for the bridge used in this investigation this would be a total variation of not to exceed ±0.0004 ohm. Each of the measurements used in calibrating the bridge was within this limit and no single measurement of the transition temperature differs from the average by more than this amount. As 0.0004 ohm represents approximately 0.004°, it appears that the transition temperature of sodium sulfate heptahydrate to anhydrous sodium sulfate and solution is 23.465 ± 0.004°.

In his work on the sodium sulfate decahydrate temperature, Richards[1] reported that lowering the outside temperature by two or three degrees lowers the temperature of the salt by about 0.01°. Dickinson and Mueller[3] found no changes exceeding 0.001° were observed by raising or lowering the outside temperature by as much as 10° if a large tube is used and if many crystals are formed by sudden cooling. In the case of the sodium sulfate heptahydrate transition point, the data show that the external temperature probably can vary by at least 3° from the equilibrium temperature without changing the temperature of the salt, but an ice-bath surrounding the tube does lower the temperature, probably by several hundredths of a degree. From experience it would seem that the sodium sulfate heptahydrate transition temperature probably is influenced slightly more by external conditions than is the transition temperature of the decahydrate.

Summary

A method has been described for preparing sodium sulfate heptahydrate and for maintaining the sodium sulfate heptahydrate transition temperature. This temperature has been determined to be 23.465 ± 0.004°. Because the salt is purified easily and the transition temperature is near the most used part of the thermometer scale, this point is proposed as a useful secondary standard in calibrating thermometers along with the sodium sulfate decahydrate transition point.

LINCOLN, NEBRASKA RECEIVED OCTOBER 11, 1937

[CONTRIBUTION FROM THE BIOCHEMICAL RESEARCH FOUNDATION OF THE FRANKLIN INSTITUTE]

An Ultracentrifugal Study of Gelatin

BY EDWARD B. SANIGAR, LAURA E. KREJCI AND ELMER O. KRAEMER

Introduction

Krishnamurti and Svedberg[1] some years ago carried out an ultracentrifugal investigation of a photographic gelatin at 20°. In the range of *p*H 4.6-6.0 they found the sedimentation velocity to be high and difficult to reproduce, and to increase rapidly with aging of the solution, the increase being greater the nearer the *p*H was to the isoelectric point. At *p*H's 4.0 and 7.5, the sedimentation velocity was low, corresponding approximately to that of a protein like egg albumin, and was independent of time. According to the evidence provided by the light scattering capacity of gelatin, as given by Kraemer and Dexter[2] and by Krishnamurti,[3] it appears that the molecular aggregation associated with gelation does not occur at these *p*H's even at 20°. On the other hand, the results given by Kraemer[4] on the viscosities of dilute gelatin solutions with these *p*H's at temperatures below 30° reveal quite definitely the fact that aggregation and gelation do occur under these conditions. It seemed desirable, therefore, to extend the ultracentrifugal study to higher temperatures where the effects of gelation would be more definitely repressed. From previous work[2,4,5] it appeared that a temperature definitely above 30° would be desirable.

Experimental Procedure

Four commercial gelatins were investigated: Coignet Gold Label Gelatin (ossein) (Gelatin A), Grayslake Gelatin (pigskin) (Gelatin B), Eastman De-ashed Gelatin (calfskin, first extraction) (Gelatin C), Atlantic Super-X Gelatin (calfskin, first extraction) (Gelatin D). Each gelatin was studied at two different concentrations, *i. e.*, 2 g. and 0.4 g. of anhydrous gelatin per 100 cc., respectively.

For the preparation of the more concentrated solution, 5 g. of air-dry gelatin (moisture content *ca.* 20%) was allowed to swell for about an hour in 25 cc. of water containing 0.5 g. of phenol, and solution was completed at 37.4°

(1) Krishnamurti and Svedberg, THIS JOURNAL, **52**, 2897 (1930).
(2) Kraemer and Dexter, *J. Phys. Chem.*, **31**, 764 (1927).
(3) Krishnamurti, *Proc. Roy. Soc.* (London), **A129**, 490 (1930).
(4) Kraemer, "Colloid Symposium Monograph," Vol. IV, 1926, p. 102.
(5) Kraemer and Fanselow, *J. Phys. Chem.*, **32**, 894 (1928).

