idge[16] and Buckley and Taylor[17] have observed abnormally high values of the quantity $i_d/Cm^{2/3}t^{1/6}$ at drop times less than three seconds unless a suppressor, such as gelatin, was present.

The use of some other supporting electrolyte with a higher solubility was not attempted because of the difficulty involved in finding one which was not reduced at very negative potentials and which could also be freed easily from traces of the alkali metal ions. In all probability, better agreement between the observed and calculated values of the diffusion current would be obtained if higher ratios of non-reducible to reducible ion were to be used.

(16) J. J. Lingane and B. A. Loveridge, THIS JOURNAL, 66, 1425 (1944).

(17) F. Buckley and J. K. Taylor, *J. Research Nat. Bur. Standards*, 34, 97 (1945).

Summary

The half-wave potentials of the alkali metal ions in liquid ammonia were found to agree with those calculated theoretically, and the reduction process appeared to be reversible.

The diffusion currents of the alkali metal ions were measured and compared with those calculated from the Ilkovic equation.

URBANA, ILLINOIS RECEIVED MARCH 4, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Mechanical Properties of Substances of High Molecular Weight. IV. Rigidities of Gelatin Gels; Dependence on Concentration, Temperature and Molecular Weight[1]

BY JOHN D. FERRY

Earlier measurements[2] have shown that the rigidity of a gelatin gel is approximately proportional to the square of the concentration, and it is well-known that the rigidity decreases with increasing temperature[3] or with degradation.[4] Previous work has, however, been confined to samples of unknown molecular weight. The series of degraded gelatins described by Scatchard, Oncley, Williams and Brown[5] has now afforded the opportunity of studying samples of known average molecular weight and molecular size distribution. The method of propagation of transverse vibrations[6] permits absolute measurements of rigidity to be made conveniently at concentrations lower than those usually employed by previous investigators. This paper reports rigidity measurements by the transverse vibration method on certain of the gelatin samples studied by Scatchard, Oncley, Williams and Brown, together with several other gelatins from different sources.

Materials and Method

The following gelatin samples were employed: four of the series of degraded ossein gelatins,[5] originally furnished through the kindness of Dr. D. Tourtellotte of the Knox Gelatin Company; one sample each of ossein (A-O), porkskin (A-P), and calfskin (A-C) gelatin, furnished by the Atlantic Gelatin Company; and one calfskin gelatin (EK-120), purchased from the Eastman Kodak Company. Table I lists the values of number-average molecular weight, M_n, derived from osmotic pressure and viscosity measurements.[7] Values of α, the fraction of bonds broken in the parent molecule, and M_w, the weight-average molecular weight, as calculated from the statistics of degradation,[5] are also included for the degraded series. They are omitted for the other samples because it is not certain that the details of the statistical treatment are applicable to those.

TABLE I

MOLECULAR WEIGHTS OF GELATIN SAMPLES

Sample	α	$M_n \times 10^{-3}$	$M_w \times 10^{-3}$
Degraded Series (Ossein)			
L1–00	0.00125	45	74
L1–80	.00235	29	53
L1–180	.00345	22	41
P7–180	.00470	17	33
Additional Samples			
A–P		47	
A–C		47	
A–O		39	
EK–120		37	

The degraded samples were furnished as sterile stock solutions, at a concentration of about 60 g./l., in 0.15 *M* sodium chloride at *p*H 7; the others, furnished in solid form, were dissolved in 37° and adjusted to the same *p*H and salt concentration, unless otherwise specified. Stock solutions were kept at 0°, except for occasional brief warming to 37° to withdraw samples, with sterile precautions. From studies of the rate of degradation,[5,7] no perceptible change in molecular weight would be expected for several years under these conditions. The measurements described here were completed in less than two years. Recent unpublished measurements on similar samples indicate that there is no change after five years.

The stock solutions, after warming at 37° for one hour, were diluted with 0.15 *M* sodium chloride (unless otherwise specified) to the desired concentrations and transferred to rectangular glass cells provided with clamps[8] to hold the

(1) Part of this work was carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University. Presented in part at meetings of the Society of Rheology, New York, N. Y., Oct. 30, 1943, and Nov. 17, 1944.

