[CONTRIBUTION FROM THE FOOD INVESTIGATION LABORATORY OF THE BUREAU OF CHEMISTRY.]

THE MUTAROTATION OF GELATIN AND ITS SIGNIFICANCE IN GELATION.

BY C. R. SMITH.

Received November 6, 1918.

Gelatin when dissolved (dispersed) in water is characterized by its power of jellying and liquefying reversibly within a comparatively narrow range of temperature. This change appears to be capable of indefinite repetition, if high temperatures are avoided in bringing about liquefaction.

When the solution is heated above 60 to 70° the power of jellying, when cooled, is gradually destroyed, the more rapidly the higher the temperature. Heating for a few minutes at 140° under pressure suffices to destroy the jellying power completely. Gelatin thus changed (probably hydrolyzed) is known as β-gelatin. β-Gelatin does not show mutarotation. Ordinary gelatin, on the other hand, does possess this property and, so far as is now known, is the only protein which does so.

Comparatively little study of the rotatory power of gelatin appears to have been made. Trunkel,[1] in a report of his study of the optical rotation of solutions, or sols, of gelatin (Leim) states, referring to work of de Bary,[2] Krüger,[3] and Framm[4] that the extent of our previous knowledge on the subject may be summarized by the statement that the specific rotatory power of gelatin changes with the temperature and that continued heating at 100° gives a product β-gelatin, the specific rotatory power of which is lower than that of ordinary gelatin.

Trunkel employed solutions of gelatin varying in concentration from 0.22 to 0.90%, prepared by heating for 6 to 8 hours at 80°, and it is probable, as he himself states, that some change in the composition of his material may have been produced by this treatment. Moreover, in some cases his readings were made uncertain by opalescence even after filtration. It is, therefore, not surprising that his measurements were variable. Trunkel did not find constant specific rotation at low temperatures. Especially significant, however, are his conclusions:

(1) That the specific rotatory power of a gelatin solution at temperatures between 30° and 80° is practically constant; (2) that when such a solution is cooled to a temperature of 10° to 15° an increase in levorotation takes place, but that this change takes place gradually and constant rotation is not reached for some time; and (3) that the change in rotation

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[1] *Biochem. Z.*, **26**, 493 (1910).

[2] Hoppe-Seylers "Medizinisch-Chemische Untersuchungen," **1**, 71 (1866).

[3] Mayls "Jahresberichte über die Fortschritte der Tierchemie," **1889**, p. 29.

[4] *Arch. ges. Physiol.*, **68**, 144 (1897).

is reversible with the temperature, provided long-continued heating at high temperatures is avoided.

The writer, in the course of the examination of a large number of samples of gelatins was led to investigate more fully the significance of this change in the rotatory power of gelatin solutions.

Measurements of the Specific Rotation of Gelatin.

No method for the preparation of chemically pure gelatin has yet been discovered. Methods of purification based upon repeated precipitations with alcohol are not satisfactory. After a considerable study of this and various other methods of purification had been made, it became evident that commercial samples would have to be used for the purpose of this investigation. The greater part of the material used was from imported gelatins said to have been made from ossein. The data given in the tables were obtained on such gelatins. A few samples of hide and Russian isinglass gelatins were prepared either by the author in the laboratory or under his direction in gelatin manufacturing plants in this country. It is believed, however, that if it had been possible to prepare and use chemically pure gelatin the conclusions drawn would not have been appreciably affected.

The solutions (or sols) were prepared from the powdered air-dried samples by soaking for a few minutes with water in a graduated flask, heating on the steam-bath for 10 minutes, cooling, and making up to volume at 35°. Prolonged heating and temperatures higher than 60° were

TABLE I.

Rotations of Solutions of Commercial Gelatins Kept at 15° for 6 Hours.

Concentration (g. per 100 cc.).	Angular rotation.	Specific rotation. $[\alpha]_D$.	Concentration (g. per 100 cc.).	Angular rotation.	Specific rotation. $[\alpha]_D$.
	Sample 806.			Sample 705.	
1	—1.59°	—159.0°	1	—1.96°	—196.0°
2	3.31	166.0	2	4.07	204.0
3	5.09	170.0	3	6.24	208.0
5	8.65	173.0	5	10.34	207.0
7	12.13	173.0	7	14.69	210.0
	Sample 805.			Sample 803.	
1	—1.90°	—190.0°	1	—2.44°	—244.0°
2	3.90	195.0	2	4.94	247.0
3	6.03	201.0	3	7.50	250.0
5	10.22	204.0	5	12.40	248.0
7	14.38	205.0	7	17.32	247.0
			10	24.60	246.0
	Sample 407.			Sample 670.	
1	—2.44°	—244.0°	1	—2.46°	—246.0°
2	4.92	246.0	2	5.00	250.0
4	9.89	247.0	3	7.57	252.0
5	12.35	247.0	5	12.61	252.0
7	17.15	245.0	7	12.57	251.0

avoided. The solutions so prepared were clear even with concentrations as high as 7 to 10 g. per 100 cc. Most of the readings were made through the gels which do not appear to influence the reading in any way. No corrections have been made for moisture, ash, or variations in specific gravity. The light used in all work with the polariscope was filtered through a solution of potassium dichromate.