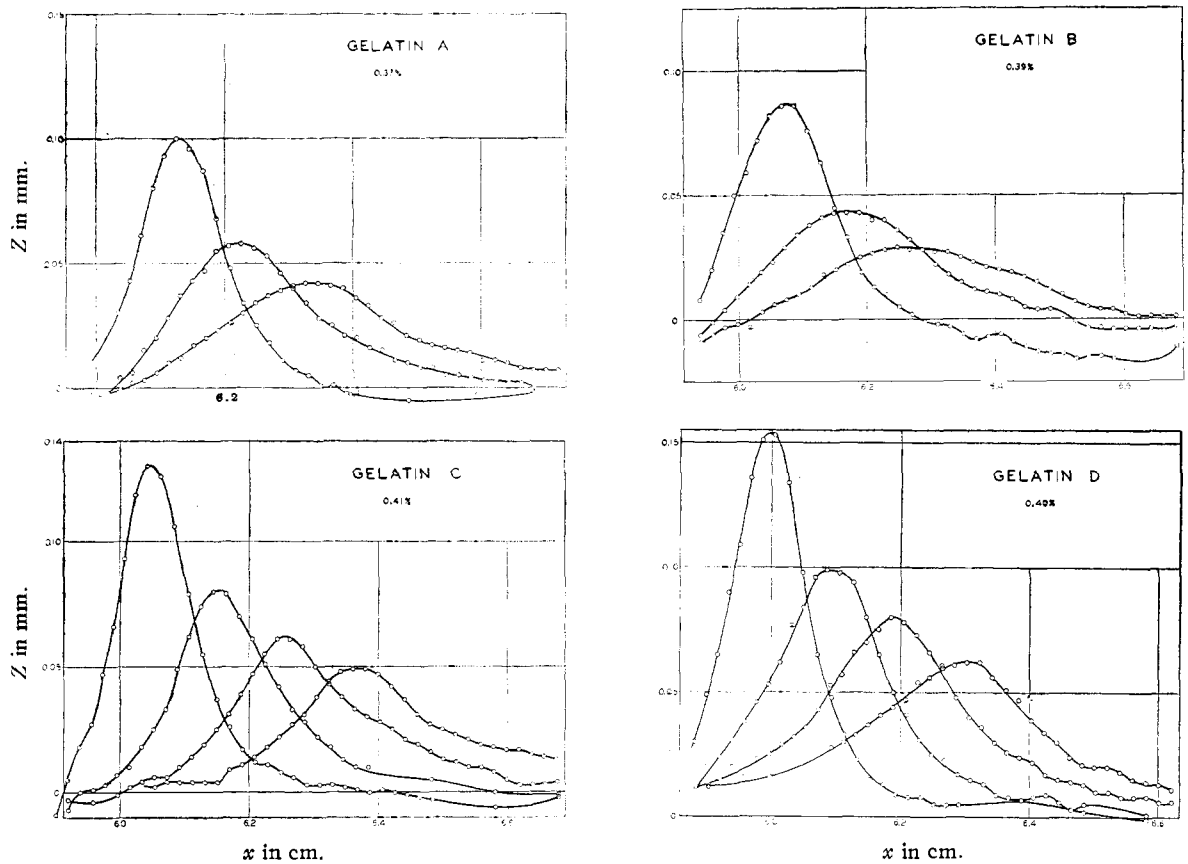


Fig. 1.—Ultracentrifugal sedimentation diagrams for dilute gelatin solutions. The abscissas represent distances from the center of rotation, and the ordinates scale-line displacements. The first exposure in each series was taken approx. twenty minutes after final speed was reached. Interval between exposures, thirty minutes. Cell thickness 3 mm. Scale distance 10 cm. Speed (approx.) 50,000 r. p. m. Centrifugal force (approx.) 200,000 g. Temperature (approx.) 34°.

The resulting solution was titrated with 0.1 *N* sodium hydroxide to a *p*H of approximately 7.5, using brom thymol blue as external indicator. It was then made up to 50 cc. with distilled water, diluted with 50 cc. of 0.1 *M* phosphate buffer of *p*H 7.5, and replaced in the thermostat overnight. The next morning the solution was centrifuged in a laboratory centrifuge to remove the slight precipitate which had formed on standing. The clear, centrifuged solution was diluted with an equal volume of 0.85% sodium chloride solution, and stored in a refrigerator at 3°.