(2) A. Leick, *Ann. Physik*, 14, 139 (1904); S. E. Sheppard and S. S. Sweet, THIS JOURNAL, 43, 545 (1921).

(3) M. L. Sheely, *Ind. Eng. Chem., Anal. Ed.*, 2, 348 (1930); J. C. Derksen, Thesis, Amsterdam, 1935.

(4) E. T. Oakes and C. E. Davis, *J. Ind. Eng. Chem.*, 14, 708 (1922).

(5) G. Scatchard, J. L. Oncley, J. W. Williams and A. Brown, THIS JOURNAL, 66, 1980 (1944).

(6) J. D. Ferry, *Rev. Sci. Instruments*, 12, 79 (1941); THIS JOURNAL, 64, 1323 (1942).

(7) Unpublished measurements by Drs. G. Scatchard and A. Brown, Massachusetts Institute of Technology.

(8) Designed by Dr. S. H. Armstrong, Jr.

vibrator plates in place until rigidity had developed. The solutions were usually covered with paraffin oil to prevent evaporation. The velocity of propagation of transverse waves, V, was measured as previously described.[6] The modulus of rigidity is given by $G = V^2\rho$, where ρ is the density. The latter value was calculated from the density of the salt solution and an assumed value of 0.70 for the partial specific volume of gelatin. Damping was not measured. For each gel at each temperature, measurements of V were made at four or five different frequencies, covering a range of about twofold; these values usually agreed within 1–2% of the mean for the strongest gels and within 5% for the weakest. The rigidity was calculated from the mean value in each case. Measurements on three separate series of gels prepared from the same stock solution showed agreement to about the same extent (represented in Fig. 2). Since one year had elapsed between the first and the third series, this agreement confirms absence of degradation in storage.

There was no evidence of dispersion of the rigidity; the frequency ranges employed varied from about 1250–2500 cycles for the strongest gels to about 320–630 cycles for the weakest. Absence of dispersion indicates that the rigidity thus measured dynamically can be taken as identical with that which would be measured by the static methods used by previous investigators[2]; it is certainly of the same order of magnitude. A direct comparison of dynamic and static rigidities will be reported subsequently.[9]

Results

Changes of Rigidity with Time at Constant Temperature.—When a solution was quickly cooled from 37 to 15° and held at the latter temperature, gelation occurred within half an hour, with development of a measurable rigidity. The rigidity increased rapidly at first and continued to rise slowly for many hours without attaining a constant value. However, when a solution was first chilled at 0° for a day or more, and then quickly warmed to and held at 15°, the rigidity fell rapidly from its value characteristic of the lower temperature and reached an essentially constant value, usually within five hours. These changes are illustrated in Fig. 1 for sample EK-120 at a concentration of 25.7 g./l. and pH 5.4 (no salt). Similar measurements on several other samples at various concentrations showed the same behavior; when a chosen temperature was approached from above, the rigidity failed to attain a constant value in as long a period as fifty hours, but when it was approached from below, the rigidity became constant within five hours. These observations agree with earlier studies of the optical rotation,[10] specific volume,[11] and light scattering[12] of gelatin gels, in which equilibrium was always more rapidly attained after precooling.

Accordingly, the following procedure was adopted for all subsequent experiments: each solution, after introduction into the cell, was quickly cooled and kept at 0° for about twenty-four hours; the rigidity of the gel at 0° was measured; the temperature was raised a few degrees and kept constant (±0.1°) for five to twelve hours, and the rigidity was again measured; and this process was repeated until the gel melted. Since the initial measurement does not involve approach from a lower temperature, it cannot be compared with the others; and even at 10° the rigidity values are slightly higher when the chilling at 0° is prolonged for several weeks instead of limited to twenty-four hours. However, above 10° the results appear to be independent of the time of chilling. All the values are quite closely reproducible when the above procedure is followed.