A few measurements obtained in preliminary work on different samples are given in Table I. These measurements show that the specific rotation tends to approach a constant value which is different for the different samples, probably because of the different degree of purity or "strength" of the samples.

More extended measurements of rotation were made between 10° and 45° on sample 670. The results are given in Table II. Sufficient time was allowed for the readings to become constant at the given temperature. Above 33° constant rotation is quickly attained if the solution after a preliminary warming to 37° to 40°, is brought to the desired temperature. To obtain constancy between 17° and 33° it is necessary either to maintain the solution for 10 to 15 hours at the desired temperature or, better, to cool about 2° to 3° below, until the calculated rotation is reached and then maintain at the desired temperature. Below 17° maintenance of the desired temperature for 10 to 12 hours is required. Constant rotation is more quickly obtained in the more concentrated solutions. In the higher concentrations about 90% of the change takes place in 6 hours. With concentrations below 1 g. per 100 cc. more time is required than that stated above.

TABLE II.

Specific Rotations of Gelatin at Various Temperatures. Sample 670.

Concentrations (g. per 100 cc.).	Specific rotations at temperatures.						
	10°.	15°.	16°.	17°.	18°.	19°.	20°.
1....	—260.0°	—256.0°	—250.0°	—241.0°	....	—226.0°	—215.0°
2....	265.0	263.0	251.0	246.0	....	230.0	224.0
3....	266.0	265.0	253.0	248.0	....	233.0	227.0
5....	267.0	266.0	254.0	248.0	....	236.0	229.0
7....	266.0	266.0	253.0	246.0	....	234.0	230.0
	22°.	23°.	24°.	26°.	26°.	27°.	29°.
1....	—201.0°	—188.0°	—180.0°	—164.0°	—156.0°	—142.0°	—123.0°
2....	212.0	201.0	195.0	183.0	173.0	156.0	126.0
3....	215.0	207.0	202.0	187.0	175.0	164.0	129.0
5....	218.0	211.0	208.0	197.0	186.0	176.0	138.0
7....	220.0	215.0	211.0	206.0	196.0	187.0	144.0
	32°.	35°.	40°.	45°.			
1....	—120.0°	—120.0°	....	....			
2....	121.0	120.0	....	....			
3....	121.0	121.0	119.0	117.0			
5....	124.0	121.0	118.0	116.0			
7....	125.0	121.0	....	....			

The behavior shown by the gelatin represented by Sample 670 is exhibited by all gelatins of the best manufacture. At and above 35° the specific rotatory power is practically constant even with widely varying concentrations except for such changes with the temperatures as are usually observed with optically active substances. Another and quite different specific rotation is shown at 15° which is constant at this and lower temperatures for widely varying concentrations except that it changes slightly, as might be expected, with progressive lowering of the temperature.

To determine more definitely what the specific rotations are at these temperatures, a considerable number of the finest gelatins were picked out by appearance, noting particularly the color and fracture of the samples. The results are given in Table III. For comparison, the values for the specific rotation at an intermediate temperature, namely, 25° are given.

TABLE III.

Specific Rotatory Power.

(Concentration, 3 g. per 100 cc.)

Sample number.	Temperature.		
	35°.	25°.	15°.
803	—121.8°	—189.4°	—266.0°
670	120.6	188.2	265.7
401	121.2	188.8	270.0
562	123.0	189.4	270.2
193	121.2	188.8	270.2
863	123.0	189.4	271.3
227	121.2	189.4	270.2
616	121.2	189.4	270.2
393	123.0	191.7	271.3
857	123.0	190.0	271.6
544	123.5	190.0	271.6

These values show a remarkable uniformity. The acidity of these gelatins was nearly constant, varying between 3.2 and 4.0 cc. of 0.01 *N* alkali for 3 g. of the sample, using phenolphthalein as indicator. Three cc. of a 10% solution of sodium acetate was added to all samples to reduce the hydrogen-ion concentration since the effect of hydrogen ions is to reduce the rotation, although slight difference is noted when the concentration of the hydrogen ion is no greater than in these samples.

