[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

The Molecular Weight of Beta-Amylose from Corn Starch by Means of the Ultracentrifuge[1]

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The chemical and physical methods that have been used to estimate the particle size of β-amylose yield values which sometimes differ from one another by orders of magnitude. In addition to the criticism that various authors have used methods of varying intensity to disrupt the starch granule and thereby degraded the starch to different extents, there is the further criticism that the methods used for determining particle size are at present unsound theoretically or experimentally or both, and, further, do not give any indication of the heterogeneity of the starch product.

Haworth and collaborators[2] conclude from their methylation studies (end-group assay) that the molecule of starch contains 30 glucopyranose units, corresponding to a molecular weight of 5000, and reconcile their results with the higher values (of the order 10^5) found by Samec[3] from osmotic pressure measurements, by Staudinger[4] from viscosity methods and by others from the determination of free aldehydic groups by analytical methods, by assuming the associative force of the hydroxyl group. This hypothesis is supported by the work of the late Professor T. C. Taylor and his collaborators[5] of this Laboratory who have found that gentle disruptive methods like dry grinding and neutral salt gelatinization are effective in reducing the particle size as determined by the "alkali-labile" value,[6] an index which is only relative, to be sure, but whose practical usefulness is established by the present work. The recent reëxamination of the methylation technique by Hess and Lung,[7] which indicates that the chain length of starch is 52 glucopyranose units (molecular weight, 8500), does not change the above argument.

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(2) W. N. Haworth, *Chemistry and Industry*, **54**, 859 (1935).

(3) M. Samec, "Kolloidchemie der Stärke," Verlag von Theodor Steinkopff, Dresden und Leipzig, 1927, p. 251.

(4) H. Staudinger, *Naturwissenschaften*, **25**, 673 (1937).

(5) T. Clinton Taylor, "The Chemistry of Certain Amylose Transformations," Presented at the 89th Meeting of the American Chemical Society, New York, N. Y., 1935.

(6) T. C. Taylor, H. H. Fletcher and M. H. Adams, *Ind. Eng. Chem., Anal. Ed.*, **7**, 321 (1935).

(7) K. Hess and K. H. Lung, *Ber.*, **70**, 1259 (1937).

However, Staudinger's[4] hypothesis that the 30 unit (or 52 unit) chains are linked through primary valence bonds cannot be discarded in view of his finding that the molecular weights of a starch preparation, the corresponding starch triacetate and the starch regenerated by deacetylation are practically identical when determined by osmotic pressure and viscosity measurements.

The weaknesses inherent in both methods of attack make a decision in favor of one hypothesis or the other impossible at the present time. On the one hand, the method of end-group assay depends on the methylation of starch which is never complete. The subsequent hydrolysis to the methyl glucoses invariably results in the formation of a non-crystallizable residue which may contain dimethyl glucose and which is neglected in the calculations. It is assumed that the end-groups are "under-methylated" to the same extent as the chain groups. On the other hand, the two physical methods most often used, namely, osmotic pressure and viscosity, suffer from the fact that the former, although theoretically sound, is burdened with large experimental error when applied to high molecular weight substances, while the latter, which is experimentally precise, has a doubtful theoretical foundation. In fact, Hess and Lung[7] point out that the molecular weight of their methylated starch is 30–60 times greater when measured by the viscosity of its solutions than when measured by the end-group assay, while for methylated cellulose the situation is reversed, the end-group assay giving a value 5–10 times greater than the viscosity method.

Perhaps the most satisfactory of all are the ultracentrifugal methods which have been used in only one other instance on starch. These methods give estimates not only of particle size, but of particle shape and, for mixtures, the distribution of particle sizes. Thermodynamically, the sedimentation equilibrium method of Svedberg[8] is almost ideal but long and tedious, frequently re-

(8) The Svedberg, "Colloid Chemistry," 2d ed. (A. C. S. Monograph No. 16), The Chemical Catalog Co., New York, N. Y., 1928.

quiring days to complete. Sedimentation velocity measurements, on the other hand, are not on as sound a theoretical basis but may be performed in a few hours and still give very satisfactory results. Furthermore, they give better information regarding the degree of heterogeneity of the sample. Hence, since Beams[9] successfully adapted the principle of the air driven top of Henriot and Haugenard[10] to the construction of simple and relatively inexpensive optical ultracentrifuges, recourse was had to this method.