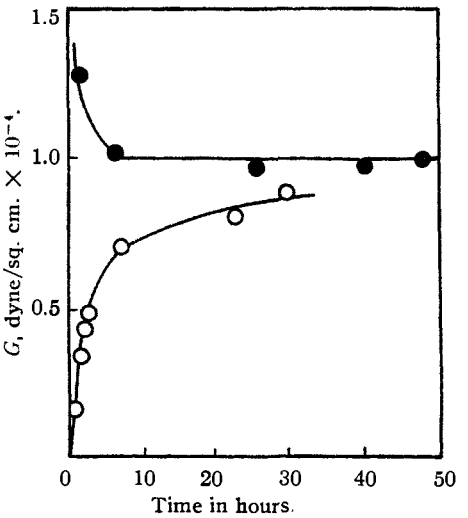

Fig. 1.—Change of rigidity with time at 15°, sample EK-120, concentration 27.4 g./l., pH 5.4: ●, after warming from 0°; O, after cooling from 37°.

Dependence of Rigidity on Concentration.—The rigidities of gels of sample L1-00 are plotted against the square of the concentration in Fig. 2 for several temperatures. Close proportionality is observed up to a concentration of about 60 g./l.; the values of G/c^2 in (dyne/sq. cm.)(g./l.)$^{-2}$ are 44 at 0°, 23 at 15°, and 12 at 21.8°. For a sample of similar molecular weight, A-O, over a much higher concentration range (up to 160 g./l.), there is slight downward curvature (Fig. 3); while for a sample of lower molecular weight, Ll-80, in the more dilute range there is slight upward curvature (Fig. 4).

Dependence of Rigidity on Temperature.—Values of G/c^2 for sample L1-00 are plotted against temperature in Fig. 5. The rigidity falls rapidly with increasing temperature, and vanishes at about 30°. It follows from Fig. 2, of course, that the curves for several different concentrations coincide. Nevertheless, these curves presumably diverge just below the melting point (where the rigidity disappears), because the latter is a function of concentration. For a gelatin with this molecular weight, the melting point, determined[13] after chilling for twenty-four hours at 0°, varies from 28.2° at a concentration of 20 g./l. to 30.6°

(9) J. E. Eldridge and J. D. Ferry, unpublished experiments at the University of Wisconsin.

(10) C. R. Smith, THIS JOURNAL, **41**, 135 (1919).

(11) E. Heymann, *Trans. Faraday Soc.*, **32**, 1, 462 (1936).

(12) W. Heller and E. Vassy, *Compt. rend.*, **207**, 157, 991 (1938).

(13) R. S. Gordon, Jr., and J. D. Ferry, *Federation Proc.*, **5**, 136 (1946).

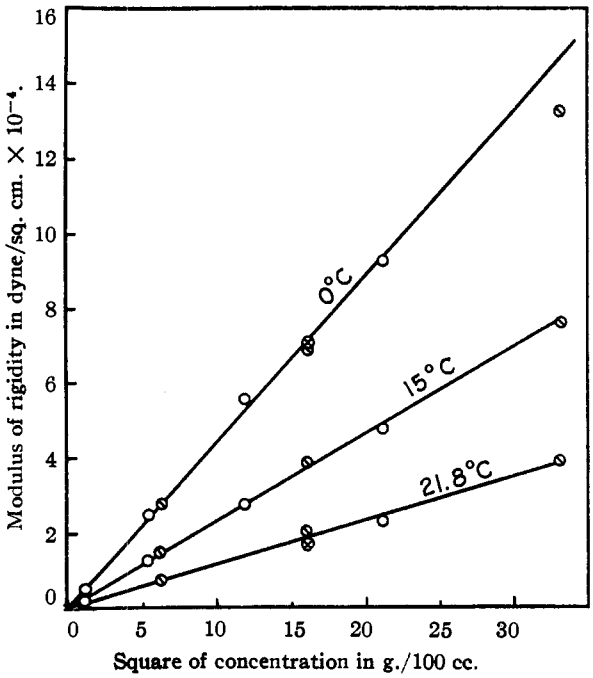

Fig. 2.—Rigidities of sample L1-00, plotted against square of concentration at different temperatures; age of stock solution: O, six months; ⊖, seven months; ⊗, eighteen months.

at 55 g./l. The rigidities of gels in the immediate vicinities of their melting points will be reported subsequently.[9]

