, 19, 289 (1906).

could be determined. Selecting the values for an age of solution of 85 minutes the viscosity- $p_H$  values for 0.25 to 0.5% and 1% solution of No. 3 gelatin were plotted in Fig. 6. In Fig. 7 the corresponding values for 1% solutions of No. 1 and No. 2 are plotted with the values for 0.25% solutions of No. 3 repeated for comparison. 1% solutions of No. 3 gelatin are increasing in viscosity so rapidly that the gelation factor has entirely masked the effect of hydrogen-ion concentration. The other curves show a distinct maximum at  $p_H$  3.0 to 3.5 when the viscosity is taken at an age of solution of 85 minutes and 25°. On the alkaline side of the iso-electric point the corresponding viscosities plot a straight line until such a concentration of hydroxyl ion is reached as will catalyze the hydrolysis of the gelatin as described later. On the acid side the curves are essentially the same as described by Loeb.<sup>1</sup> On the alkaline side the type of curve is quite different since Loeb records a maximum at  $p_H$  11 approximately. Since Loeb<sup>7</sup> used an entirely different procedure undoubtedly a difference in the ages of the solutions accounts for the difference in the types of the curves. The maximum in the curve at  $p_H$  3 to 3.5 is unaccounted for except on the assumption of the formation of a compound at this point, data included in a paper in the course of preparation support this view.

### Effect of Hydrolysis on Viscosity.

The effect of hydrolysis of the gelatin resulting from boiling the solution has been discussed. The abnormal drop in viscosity recorded in Figs. 6 and 7 at  $p_H$  values greater than 9 suggests that we are encountering an

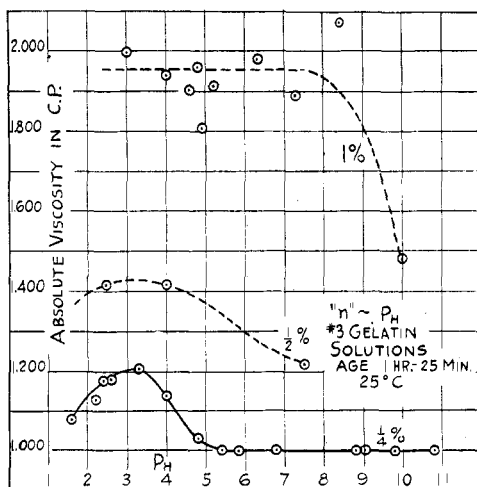


Fig. 6.

<sup>7</sup> This work was done in the summer of 1920, before Loeb's results were published in September (1920).

effect other than that of the alkali combining with the gelatin to form a metal gelatin. It appeared that the hydroxyl ion at this concentration was catalyzing the hydrolysis of the gelatin to such an extent as to lower the viscosity to the extent indicated in Fig. 7, the actual values being 1.251 at  $p_H$  values 6.4, 7.4, 9, and 1.047 at  $p_H$  12.9.

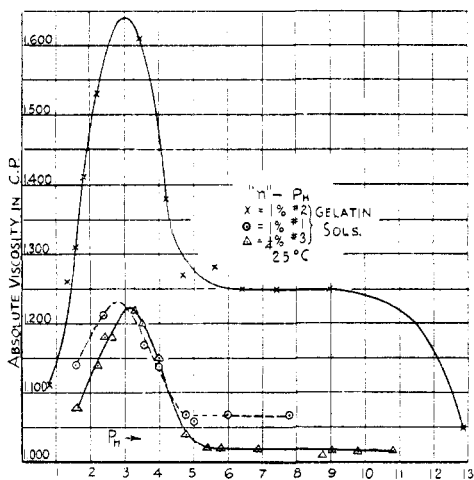


Fig. 7.

In order to test this point 1% solutions of No. 2 gelatin were made up in duplicate. Solution B1 was made up exactly as described heretofore. Two g. of gelatin was dissolved in 200 cc. of 0.2 M sodium hydroxide

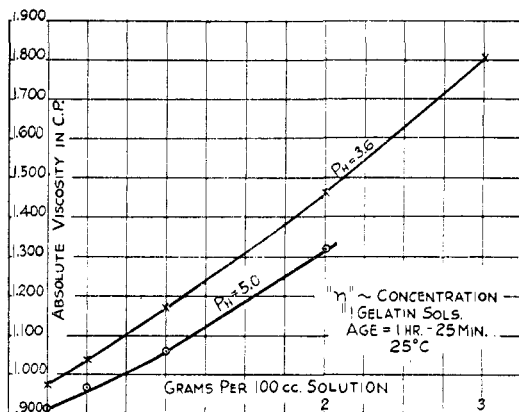


Fig. 8.

solution, heated for 20 minutes, when a temperature of 75° was reached, allowed to cool, filtered and placed in the thermostat. At the same time Solution B2 was prepared as follows. Two g. of the gelatin was added

to 190 cc. of water and the solution accomplished exactly as in the case of Sample B1, the two solutions being carried along together. After B2 was removed from the hot plate and allowed to cool 10 cc. of a solution containing 1.6 g. of sodium hydroxide was added. The  $p_H$  value of these two solutions, B1 and B2 was 12.9. Viscosity of these solutions was found to be as follows.