At 35° the specific rotatory power $[\alpha]_D$, of the purest gelatins obtainable on the market at the present time, is therefore found to be —123°. Assuming a moisture and ash content of 11.4% and 1.6%, respectively, which are perhaps average values, $[\alpha]_D$ at 35° becomes —141° on a moisture- and ash-free basis. When cooled to 15° or below, $[\alpha]_D$ is found to be about —272°, or —313° when calculated to a moisture- and ash-free basis.

The ratio between $[\alpha]_D$ at 35° and 15° is 2.21 : 1. This ratio is about the same for the best grades of commercial gelatin whether obtained from bones, hides, or Russian isinglass. Gelatins from other sources have not as yet been investigated.

The behavior of gelatin under the conditions described above leads to the conclusion that we are dealing with two chemical forms; one stable above 33° to 35°, which we shall call the sol Form A, and another form which will be called the gel Form B, existing at lower temperatures. It is believed, as will be discussed later, that it is the Form B which produces gelation.

The probability of the existence of two forms of gelatin is strengthened by the appearance of the graphical representation of the data given in Table II, as shown in Fig. 1. The dotted line is the line of separation between the sols and the gels. Above the line, the readings were through gels, and below, through the sols. The method of distinguishing between sol and gel is given later. This figure also shows that in the higher concentrations the change in rotation produced by the transformation of Form A into Form B takes place more quickly than in lower concentrations and that the transformation is practically complete at 15°. The condition at any intermediate temperature between 35° and 15° is one of equilibrium, which may be represented by the equation

$$\text{Sol Form A} \rightleftarrows \text{Gel Form B.}$$

It has already been stated that Trunkel found the change in rotation to be reversible with the temperature. This has been fully corroborated by the writer. A further investigation of the nature of the reaction has been made, based upon the assumption that the change in rotation is proportional to any chemical change involved.

Velocity of Mutarotation.

The velocity of mutarotation has been followed polarimetrically by rapidly cooling the gelatin solutions from 35° to 20° or below in a 2 dcm. water-jacketed tube. The readings given in Tables IV, V and VI were begun after 2 minutes' cooling to the specified temperature at which time $t = 0$. The time (t) is given in minutes. The readings given for $t = \infty$, in Tables IV and V, is the value obtained by approaching the condition of equilibrium from both sides, that is, by cooling to the specific temperatures and by cooling somewhat below it and then bringing the temperature up to that specified in the tables.

The values which have been calculated for 100 k and given in Table VI were obtained by assuming that —240° represents the specific rotation at the end of the bimolecular reaction before the effect of any secondary reaction is appreciable. The rotations for the 2 and 3 g. concentrations would be —4.80° and —7.20°, respectively, and these rotations have been used in Table VI instead of the rotations at $t = \infty$ given in Tables IV and V.

TABLE IV.

Velocity of Mutarotation at 20°.

Sample 670.

Conc. 1 g. per 100 cc.

t.	Rot.	100 k.
0	—1.28°	...
2	1.32	...
4	1.37	3.0
8	1.42	2.6
14	1.51	2.8
20	1.58	2.8
28	1.64	2.7
44	1.74	2.7
56	1.79	2.6
66	1.82	2.5
84	1.87	2.6
100	1.92	2.5
126	1.96	...
141	1.98	...
∞	2.18	...
(a = 0.90)		...

Conc. 2 g. per 100 cc.

t.	Rot.	100 k.
0	—2.61°	..
2	2.77	..
4	2.91	2.74
6	3.02	2.69
9	3.15	2.61
12	3.27	2.64
16	3.39	2.63
19	3.46	2.58
27	3.64	2.71
35	3.78	2.64
48	3.90	2.87
64	3.98	2.70
79	4.07	2.90
∞	4.42	..
(a = 1.81)		..

Conc. 3 g. per 100 cc.

t.	Rot.	100 k.
0	—4.07°	..
2	4.39	2.76
3	4.54	2.90
4	4.66	2.86
5	4.75	2.78
6	4.85	2.80
8	4.99	2.70
10	5.12	2.66
12	5.24	2.66
14	5.33	2.65
18	5.49	2.63
22	5.63	2.70
25	5.70	2.66
31	5.80	2.55
58	6.13	2.65
71	6.20	2.59
∞	6.65	..
(a = 2.58)		

Conc. 4 g. per 100 cc.

t.	Rot.	100 k.
0	—5.47°	..
2	6.01	2.78
4	6.42	2.85
6	6.67	2.69
7	6.79	2.67
9	7.01	2.70
10	7.12	2.77
11	7.17	2.68
12	7.24	2.66
14	7.38	2.70
16	7.48	2.66
20	7.65	2.63
23	7.76	2.65
32	8.02	2.76
47	8.22	2.65
∞	8.87	..
(a = 3.40)		

Conc. 5 g. per 100 cc.

t.	Rot.	100 k.
0	—6.89°	..
1	7.32	2.84
2	7.70	2.89
3	7.96	2.78
5	8.40	2.74
7	8.75	2.82
8	8.89	2.78
9	9.02	2.80
11	9.26	2.90
14	9.46	2.78
18	9.73	2.90
22	9.85	2.70
28	10.01	2.76
30	10.09	2.66
36	10.24	2.75
44	10.32	2.57
70	10.59	2.77
∞	11.05	..
(a = 4.16)		

TABLE V.