The solutions containing 0.4 g. per 100 cc. were prepared by diluting portions of the corresponding 2% solutions with four volumes of a solvent containing the same salt concentrations (0.025 *M* phosphate, 0.425% sodium chloride, 0.25% phenol and 0.005 *N* sodium hydroxide).

Micro-Kjeldahl analyses were made in duplicate on each solution, using the Parnas and Wagner distillation apparatus.[6] The gelatin content was calculated from the micro-Kjeldahl results on the basis of 17.9% nitrogen content.

Measurements of Density and Viscosity.—The density and viscosity of each solution were determined at 31.1, 34.0 and 37.4°. The density measurements were made in 10-cc. Sprengel pycnometers; viscosities were measured in Ostwald viscometers.

To obtain a clearer insight into the effect of gelatin concentration on the viscosity, and of the variation between the four gelatin samples, the data have been expressed in several ways and tabulated in Table I.

TABLE I

VISCOSITY OF GELATIN SOLUTIONS

Absolute viscosity of solvent: 0.0076 at 34°, 0.0071 at 37.4°.

Gelatin	Concn.[a]	η/η_0 at 34°	η/η_0 at 37.4°	$[\eta]$ at 34°	ϕ/cV at 34°
A	0.37	1.13	1.14	0.33	21
	2.10	2.24	2.27	..	..
B	0.39	1.20	1.16	.46	30
	1.94	2.55	2.21	..	..
C	0.41	1.29	1.30	.62	42
	2.09	3.54	3.27	..	..
D	0.40	1.24	1.24	.53	35
	2.00	3.21	3.07	..	..

[a] In g./100 cc. at room temperature.

The relative viscosity, η/η_0 or η_r, is the ratio of the viscosity of the solution to that of the solvent

(6) Parnas and Wagner, *Biochem. Z.*, **125**, 253 (1921).