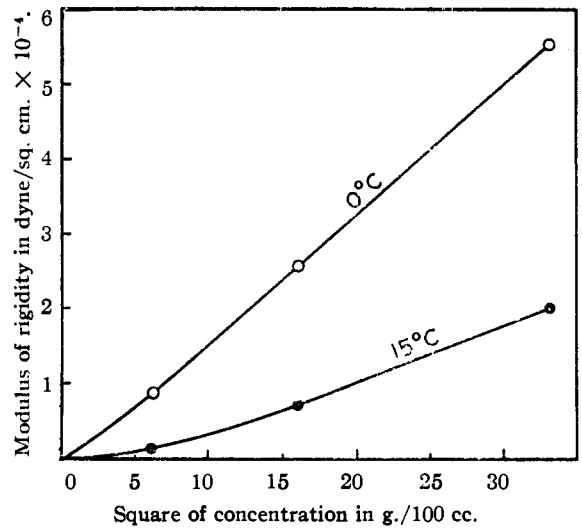

Fig. 4.—Rigidities of sample L1-80, plotted against square of concentration at different temperatures.

Since the values at 0° do not represent equilibrium approached from a lower temperature, and

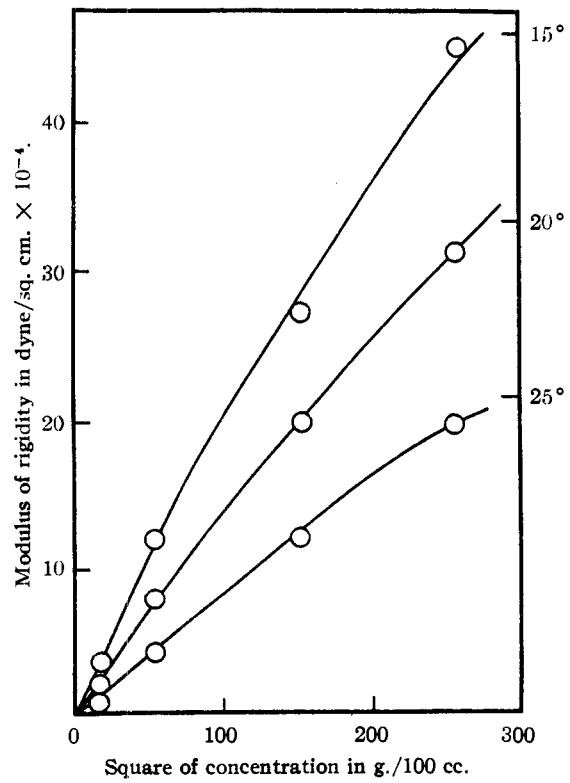

Fig. 3.—Rigidities of sample A-O, plotted against square of concentration at different temperatures.

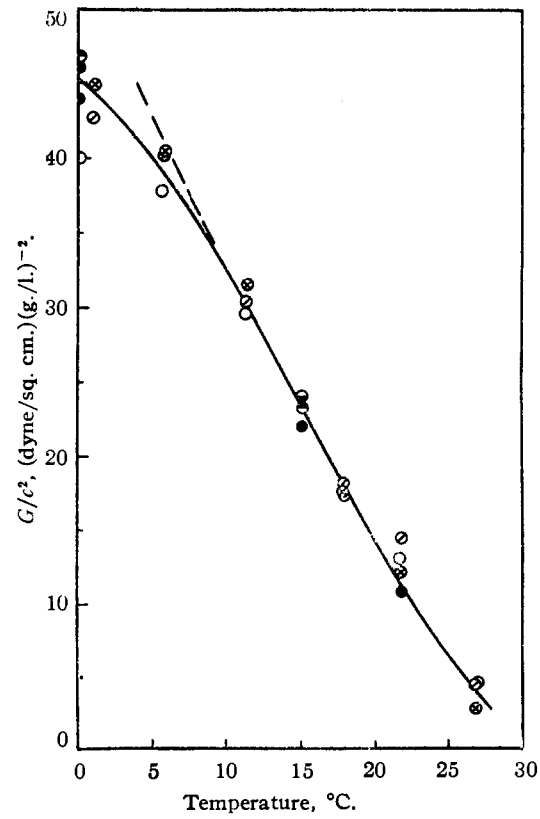

Fig. 5.—G/c^2 plotted against temperature, for sample L1-00; concentrations in g./l.: ○, 57.5; ●, 46; ⊘, 40; ⊖, 34.5; ⊗, 25; ⊜, 23.

are undoubtedly too low, it is possible that the inflection point in Fig. 5 is an artifact and the curve representing fully developed rigidity should more nearly follow the dashed line.