Velocity of Mutarotation at 19°.

Sample 393.
Conc. 3 g. per 100 cc.

t.	Rot	100 k.
0	—4.25°	..
1	4.45	..
2	4.61	..
3	4.76	2.77
4	4.92	2.86
5	5.04	2.85
6	5.14	2.82
7	5.23	2.80
9	5.42	2.90
10	5.49	2.89
11	5.54	2.84
12	5.59	2.80
15	5.78	2.93
17	5.85	2.87
19	5.89	2.73
24	6.06	2.80
30	6.18	2.72
40	6.34	2.52
45	6.37	..
52	6.45	..
60	6.51	2.60
70	6.56	..
80	6.61	2.53
90	6.63	..
115	6.72	..
150	6.82	..
210	6.89	..
∞	7.03	..
	(a = 2.78)	

Sample 562.
Conc. 3 g. per 100 cc.

t.	Rot.	100 k.
0	—4.29°	..
1	4.48	..
2	4.66	..
3	4.80	2.67
6	5.18	2.80
7	5.26	2.74
9	5.42	2.71
11	5.56	2.73
13	5.68	2.75
14	5.73	2.75
16	5.82	2.73
17	5.85	2.70
20	5.95	2.70
25	6.10	2.65
30	6.18	2.52
40	6.35	2.54
45	6.39	..
50	6.46	2.53
60	6.55	..
70	6.60	..
90	6.68	2.40
120	6.77	..
150	6.84	..
183	6.89	..
210	6.93	..
245	6.98	..
∞	7.08	..
	(a = 2.79)	

From the data in Table IV we may calculate the time required for half the total change in rotation. For the different concentrations it is as follows:

Concentration, g. per 100 cc.	Approximate time required for 1/2 the total change in rotation. Minutes.
1	44
2	22
3	15
4	11
5	9

The time varies inversely as the concentration which leads to the conclusion that the reaction taking place is bimolecular. The simplest explanation of this is believed to be the assumption that two molecules of the sol Form A combine to form one molecule of the gel Form B. The

TABLE VI.
Velocity of Mutarotation at 15°.
Sample 393.

Conc. 2 g. per 100 cc.		
t.	Rot.	100 k.
0	—2.96°	. . .
1	3.15	6.2
2	3.29	6.0
3	3.40	5.7
4	3.52	6.0
5	3.64	6.4
6	3.72	6.5
7	3.79	6.4
8	3.86	6.5
9	3.92	6.6
10	3.98	6.7
11	4.03	6.8
12	4.09	7.2
14	4.16	7.3
16	4.24	7.7
18	4.28	7.6
20	4.33	8.0
22	4.38	. . .
24	4.42	. . .
26	4.44	. . .
30	4.50	. . .
35	4.59	. . .
40	4.63	. . .
50	4.68	. . .
60	4.75	. . .
70	4.78	. . .
80	4.80	. . .
100	4.85	. . .
150	4.95	. . .

(a = 1.84)

Conc. 3 g. per 100 cc.		
t.	Rot.	100 k.
0	—4.57°	. . .
1	4.92	. . .
2	5.20	7.3
3	5.44	6.3
4	5.61	6.3
5	5.77	6.4
6	5.89	6.3
7	6.03	6.8
8	6.12	6.8
9	6.18	6.7
10	6.25	6.7
12	6.39	7.1
14	6.50	. . .
17	6.62	. . .
20	6.74	. . .
22	6.77	. . .
25	6.84	. . .
30	6.93	. . .
35	7.00	. . .
45	7.10	. . .
60	7.20	. . .
75	7.27	. . .
90	7.32	. . .
120	7.36	. . .
150	7.41	. . .
180	7.46	. . .

(a = 2.63)

velocity of mutarotation has been found to be represented by the usual equations for a bimolecular reaction in which the two reacting substances are present in equivalent proportions. For a reaction of this kind

$dx/dt = k(a - x)^2$ which when integrated gives $k = 1/t \,.\, x/a(a - x)$.