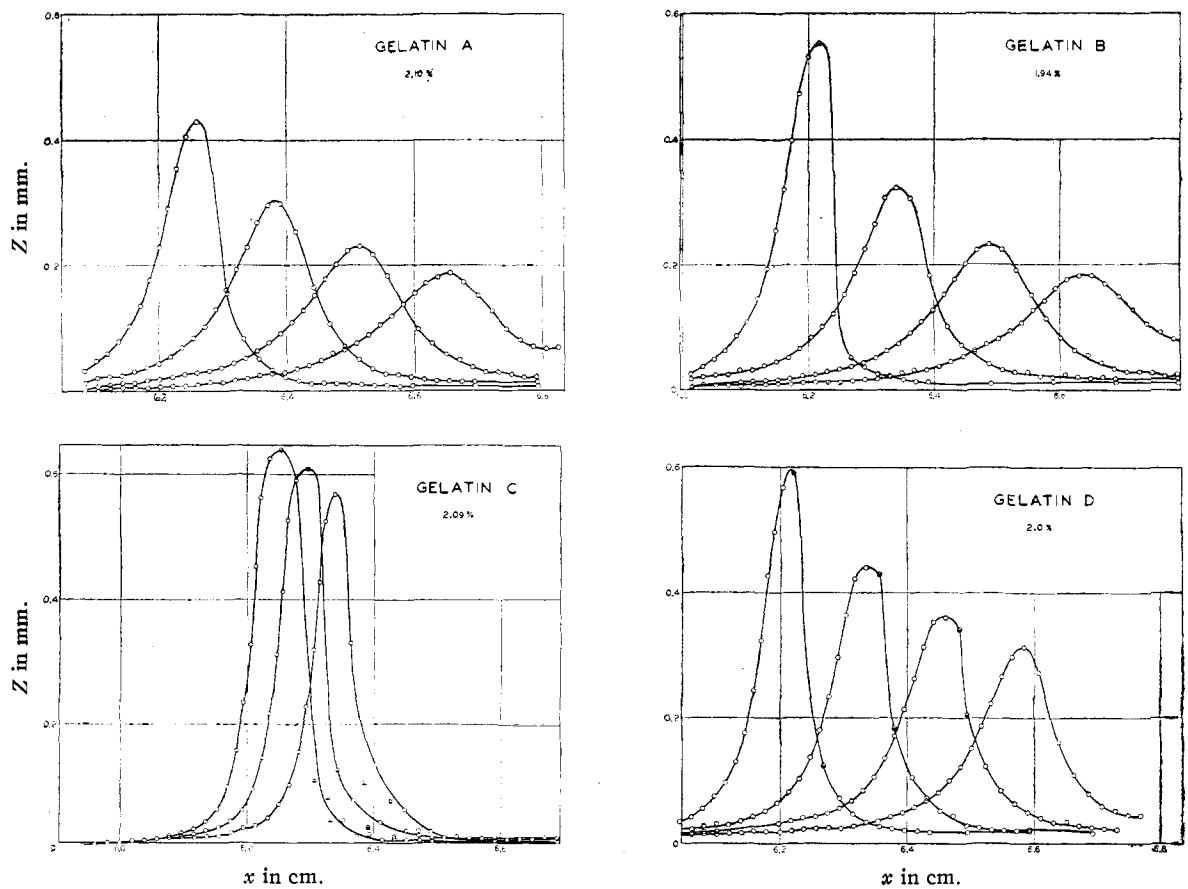


Fig. 2.—Ultracentrifugal sedimentation diagrams for concentrated gelatin solutions. The abscissas represent distances from the center of rotation, and the ordinates scale-line displacements. The first exposure in each series was taken approx. 70 minutes after final speed was reached, except for Gelatin C. Interval between exposures, 60 minutes except for Gelatin C, where the exposures are those taken at approx. 160, 190 and 220 minutes after reaching speed. Cell thickness 3 mm. Scale distance 5 cm. Speed (approx.) 50,000 r. p. m. Centrifugal force (approx.) 200,000 g. Temperature (approx.) 34°. ◒. Indicates values given by double lines on photographs.

(composition as above) at the same temperature. The intrinsic viscosity, $[\eta]$, is defined by the equation[7]

$$[\eta] = \lim_{c \to 0} \frac{\eta_r - 1}{c} = \frac{\ln \eta_r}{c}$$

and represents the increase of relative viscosity, η_r, with concentration (c) at infinite dilution. The ratio ϕ/cV is defined in terms of an extended form of Einstein's equation, according to which the hydrodynamic volume ϕ of spherical, unsolvated particles is equal to cV, the product of the concentration c in grams per cc. and the partial specific volume V of the particles.[8] When the particles are not spherical or are solvated, $\phi > cV$, and the deviation of ϕ/cV from unity provides a measure of the extent to which the viscosity is non-ideal. Gelation would be expected to give large values of ϕ/cV, but since the relative viscosity at 37.4° is so nearly the same as at 34°, it is not believed that the normal, temperature-reversible gelation is responsible for the observed high values of ϕ/cV.

Sedimentation Velocity Measurements.—Measurements of sedimentation velocity were made, using the refractive index method,[9] in a standard Svedberg oil-turbine ultracentrifuge at a speed of 50,000 r. p. m., corresponding to a centrifugal force of about 200,000 times gravity at the center of the cell. A 3-mm. thick cell was used, with a scale distance of 5 cm. for the concentrated solutions, and 10 cm. for the dilute solutions. The temperature in the cell was maintained at 34–35° as closely as possible:

(7) Kraemer and Lansing, *J. Phys. Chem.*, **39**, 153 (1935).
(8) Kraemer and Sears, *J. Rheol.*, **1**, 231 (1930).
(9) (a) Lamm, *Z. physik. Chem.*, **A138**, 313 (1928); (b) Lamm, *ibid.*, **A143**, 177 (1929); (c) McFarlane, *Biochem. J.*, **29**, 407 (1935); (d) Pedersen, *ibid.*, **30**, 948 (1936).

during any one run the variation in temperature from the time of attaining full speed did not exceed 0.6° except in two instances (Gelatin A and Gelatin B, 0.4% solutions). The precaution was observed of making solvent runs under identical centrifuging conditions (see also Pedersen),[9d] the scale photographs so obtained being used as the standard reference scales from which the deviations, caused by sedimentation of the gelatins, were measured.

The line positions in the scale photographs were measured to the nearest micron; readings were reproducible to ±2 microns. The sedimentation curves for the gelatins studied, obtained by plotting the line displacement Z (in mm.) against the distance (in cm.) from the center of rotation, are shown in Figs. 1 and 2.