Rigidities for different gelatin samples, all at a concentration of 40 g./l., are plotted against the temperature in Fig. 6. The marked decrease in rigidity with increasing temperature is seen in each case; the curves form a coherent family except for that of sample A-C. Below 10° the curves are broken because, as pointed out above, they probably do not represent equilibrium values.

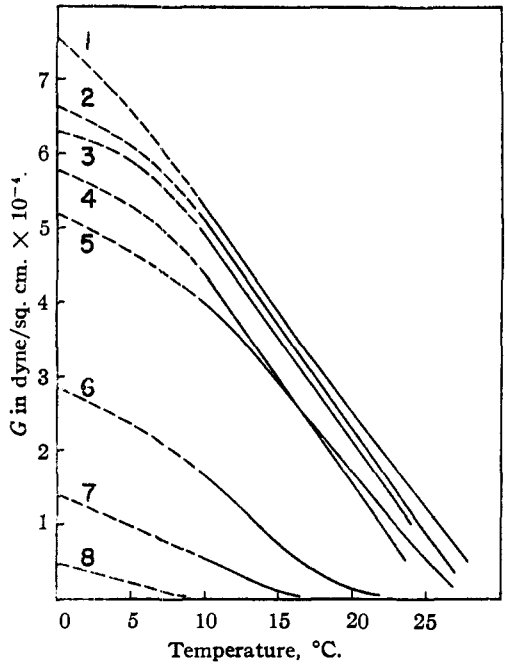

Fig. 6.—Rigidity plotted against temperature, at a concentration of 40 g./l.: 1, L1-00; 2, A-P; 3, A-O; 4, EK-120; 5, A-C; 6, L1-80; 7, L1-180; 8, P7-180.

Dependence of Rigidity on Molecular Weight.—Values interpolated from Fig. 6 at 5° and 15° are given in Table II, and are plotted against the number-average molecular weight in Fig. 7. The

TABLE II

RIGIDITIES OF VARIOUS SAMPLES AT GEL CONCENTRATIONS OF 40 G./L.

Sample	$M_n \times 10^{-3}$	$G \times 10^{-4}$, 5°	$G \times 10^{-4}$, 15°
	Degraded Series		
L1-00	45	6.6	3.9
L1-80	29	2.35	0.7
L1-180	22	1.0	0.1
P7-180	17	0.2	0
	Additional Samples		
A-P	47	6.2	3.7
A-C	47	4.7	2.9
A-O	39	5.8	3.55
EK-120	37	5.6	3.0

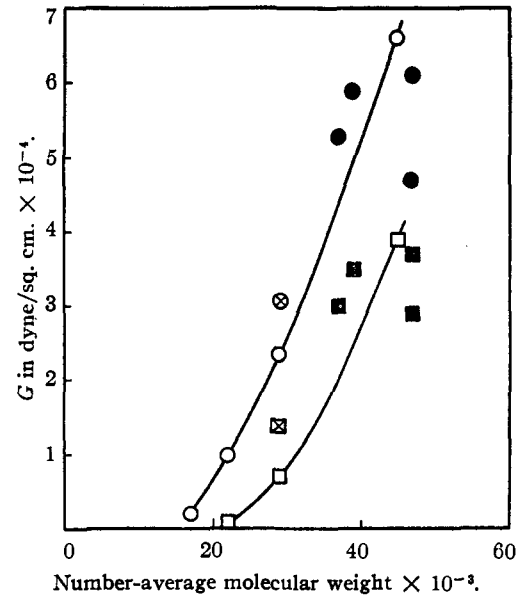

Fig. 7.—Rigidity plotted against number-average molecular weight, at 5° (circles) and 15° (squares): open points, degraded series; crossed points, mixture; solid points, additional samples.