At temperatures between 35° and 15° we probably have a condition of equilibrium which strictly speaking, should be represented by the equation

$$dx/dt = k(a - x)^2 - k'x.$$

The simpler equation has been used for calculating the velocity constant. This constant will be nearly equal to the sum of the constants k and k'. Multiplied by 100 the values so obtained for this velocity constant at 20° and 19° are given in Tables IV and V, respectively. They show a fair degree of constancy. The value of this constant is approximately 0.027

in both cases. Since at 19° there must be a displacement of the equilibrium in favor of the formation of more of Form B than is formed at 20°, the fact that the constant is nearly the same in both cases is probably due to the increase in k being about equal to the decrease in k'.

At 15° the velocity of the reaction is so great that the errors in reading are large. The values for 100 k obtained show a degree of constancy sufficient to lead to the belief that at 15°, also, the principal reaction involved is the bimolecular reaction of the formation of Form B from Form A. Apparently at this temperature the bimolecular reaction is disturbed to some extent by some other reaction, possibly of a monomolecular nature, which takes place at the same time. At 17° the values for the specific rotations of gelatin in varying concentrations first come into approximate agreement at about —240°. A comparison of the change in rotation at 15° and 10° for various concentrations given in Table VIII below shows that at this temperature the values for the specific rotation likewise first come together at about —240° and then slowly increase with time, but remain together for the different concentrations.

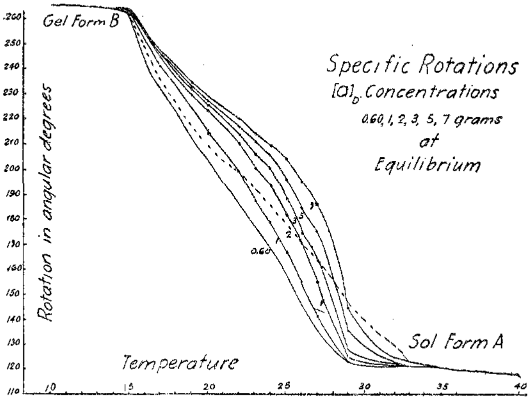

Fig. 1.

TABLE VII.

Change of Specific Rotation with Time for Different Concentrations at 15°.
(Sample 393. Specific Rotation at 35° = —123°.)

Concentration (g. per 100 cc.).	Specific rotations.				
	20	30	60	90	150 minutes.
2	—216.5°	—225.0°	—238.2°	—242.5°	—247.6°
3	224.0	231.0	240.1	242.6	248.5
4	226.0	231.0	241.6	243.3	248.5
5	228.0	232.0	240.8	244.2	247.6

TABLE VIII.

Change of Specific Rotation with Time for Different Concentrations at 10°.
(Sample 393.)

Concentration (g. per 100 cc.).	Specific rotations.				
	30	80	140	200	350 minutes.
1	—242.5°	—251.1°	—256.2°	—260.0°	—263.3°
2	251.1	256.2	260.0	262.4	266.7
3	254.5	259.9	262.4	264.1	266.0
5	256.2	259.9	261.0	264.0	266.7

Conditions of Equilibrium.

In view of the probable existence of two forms of gelatin, an assumption which is strongly supported by the data given in the preceding pages, we may apply the mathematical expression for the condition of equilibrium, which in this case is $k(a - x)^2 = k'x$, and expect the relation $(a - x)^2/x = k'/k = K$, where K is the equilibrium constant, to hold for different concentrations at any given temperature between 17° and 33° to 35°. The temperatures 17° and 35° represent nodes of the specific rotation, since the specific rotation is practically constant beyond these points (see Fig. 1).

Temperatures near the belly of the curve (about 25°) were selected and careful measurements of the equilibrium rotations made, using 0.10 g. of sodium acetate per 100 cc. per g. concentration to reduce the hydrogen-ion concentration.

That this relation does hold is shown by the data given in Table IX (*a*), the difference between the rotations produced by one gram of gelatin at 33° to 35° (about —1.20°) and at 17° (about —2.40°), is 1.20. x is the difference in rotation between that at 33° and 35° and that at the specified temperature. x is assumed to be proportional to the percentage of Form B and $(a - x)$ proportional to the percentage of Form A present when the two forms are in equilibrium.

TABLE IX.

Equilibrium between Forms A and B at Temperatures 24°, 25°, 26° and 27°.

Concentration, g. per 100 cc.	Equilibrium rotation.	x.	$a - x$.	$(a - x)^2/x = K$.
24°.				
1	—1.80°	0.60	0.60	0.60
2	3.89	1.49	0.91	0.57
3	6.06	2.46	1.14	0.53
5	10.38	4.38	1.62	0.60
25°.				
1	—1.68°	0.48	0.72	1.08
2	3.66	1.26	1.14	1.03
3	5.66	2.06	1.54	1.15
5	9.87	3.87	2.13	1.17
26°.				
1	—1.56°	0.36	0.84	1.96
2	3.45	1.05	1.35	1.73
3	5.35	1.75	1.85	1.96
5	9.32	3.32	2.68	2.16
27°.				
1	—1.42°	0.22	0.98	4.4
2	3.12	0.72	1.68	4.0
3	4.92	1.32	2.28	4.0
5	8.80	2.80	3.20	3.7

Other Evidence of the Existence of Two Forms of Gelatin.