The sedimentation constants[10] were calculated from the equation

$$s_T = \Delta x/\Delta t \times 1/\omega^2 x_m$$

and, for comparison purposes, reduced to the basis of sedimentation in water at 20° by the expression

$$s_{20} = s_T \cdot \eta_T/\eta_{20} \cdot (1 - V\rho_{20})/(1 - V\rho_T)$$

where Δx = the distance moved (in cm.) by the sedimenting boundary in time Δt sec.; ω = the angular velocity; x_m = the mean distance (in cm.) from the boundary to the center of rotation; η_T, ρ_T = the viscosity and density, respectively, of the solution at the temperature of centrifuging; η_{20}, ρ_{20} = the viscosity and density, respectively, of water at 20°; V = the partial specific volume of gelatin, 0.682[1,11]

Inspection of the values of s_{20} (Table II) shows that in the dilute solutions, where conditions are more favorable for ideal sedimentation, the sedimentation constants increase in the order of increasing viscosity, while in the concentrated solutions there is an apparent discrepancy which is considered further in the discussion of the results. The values are greater at the higher concentration, suggesting aggregation.

The data of Krishnamurti and Svedberg[1] for gelatin solutions of the same concentration and *p*H, as well as the sedimentation constant of egg albumin,[10b] have been included in Table II for comparison. The sedimentation constants which Krishnamurti and Svedberg obtained for gelatin are higher than those found in the present investigation, but, in view of the variation of these four gelatins, the discrepancy is not marked, and may be a consequence of the effect of lower temperature of centrifuging (20°) on the state of aggregation.

TABLE II[a]

Scale distance: 5 cm. for the concentrated solutions, 10 cm. for the dilute solutions. Exposures: every thirty minutes for the concentrated solutions, every fifteen minutes for the dilute solutions. Duration of run: concentrated solutions, four hours; dilute solutions, two and one-half hours.

Gelatin	Concn.[b]	Mean temp., °C.	$s_T \times 10^{13}$ in cm./sec. dyne	$s_{20} \times 10^{13}$[c] in cm./sec. dyne	(s_{20})adj. $\times 10^{13}$ in cm./sec. dyne
A	0.37	34.2	3.04	2.60	2.57
	2.10	34.6	1.98	3.36	3.10
B	0.39	34.0	3.09	2.82	2.78
	1.94	34.9	2.21	4.07	3.63
C	0.41	34.8	3.40	3.30	3.20
	2.09	34.8	1.69	4.41	4.05
D	0.40	34.8	3.23	2.98	2.88
	2.00	34.6	1.89	4.52	4.03
Krishnamurti and Svedberg,[1] Table II:[d]					
Run 13	0.4	*ca.* 20	..	3.64	..
Run 14	.4	*ca.* 20	..	3.43	..
Egg albumin[10b]				3.55	

[a] All the solutions used in the present investigation had been aged at least five days. This should have been long enough to complete any significant changes in particle size which might be expected to occur on aging, especially since aging appears to cause little change in the properties of gelatin solutions at this *p*H. [b] In g./100 cc. at room temperature. [c] It should be emphasized that the values of s_{20} express the measured rate of sedimentation of the gelatin *at 34–35°*, corrected to 20° in terms of the density and viscosity of the medium only, to facilitate comparison with the sedimentation constants of other substances. They do not necessarily represent the values which would be obtained by centrifuging the same solutions *at 20°*. Agreement could be expected only if change of temperature caused no change in the state of aggregation. This appears to be true for most proteins, but would hardly be expected to be invariably true for gel-forming systems. [d] Run 13 represents a freshly prepared gelatin solution at *p*H 7.5, run 14 the same solution stored for two days in a refrigerator at 0°.

Boundary Spreading Measurements.—The apparent diffusion coefficients were calculated in the conventional manner[9b,12] by measuring the half-width (u) of each curve at height h where

$$h = \frac{\text{maximum height of curve}}{\sqrt{e}} = 0.606 \text{ max. ht.}$$

and plotting the square of this value (u^2) against the time of centrifuging (t). The best smooth curve was drawn through the points, and the slope of the curve was measured at times corresponding to each of the plotted points. From

(10) (a) Svedberg, *J. Biol. Chem.*, **103**, 311 (1933); (b) Svedberg, *Nature*, **139**, 1051 (1937).