points for the degraded series fall on a smooth curve. The scatter of the other points indicates that gelatins from various sources have roughly similar rigidities when compared on the basis of number-average molecular weight, but that the exact value of the rigidity is influenced by the details of preparative procedure. These data suggest further that the rigidity may depend more on the details of preparation than on the nature of the tissue source (ossein, porkskin or calfskin).[14]

Rigidities of a Mixture of Samples.—Two samples of widely different average molecular weight, L1-00 and L1-180, were combined in equal proportions by weight, yielding a mixture with M_n = 29,500 and M_w = 57,500, The rigidities of the mixture are compared with those of the original samples, all at a concentration of 57.5 g./l., in Fig. 8. (The points for L1-180 represent measurements at 40 g./l., corrected by the factor $(57.5/40)^2$.) The rigidity of the mixture does not correspond to the arithmetic mean of values for the individual samples, $(G_1 + G_2)/2$; instead, it

(14) A few measurements were made on several fractionated calfskin gelatin samples, sent by Dr. S. E. Sheppard of the Eastman Kodak Company to Professor Scatchard, in which the distribution of molecular weights was presumably far sharper than in the degraded series described here. The temperature dependence of rigidity for the fractionated samples was very similar to that shown Figs. 5 and 6, but the dependence on number-average molecular weight was quite different from that shown in Fig. 7. For M_n ranging approximately from 80,000 to 120,000, as estimated by Mr. R. H. Wagner in Dr. Sheppard's laboratory, the rigidity was almost independent of molecular weight and slightly less than that of our sample L1-00 at comparable concentration and temperature. This behavior was in agreement with unpublished measurements of the rigidity of these samples obtained by Dr. R. C. Houck in Dr. Sheppard's laboratory. The relation of these results to the measurements reported in this paper is not clear.

agrees closely with values calculated as $[(\sqrt{G_1} + \sqrt{G_2})/2]^2$, given by the dashed curve. Thus the square roots of the rigidities, rather than the rigidities themselves, are additive.

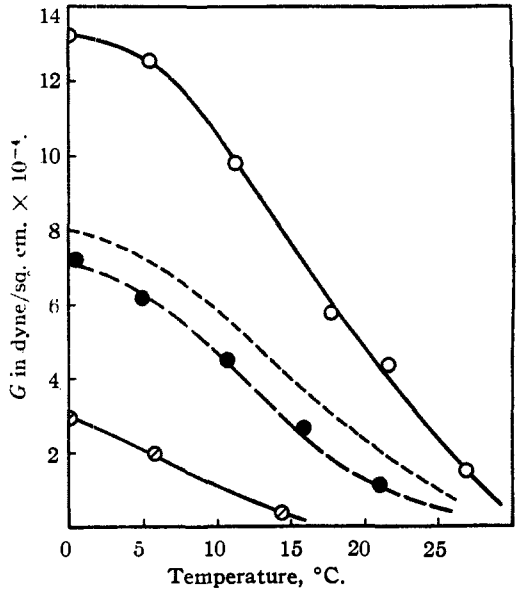

Fig. 8.—Comparison of rigidities of a mixed sample and of its components, at a concentration of 57.5 g./l.: O, L1-00 (G_1); ⊘, L1-180 (G_2); ●, mixture of equal parts by weight. ---, $(G_1 + G_2)/2$ (calculated); — — —, $[(\sqrt{G_1} + \sqrt{G_2})/2]^2$ (calculated).

Rigidities of the mixture at 5 and 15°, corrected to a concentration of 40 g./l., are included in the plot against M_n in Fig. 7; they do not fall on the curve for the individual degraded samples. However, when M_w is taken as the independent variable, points for the mixtures fall on the curves for the individual samples. Moreover, the square root of the rigidity is found to be a linear function of the weight average molecular weight (Fig. 9). It can easily be shown from the definition[5] of M_w that additivity of $\sqrt{G}$ for different samples on a weight concentration basis, as shown in Fig. 8, is a necessary consequence of the linear relation exhibited in Fig. 9.