In solutions of gelatin at temperatures below 30°, the gelatin is either precipitated or the solutions made opalescent by the addition of alcohol to the extent of 15%. All commercial gelatins tried behave in this way. If the gelatin concentration is high an opalescent jelly is produced. When opalescence only is produced, it is probable that coalescence is prevented by electrical charges on the particles, since a drop of a solution of alum or a feeble electric current has been found to produce coagulation in such cases. Such gelatins as coagulated without this treatment probably contained an appreciable quantity of some electrolyte.

Above 35°, precipitation requires very much larger proportions of alcohol, about 45 to 50%, unless a comparatively large amount of some electrolyte is added.

Moore and Roaf,[1] as the result of their measurements of the osmotic pressure of gelatin solutions, conclude that "when the temperature is lowered, so that it lies just above the point at which a jelly is formed, there is no sudden fall in pressure, but if it is kept for some days at this temperature, a very marked continuous fall is observed, showing that, in the neighborhood of the temperature of formation of the hydrogel, a rapid aggregation to form much larger solution aggregates occur."

Similar conclusions may be drawn from the viscosity measurements of von Schroeder,[2] and from observations made by W. Menz[3] in connection with the determination of the "gold numbers" of gelatin.

Conditions of Gelation in Aqueous Solution.

Since gelatin is favored by low temperature we might expect a more or less definite concentration of Form B, stable at low temperatures, to be requisite for the production of gel, and that the formation of this amount of Form B would be accompanied by a corresponding change in rotation from that observed at 35°.

0.55 g. of gelatin in 100 cc. of water is the minimum quantity of gelatin which produces a gel near 0°. For increasing concentration of gelatin there are maximum temperatures above and below which sol and gel, respectively, are stable for any length of time. The writer considers these temperatures to be the true melting points. The melting points of gels, as ordinarily determined, are temperatures at which the gels melt within certain arbitrary short periods of time, and hence are not identical with these maximum temperatures. For this reason the melting point, as ordinarily determined, does not agree with the "setting temperatures" and statements are found in text-books and elsewhere to the effect that a solution of gelatin sets at, say 21°, and that the gel melts at, say 26°.

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[1] *Biochem. J.*, **2**, 52 (1907).

[2] *Z. physik. Chem.*, **45**, 75–117 (1903).

[3] *Ibid.*, **66**, 129–137 (1909).

If maximum gelation temperatures are taken as melting points, then setting temperature and melting point become the same.

For the determination of maximum gelation temperatures the expedient was adopted of cooling the sol 2 to 3° below the expected temperature, maintaining the temperature at this point until gel was produced and then transferring to a constant temperature bath held at the expected temperature. At this temperature the gel should show continuously for several (3 or 4) hours the selected condition of viscosity. In these experiments polariscope tubes were used and the viscosity selected to mark the transition from sol to gel was such that a bubble of air about 4.5 mm. in diameter admitted to the tube moved vertically with a scarcely perceptible motion of about one centimeter in 4 seconds. Data obtained in this way are given in Table X.

TABLE X.

Maximum Gelation Temperatures.

Concentration, g. per 100 cc.	Maximum gelation temp. Degrees.	Polarization at at 35° C. Degrees of rotation.	Polarization at gelation temp. Degrees of rotation.	Difference. Degrees of rotation.
0.55	0.0	—0.70°	—1.51°	0.81°
0.60	15.0	0.73	1.53	0.80
1.00	22.0	1.21	2.01	0.80
2.00	26.2	2.42	3.22	0.80
2.73	27.0	3.30	4.16	0.86
4.00	27.5	4.84	5.77	0.93
5.00	28.0	6.05	7.09	1.04
6.00	28.4	7.26	8.50	1.24
7.00	29.5	8.47	..	..
8.00	29.7	9.68	11.07	1.39
10.00	30.0	12.10	13.66	1.56
15.00	30.5	18.15	..	..
20.00	31.5	24.20	26.47	2.27

It is apparent that the maximum gelation temperatures approach 33°, the upper limit for Form B. This is shown graphically in Fig. 2. It is also to be noted that for concentrations of gelatin between 0.60 and 4 g. per 100 cc. there is a change of about 0.85° in rotation corresponding to a definite quantity of gelatin, about 0.60 g., which produces a gel when completely transformed into Form B. As the concentration increases to 15 or 20 g. the differences in rotation are larger.