(11) Krishnamurti and Svedberg[1] found the partial specific volume of gelatin to be substantially independent of temperature and concentration over the ranges 20–30° and 1–2%, respectively.

(12) Kraemer and Lansing, THIS JOURNAL, **55**, 4319 (1933).

these slopes the values of the apparent diffusion coefficient (D) were calculated by means of the equation

$$D_T = \Delta(u^2)/2\,\Delta t$$

These values were corrected to the basis of diffusion in water at 20° by means of the equation

$$D_{20} = D_T(T_2/T_1)\eta_1/\eta_2$$

where η_1 is the viscosity of the gelatin solution at the temperature of centrifuging, T_1 (absolute temperature), and η_2 is the viscosity of water at 20° (T_2, absolute temperature). The D_{20} values were plotted against time of centrifuging, and are shown in Fig. 3.

Discussion of Results

Sedimentation Constant.—The gross departure of the ratio ϕ/cV from unity indicates that gelatin, even at 34° and in dilute solution, does not behave like egg albumin, hemoglobin and other globular proteins, and is evidently, therefore, not a compact, spherical, unsolvated molecule. Svedberg's results on density and hydration in gelatin solutions and gels[13] show there may be as much as half a gram of water attached to each gram of gelatin. However, hydration even several times greater than this would not account for the viscosity data (see last column, Table I), and it must therefore be assumed that dissolved gelatin particles are also non-spherical or porous or both. When, in addition, it is considered that gelatin is undoubtedly heterogeneous, calculations of molecular weights (or particle weights) from sedimentation velocity data alone are of no value.

The present results show that an increase of concentration from 0.4 to 2% causes a rise in the sedimentation constants of the gelatins. This would indicate larger particle size at the higher concentration, which is contrary to the conclusions of Krishnamurti,[3] based on measurements of the depolarization of scattered light.

Several factors may operate to give falsely high values for s_{20} in concentrated solutions. One is the progressive dilution below the boundary caused by centrifuging in a sector-shaped cell in a field of varying intensity: this is given[14] by the equation

$$c_t = c_0(x_0/x_t)^2$$

where c_t = concentration of the solution below the boundary after centrifuging for t seconds;

(13) Svedberg, THIS JOURNAL, **46**, 2673 (1924).
(14) Svedberg and Rinde, *ibid.*, **46**, 2677 (1924).

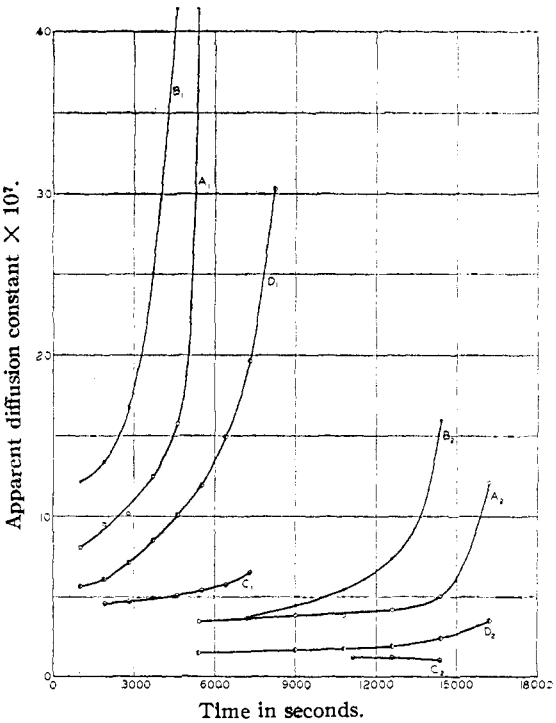


Fig. 3.—Subscript 1 refers to dilute solutions and subscript 2 to concentrated solutions.

c_0 = initial (analytical) concentration; x_0 = distance from the meniscus to the center of rotation, and x_t = distance from the center of rotation to the boundary at time t sec. The reduction in viscosity accompanying this dilution, which is negligible in dilute solutions of normal viscosity, amounts in the concentrated gelatin solutions to a factor of 8–11% (see (s_{20})adj., Table II). The increase of sedimentation constant with increase in concentration persists even when this correction is applied.

The above equation is valid only for solutions of homogeneous particles. In heterogeneous solutions the dilution is greater because of the more rapid removal of particles heavier than the main constituent. The relatively steep slopes of both sides of the curves in the sedimentation diagrams, however, show that the effect of this factor must here be negligible.