Age of solution.		C. p. B1 absolute	C. p. B2 absolute
Hours.	Min.	viscosity.	viscosity.
1	25	1.009	1.273
5	30	0.987	1.100
22		0.979	1.019
27		0.976	1.007
49		...	0.996

In exactly the same manner the effect of acids on the hydrolysis of gelatin solutions was determined. Solutions A1 and A2 were made up at a  $p_H$  1.4. In the case of A1, 2 g. of gelatin was dissolved in 160 cc. of water to which 40 cc. of 0.2 *M* hydrochloric acid had been added. A2 solution did not have the acid added until after the gelatin had been dissolved and the solution cooled. On these two solutions the following results were obtained.

Age of solution.		C. p. A1 absolute	C. p. A2 absolute
Hours	Min.	viscosity, $p_H$ 1.4.	viscosity, $p_H$ 1.4.
1	40	1.379	1.424
22	"	1.449	1.515
28		1.447	1.501

These values indicate that hydrogen ions and hydroxyl ions catalyze the hydrolysis of gelatin. Hydroxyl ion catalyzes this reaction to a very great extent at  $p_H$  values greater than 9. The effect of hydrogen ion up to  $p_H$  1.4 is slight compared to the hydroxyl-ion effect. In order to avoid the hydrolysis effect the acid and the alkali should not be added to the solution until after it has been cooled. The procedure would then be to dissolve the gelatin in pure water and after the solution has cooled add the required amount of alkali or acid to bring the solution of gelatin to the required hydrogen-ion concentration making the total volume such as will give the desired gelatin concentration. This would only be necessary where the hydrogen-ion concentration of the resulting solution was outside the range of  $p_H$  2.5 to 8.

### Summary.

1. Effects on the viscosity of gelatin of (a) method of effecting solution, (b) aging of solution, (c) hydrogen-ion concentration, (d) concentration of gelatin, and (e) hydrolysis of gelatin, have been described for three different kinds of gelatin.

2. A method of determining viscosity of gelatin solutions with a maximum error of 0.5% has been described.
3. Gelatin solutions increase in viscosity with age at different rates depending upon concentration of gelatin, hydrogen-ion concentration, and kind of gelatin.
4. For any given gelatin solution a maximum viscosity is attained at an age of solution of about 24 hours. A decrease in viscosity after the maximum is reached indicates bacterial decomposition.
5. Gelatin solutions show a maximum viscosity at a  $p_H$  3.0 to 3.5 at 25°.
6. The viscosity of gelatin solutions is not a simple function of concentration of gelatin.
7. Both hydrogen and hydroxyl ions catalyze the hydrolysis of gelatin solutions, the latter more rapidly than the former.
8. Excessive temperatures catalyze the hydrolysis of gelatin. Boiling gelatin solutions hydrolyzes them very rapidly.

NEW YORK, N. Y.

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[CONTRIBUTION FROM THE WOLCOTT GIBBS MEMORIAL LABORATORY, HARVARD UNIVERSITY.]

## THE COMPRESSIBILITY OF BENZENE, LIQUID AND SOLID.

BY THEODORE W. RICHARDS, EDWARD P. BARTLETT AND JAMES H. HODGES.

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Very few substances have had their compressibilities determined in both the liquid and the solid state. The comparison of the two different values is of considerable interest in relation to a study of the mechanism of the change of state in any given substance. Accordingly the present paper recounts such a comparison in the case of benzene, for which adequate data were lacking as regards the solid. The results were incidental to other researches, but seem to deserve separate publication.

Of liquid benzene, a careful determination, made in a glass piezometer, has already been described.<sup>1</sup> The object of repeating the determination was primarily to show whether or not the earlier apparatus, which had been used with a variety of substances, was adequate, by comparing a typical result with that yielded by a larger more complete piezometer free from the hysteresis to which glass is subject.

The piezometer used in the present research was the steel instrument already described in full in another previous paper.<sup>2</sup> In the case of liquid benzene, a weighed amount of about 30 cc. of this substance was placed in an inverted steel test-tube immersed under mercury (which filled the piezometer). The steel test-tube diminished the unnecessarily large volume

<sup>1</sup> T. W. Richards and J. W. Shipley, *THIS JOURNAL*, **38**, 989 (1916).

<sup>2</sup> T. W. Richards and E. P. Bartlett, *ibid.*, **37**, 470 (1915).