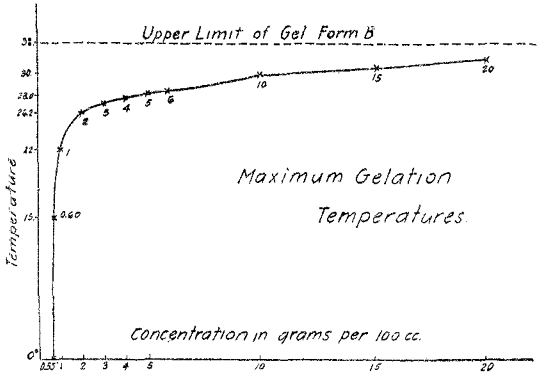

Fig. 2.

Possibly this may be due to the presence of large amounts of Form A, which serves in effect to change the character of the medium.

Temperature appears to have comparatively little influence on gelation, apart from its effect on the equilibrium, and it is unnecessary that gelation be observed at the maximum gelation temperatures to obtain the same differences or increments in rotation. Temperatures below these, at which the time required for gelation is not too short, 50 minutes or more, may be selected.

Since the increment in optical rotation necessary for gel production is nearly constant between 0.7 and 3 g. concentrations and the reaction involved has been shown to be bimolecular it is possible to derive an equation which will show the relation between the time necessary for gelation and various other factors, for concentrations of gelatin between these limits.

Let d = sp. rot. when equilibrium is established at gelation temperature minus sp. rot. at 35° (sign disregarded)

and b = increment of rotation at selected conditions of gelation viscosity,

and y = concentration in g. per 100 cc.

then $k\ 1/t \cdot b/yd(yd - b)$ and t varies as $1/yd(yd - b)$ or inversely as $y^2d - yb$ and $(y^2d - yb)t$ should be a constant quantity.

To test this expression the solutions must be cooled in a negligible fraction of time to the selected temperature and there maintained. A good procedure is to cool the gelatin, enclosed in thin metal tubes, for about 30 seconds, by immersion and agitation in a large volume of water at the selected temperature. The solution is then quickly transferred to the polariscope tube, which has been previously cooled to the same temperature, and the standard bubble formed. The principal error involved is in duplicating the standard viscosity which is represented by b. This expression would not hold if gel formation were not instantaneous with the formation of a definite quantity of Form B. Such is evidently the case as seen from the close agreement between observation and theory shown by the data given in Table XI.

TABLE XI.

Time of gelation at 15° $d = 125$; $b = 80$; t in minutes; y in g. per 100 cc.

y.	$y^2d - yb$.	t.	$(y^2d - yb)t$.
0.9	29	55	1595
1.0	45	33	1485
1.1	73	23	1669
1.2	84	18	1512
1.3	107	15.5	1658
1.4	133	12.0	1596
1.5	161	10.0	1610
1.6	208	8.1	1684
2.0	340	4.7	1598

Average, 1601 g.

TABLE XI (continued).

Time of gelation at 17° $d = 118$; $b = 80$; t in minutes; y in g. per 100 cc.

y.	$y^2d - yb$.	t.	$(y^2d - yb)t$.
1.0	38	51.0	1938
1.2	74	31.0	2294
1.4	119	20.0	2380
1.6	174	13.0	2262
2.0	312	7.2	2246

Average, 2224

Time of gelation at 19° $d = 111$; $b = 80$; t in minutes; y in g. per 100 cc.

1.0	31	108	3348
1.2	64	58	3700
1.4	105	34	3470
1.6	156	21	3276
2.0	284	13	3692

Average, 3497

Time of gelation at 20° $d = 96$; $b = 80$; t in minutes; y in g. per 100 cc.

1.0	16	243	3888
1.2	42	93	3906
1.4	76	48	3648
1.5	96	38	3648
1.6	118	32	3776
2.0	224	16.5	3696

Average, 3760

Solid Forms of Gelatin.

If we dry gelatin sols above 35° and the gels below 15° it is possible that we would obtain in the dry state the sol and gel forms which have been discussed in this paper. If the dried forms were the same as the sol and gel forms, respectively, we might expect them to show, approximately, at least, the same rotatory power.

The optical rotation of the solid, amorphous material obtained by drying sols and gels on glass plates in layers of uniform thickness under the conditions given above has been measured. Gelatin sols dried out above 35° give a rotation, calculated to a moisture- and ash-free basis, of approximately —12.0° per g. per square centimeter. If we consider a gram of the solid material to be contained in one cc., the specific rotation, based on a col. 1 dcm. in length, would be 10 times —12.0°, or —120°. The specific rotation of the sol form in various concentrations up to 20 g. per 100 cc. has been shown to be about —141° on a moisture- and ash-free basis. Assuming that all higher concentrations up to the pure sol form would have about this same rotatory power, the agreement between the specific rotatory power of the dried sol form (—120) and that of the sol form (—141°) is perhaps sufficient to indicate that the two forms may be the same.