In making the viscosity correction, a reciprocal relationship between sedimentation constant and relative viscosity has been assumed. In solutions of high relative viscosity, only part of the measured viscosity may offer resistance to sedimentation: the application of the usual correction would then represent an over-correction, and give values for s_{20} which were too high. This is

the most serious source of uncertainty, which probably cannot be elucidated until detailed data for a number of proteins over wide concentration ranges have been accumulated and studied in various ways. Consequently, it is impossible to conclude definitely from the present results whether gelatin is aggregated in solutions of 2% concentration.

The differences between the sedimentation constants for the gelatins are almost certainly real because at either concentration the viscosity corrections for all four are of similar magnitude (see relative viscosities, Table I). Among the four gelatins there is a definite correlation between sedimentation and viscosity data, as shown in Fig. 4. For the dilute solutions, the various

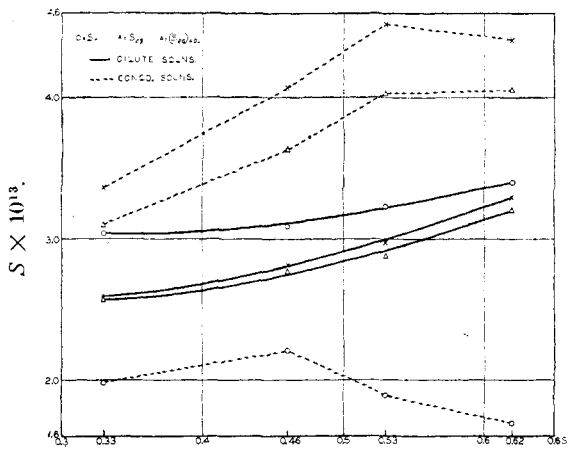


Fig. 4.

corrections are sufficiently small so that the correlation is evident for s_T, and s_{20} as well as for (s_{20})adj. For the concentrated solutions, on the other hand, where the corrections for viscosity are appreciably greater, the relationship to be expected on the basis of the behavior in dilute solution is evident only in the curve of (s_{20})adj. The fact that gelatins C and D were observed to give stronger jellies than gelatins A and B suggests that high-grade gelatins may be characterized by high sedimentation constant as well as by high viscosity.

Boundary Spreading.—The sedimenting boundary normally becomes blurred in time because of the diffusion of the sedimenting particles into the solvent above.[10b] If the sedimenting material is heterogeneous, the boundary becomes abnormally broadened owing to the progressive separation of the several species; this gives rise to a gradual increase of the apparent diffusion constant with time. When the heterogeneity is moderate, it is possible to estimate the average diffusion coefficient of the mixture by extrapolating the apparent diffusion coefficients to zero time, where the effect of spreading due to heterogeneity is minimized.[15]

In solutions where the high viscosity is due to the sedimenting particles, the spreading of the boundary is further complicated by the fact that as the particles diffuse above the boundary they enter a region of lower concentration in which they sediment more rapidly because of the decreased viscous resistance. This gives values for the diffusion constant which are too low; for a given substance the difference is greater the higher the viscosity. McFarlane[9c] (p. 413), working with homogeneous solutions of serum albumin, observed an actual sharpening of the boundary at high concentrations. If the sedimenting material is heterogeneous, the broadening of the boundary due to heterogeneity is also depressed for the same reason. However, if the heterogeneity is sufficiently pronounced, sufficient spreading may occur to give an apparent diffusion coefficient greater than the low value predicted by the effect of viscosity alone. The interplay of these various factors is illustrated by the diffusion curves shown in Fig. 3.

It will be observed that, in general, the apparent diffusion constants of the gelatin solutions vary considerably with time and, further, that the extrapolated values of D_{20} at zero time approach (with one exception, Gelatin B, 0.39%) values which increase in the order of decreasing viscosity. The peculiar effects due to the high viscosity of gelatin will be least for the more dilute solutions. In these solutions the variation of apparent diffusion constant with time is probably a qualitative measure of the relative heterogeneity of the gelatins. On this basis, therefore, Gelatin B is the most heterogeneous, the others falling in the order A, D, C, Gelatin C being the most homogeneous. In the solutions of higher concentration the mutual interference of the particles makes itself felt on the boundary spreading, as indicated by the tendency of the apparent diffusion coefficient to vary less with time, and the values of D_{20} at zero time, where the effect of heterogeneity would be reduced to a minimum, show the same

(15) (a) Svedberg and Stamm, THIS JOURNAL, **51**, 2170 (1929); (b) Lamm, *Kolloid. Z.*, **69**, 44 (1934).

order as the intrinsic viscosities. After lengthy sedimentation, however, the differences in heterogeneity assert themselves and the order of the curves becomes the same as that of the dilute solutions.