Points interpolated at other temperatures and plotted as in Fig. 9 also give straight lines of equal slope, and their intercepts follow an exponential function of the reciprocal absolute temperature. These relations can be combined in the empirical equation

$$\sqrt{G} = 0.00484(M_w - 1.20 \times 10^{10} e^{-7330/RT}) \quad (1)$$

which expresses the rigidities of every gel of the degraded series at a concentration of 40 g./l. from 5° to the melting point; the fit is within experimental error except near the melting point for the most degraded samples.

Discussion

The highly elongated shape of the largest gelatin molecules in these mixtures[4,15] makes plausible the concept of gelation as network formation by cross-linking through secondary forces of attraction.[16] Some of the bonds may be represented by regions of local crystallinity, as postulated by Herrmann and Gerngross[17] (although their X-ray evidence was based on gels far more concentrated than those described here); others may be formed by lateral association of chain segments in pairs. The stability and low internal viscosity of gelatin gels suggest that there are specific loci of attraction widely spaced along the molecules,[18] corresponding either to sequences of easily crystallizable amino acids in the polypeptide chain (a series of glycine residues, for example), or to combinations of certain side chains resulting in strong attraction. Between these loci the attractive forces must be relatively slight; otherwise highly unstable gels such as those encountered in denatured proteins or inorganic colloids would be expected.

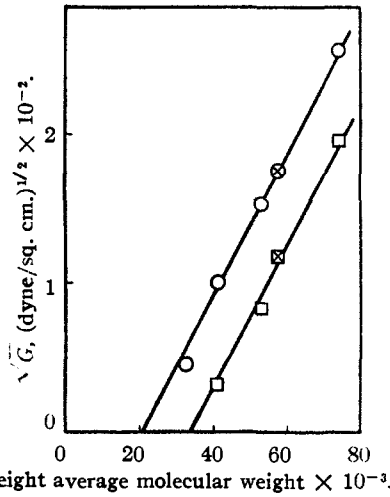

Fig. 9.—$\sqrt{G}$ plotted against weight-average molecular weight, at 5° (circles) and 15° (squares). Open points are degraded series; crossed points, mixture.

In such a network, the number of useful junctions which contribute to the rigidity is smaller than the total number of bonds or cross-links because of (a) bonds in the "sol fraction" which is not attached to the network, (b) possible cyclic structures, and (c) a number of bonds equal to the number of molecules initially present (half the number of loose ends) which will not contribute to the network.[19] Decrease in rigidity due to decreasing concentration, increasing temperature, or decreasing molecular weight may involve a decrease in the total number of bonds per cc. (n_t), an increase in the number of useless bonds per cc. (n_a), or both.

The fact that the rigidity is proportional to the

(15) E. O. Kraemer, *J. Phys. Chem.*, **45**, 660 (1941).
(16) P. J. Flory, *ibid.*, **46**, 132 (1942).
(17) K. Herrmann, O. Gerngross and W. Abitz, *Z. physik. Chem.*, **B10**, 371 (1930).
(18) J. D. Ferry, *Adv. Protein Chemistry*, Vol. IV, in press.
(19) P. J. Flory, *Chem. Rev.*, **35**, 51 (1944).

square of the concentration except near the melting point or when M_w is small indicates that, with the latter exceptions, the ratio of useless to total bonds is independent of concentration. Either $G \propto (n_t - n_a)$, as would be expected for rubberlike elasticity, and $n_t \propto c^2$, following a binary association; or $G \propto (n_t - n_a)^2$, which might correspond to a stiff strutted structure, and $n_t \propto c$, a peculiar type of association recently postulated by Doty for polyvinyl chloride solutions,[20] At present it is not possible to distinguish between these two alternatives.

Decrease in rigidity with increasing temperature must be due primarily to decrease in n_t. At the same time, the change is probably enhanced, at least near the melting point, by an increase in n_a, since the contributions to n_a from loose ends remain constant and those in the sol fraction should increase.