The gel dried out below 15° has a rotatory power of about —75.0° per g. per square centimeter on a moisture- and ash-free basis. The specific rotatory power, calculated as above, is approximately —750° as compared with —313°, that of the gel form. It therefore seems probable that these two forms are not the same.

Gelatin sols dried above 35° and gels dried below 15°, the final drying being done in a vacuum desiccator over sulfuric acid, give the same solid content. Furthermore, no change in weight takes place on heating to 100°. At 125° to 130° a loss of 1.25% is observed. According to Hofmeister[1] this heating causes regeneration of collagen. Alexander[2] suggests that there is produced by an irreversible reaction a product in which the particles are so close together that dispersion is made very difficult.

Gelatin dried at 128° I have found to swell *very slowly* and dissolve in water at 35° to 40°. When these solutions are examined with the polariscope and by the air bubble jelly test the original jellying power is found to be nearly restored. I am inclined to think that both views may be practically correct in that collagen itself may represent a form of gelatin, the dispersion of which is very difficult.

Summary and Conclusions.

1. It has been found that gelatin in solution exhibits mutarotation. The effect of temperature upon this mutarotation has been studied. It has been shown that in aqueous solutions, within the range of temperature specified, there probably exist two forms of gelatin, one, which has been designated the sol Form A, stable above 33° to 35°, and the other, the gel Form B, stable below 15°. Between these temperatures a condition of equilibrium between the two forms exists and the mutarotation observed seems to be due to the transformation of one form into the other by a reaction which is reversible with temperature.

2. The reaction involved in this transformation appears to be bimolecular, a reaction of the kind to be expected if two molecular or equivalent weights of Form A combined to form one molecular weight of Form B.

3. The relationship of the percentage quantities of the two forms present when equilibrium is established at any specified temperature between about 17° and 33° is believed to be shown by the equation

$$(a - x)^2/x = K$$

in which a is the difference, about 1.20, between the rotations produced by one g. gelatin at 33° to 35° and at 17°, x is the difference in rotation between that at 33° to 35° and that at the specified temperature, and K is a constant.

4. Increase in levorotation, signifying increasing formation of the gel Form B closely parallels increase in viscosity.

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[1] *Z. physiol. Chem.*, **2**, 299 (1878).

[2] Allen's *Commercial Organic Analysis*, 4th ed., Vol. 8, p. 586.

5. A definite quantity of Form B, that produced by cooling about 0.55 g. of high grade gelatin in ice-water for 8 hours (or longer), is necessary to form a jelly of the degree of viscosity selected as a standard in this work. The presence of this quantity, slightly increased as concentrations increase, produces the standard jelly in much higher concentration of gelatin. Maximum gelation temperatures, or melting points, approach 33 to 35° as a limit as the concentration of gelatin increases. At these maximum gelation temperatures gelation is produced by the presence of the minimum quantity of Form B, 0.60 to 1.00 g., required for the formation of a jelly. Above 35° gelation does not take place in any concentration.

6. Additional evidence of the existence of two forms of gelatin, upon which gelation, in the case of gelatin solutions, is dependent, is found in observations made by the author on the behavior of such solutions when treated with alcohol, and in measurements of osmotic pressure, viscosity and of gold numbers, to which references have been given.

7. Gelatin sols dried above 35° and gels dried below 15° give different solid forms and while the gelatin in the solid state, so prepared, might or might not be in the same form in which it exists in the material from which it was prepared, there is some indication that the solid gelatin prepared by drying sols above 35° is the form existing in the sols.

WASHINGTON, D. C.

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

RELATIONS BETWEEN DISTRIBUTION RATIO, TEMPERATURE AND CONCENTRATION IN SYSTEM: WATER, ETHER, SUCCINIC ACID.

BY GEORGE SHANNON FORBES AND ALBERT SPRAGUE COOLIDGE.

Received November 18, 1918.

When a substance is distributed between two pure solvents or two solvents, each of which dissolves a constant percentage of the other, the distributed substance being identical in both phases, the ratio of its concentrations in the two phases at any given temperature is usually assumed to be constant. The case of succinic acid in water and ether[1] has been much used as an example in elementary instruction. In this case, however, the ratio is found to be by no means constant, but varies by 7% more or less, when expressed in volume concentrations. This is hardly surprising when one considers that neither of the fundamental conditions is more than approximately fulfilled. The solvents are not pure, but each dissolves the other. If the composition of the solvents in the two layers were constant, there would be no reason to suppose that the constancy of the distribution ratio would be disturbed. But this is not the

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[1] Berthelot and Jungfleisch, *Ann. chim. phys.*, [4] 26, 396, 408 (1872).