Acknowledgment.—The authors are greatly indebted to E. I. du Pont de Nemours and Company for permission to use the oil-turbine ultracentrifuge. They also wish to express their appreciation of the help given to them during the investigation by Dr. J. B. Nichols of the du Pont Experimental Station.

Summary

1. The sedimentation constants of four different brands of commercial gelatin in 0.4% solution at *p*H 7.5, measured at approximately 34°, vary between 2.60 and 3.30, compared with 3.43, the value found by Krishnamurti and Svedberg at 20° for a gelatin solution of the same concentration and *p*H, and 3.55, the constant for egg albumin, a normal globular protein of molecular weight 43,000.

2. The sedimentation constants in the dilute solutions were found to increase regularly with the intrinsic viscosities.

3. The sedimentation constants for the 2% solutions were found to be from 29 to 52% (average 41%) higher than for the dilute solutions; from the available data it is impossible to decide whether the increase is due to aggregation, or whether it is only apparent, the result of an over-correction for viscosity.

4. All the solutions were found to be heterogeneous, to various degrees, with respect to particle size.

PHILADELPHIA, PENNA. RECEIVED JANUARY 12, 1938

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TEXAS]

Nitrogen Compounds in Petroleum Distillates. XI. Isolation of 2,3-Dimethyl-8-ethylquinoline from the Kerosene Distillate of California Petroleum[1]

BY C. L. KEY AND J. R. BAILEY

Introduction

The crude bases, used in this investigation and referred to as "kero" bases, were furnished by the Union Oil Company of California from the residual sulfur dioxide extract, obtained in refining kerosene by the Edeleanu process. Previously, the following polymethylated quinolines had been obtained from the same source: 2,3-, 2,4-[2] and 2,8-dimethylquinoline[3] along with 2,3,8-[4] and 2,4,8-trimethylquinoline.[5]

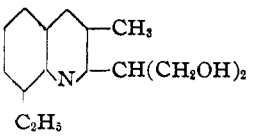
Whereas the coal-tar bases, quinoline, quinaldine, lepidine, and isoquinoline do not occur, so far as is known, in *straight run* petroleum distillates, the first two substances have been separated from *cracked* gasoline.[6]

All of the above kero quinolines are methylated at 2, the other positions of substitution being 3, 4 and 8. With the exception of the 2,3,8-homolog, they were known to synthesis, prior to being obtained from petroleum. Recently, 2,3,8-trimethylquinoline, together with quinoline, isoquinoline and lepidine, was encountered among the complex mixture of bases formed in pyrolysis of cottonseed meal.[7]

It is worthy of note that quinaldine is the only coal-tar base which yields a quinphthalone (Quinoline Yellow), whereas all of the six known kero quinolines condense readily with phthalic anhydride in the formation of quinphthalones of the same shade of color as Quinoline Yellow.

2,3-Dimethyl-8-ethylquinoline represents the only ethyl homolog so far encountered. The structure assigned was deduced from its oxidation to 2,3-dimethylquinoline-8-carboxylic acid and was confirmed by synthesis.

Through condensation with formaldehyde, it was converted to 3-methyl-8-ethyl-2-dimethylolmethylquinoline



(1) From a dissertation presented by C. L. Key to the Faculty of the Graduate School of the University of Texas in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) Biggs and Bailey, THIS JOURNAL, **55**, 4141 (1933).

(3) Lake and Bailey, *ibid.*, **55**, 4143 (1933).

(4) King and Bailey, *ibid.*, **52**, 1239 (1930).

(5) Perrin and Bailey, *ibid.*, **55**, 4136 (1933).

(6) Bratton and Bailey, *ibid.*, **59**, 175 (1937).

(7) Parker and Bailey, *ibid.*, **58**, 1102 (1936).