The decrease in rigidity with decreasing average molecular weight is clearly not due solely to the increase in loose ends, since in this case the total number of bonds should be constant and either G or $\sqrt{G}$ should be a linear function[19] of $1/M_n$. The total number of bonds evidently varies with average molecular weight, and with molecular weight distribution. Further work will be needed to explain the form of the empirical relationship given in equation (1).

(20) P. Doty, H. Wagner and S. Singer, *J. Phys. Colloid Chem.*, **51**, 32 (1947).

Summary

1. The rigidity of a gelatin gel at a given temperature reaches a constant value more rapidly if the temperature is approached from below than from above.

2. For a sample of slight degradation (M_n = 45,000), the rigidity was closely proportional to the square of the concentration up to 60 g./l. At higher concentrations, it increased somewhat less rapidly; for a sample of higher degradation, somewhat more rapidly, than with the square of the concentration.

3. For all samples and at all concentrations, the rigidity decreased gradually with increasing temperature from 0° to the melting point.

4. The rigidity decreased markedly with increasing degradation, or decreasing average molecular weight.

5. An empirical equation for the dependence of rigidity on temperature and weight average molecular weight is given.

BOSTON, MASS. RECEIVED SEPTEMBER 30, 1947

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION]

Chemical Composition and Properties of Guar Polysaccharides[1,2]

BY EILEEN HEYNE AND ROY L. WHISTLER

Endosperm of the guar seed consists principally of a galactomannan polysaccharide. It is thus analogous to the endosperm of locust beans which are widely used in commerce, and to galactomannans from other sources. Guar, a drouth-resistant legume of the genus *Cyamopsis*, is native to India where it is used for food and feed. The endosperm can be employed industrially in many ways, such as a size for paper and textiles, a dispersing agent, and a thickener. These uses have resulted in the recent growing of the guar plant in commercial quantities in the United States. As yet, however, little information is available with regard to the fundamental composition and structure of the endosperm polysaccharides. This report covers a preliminary investigation of the general composition and properties of the guar endosperm and particularly of the water soluble component which constitutes the major portion of the endosperm.

Experimental

Material.—Guar flour, produced by grinding the endosperm of the decorticated guar seed, was obtained through the kindness of General Mills, Inc. The grayish-white flour contained 0.60% nitrogen, 0.06% phosphorus, 1.06% ash and 1.5% ethanol solubles from twenty-four hours of Soxhlet extraction.

Analytical Methods.—Galactose anhydride content was determined by the following procedure: One gram of polysaccharide material ground to pass a 60-mesh sieve was dissolved in 150 ml. of 5% nitric acid by heating the mixture to 100°. After hydrolysis for three and one-half hours at this temperature, the solution was concentrated on a steam-bath to a volume of 25 ml. and 5.6 ml. of concentrated nitric acid was added to make a solution concentration of 25%. The solution was then oxidized according to the methods proposed by Tollens,[3] Van der Haar,[4] and Wise and Peterson.[5] The weight of the solution was reduced to 20 g. by heating on a steam-bath. Because of the high galactose content of the preparations, it was not found necessary to add the 500 mg. of pure mucic acid recommended by earlier works. The crystallization of mucic acid was allowed to proceed for forty-eight hours at a temperature of 0° ± 0.1° obtained with a large constant temperature bath. The solubility of mucic acid at 0° is 0.0175 g. per 100 ml. The amount of galactose anhydride equivalent to mucic acid was found by multiplying the weight of mucic acid obtained in this procedure by the factor 1.33. This factor was computed from data obtained on the analysis of a mixture of galactose and mannose combined in such proportions as to give the same yield of mucic acid as the guar samples.

(1) Journal Paper No. 319 of the Purdue University Agricultural Experiment Station.

(2) Paper presented before the Division of Sugar Chemistry and Technology at the 111th meeting of the American Chemical Society, Atlantic City, 1947.

(3) B. Tollens, *Ann.*, **227**, 223 (1885); **232**, 187 (1886).

(4) A. W. Van der Haar, *Biochem. Z.*, **81**, 263 (1917).

(5) L. E. Wise and F. C. Peterson, *Ind. Eng. Chem.*, **22**, 362 (1930).