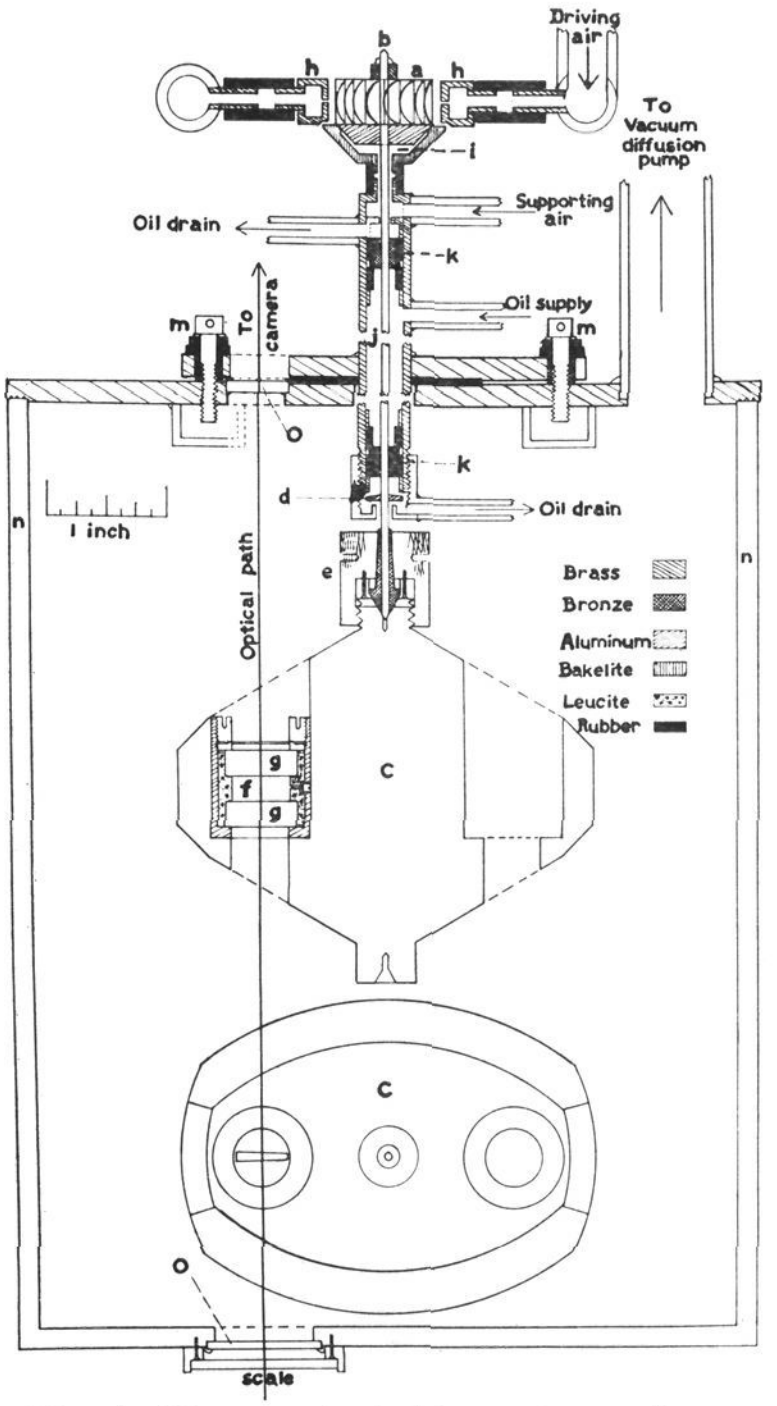


Fig. 1.—Diagram of air-driven ultracentrifuge: a, turbine[12]; b, piano wire shaft, 0.0625″ (1.6 mm.) diam.; c, rotor, major axis 3.5″ (8.9 cm.); d, oil slinger; e, chuck; f, cell compartment containing sample, 1.2 cm. in length × 0.5 cm. thickness; g, quartz plates,[15] 5 cm. thick; h, driving air manifold[9]; i, supporting air chamber; j, oil gland; k, bronze bushings individually set in duprene bushings; m, leveling screws; n, vacuum chamber; o, glass windows.

(9) J. W. Beams, *J. Applied Phys.*, **8**, 795 (1937).

(10) Henriot and Haugenard, *Compt. rend.*, **180**, 1389 (1925).

Apparatus.—The apparatus (Fig. 1) represents the application of Lamm's refractive index method[11] of determining concentration gradients to Beams' air-driven vacuum type ultracentrifuge. Since the centrifugal force is directly proportional to the radius, R, of the rotor and to the square of the angular velocity, ω, while the ultimate breaking speed of the rotor is a function of R^{-1}, the ultimate force available is a function of R^{-1}. Thus higher fields may be obtained with smaller rotors and the 3.5 inch (8.9 cm.) rotor of Beams and Pickels[12] was adopted in preference to the larger rotors of Svedberg,[13] Biscoe, Pickels and Wyckoff[14] or Bauer and Pickels.[15]

The driving mechanism differs essentially from that of Beams and Pickels[12] in the use of a conical air bearing, i, and in the individually mounted bushings, k. This latter innovation minimizes the development of standing waves in the piano wire shaft with nodes at the bushings and prevents serious bending stresses. A lead shield 2.5 inches (6.4 cm.) thick is provided for protection, encircling the vacuum chamber. The cells were constructed with no essential changes from the design of Bauer and Pickels.[15]

The vacuum system is of the conventional type: an oil diffusion pump backed with a high capacity rotary oil pump providing a vacuum of 10^{-5} mm. mercury as indicated by a McLeod gage. De Khotinsky cement serves to seal the glass portion of the system to the metal chamber. If the temperature of the latter is not allowed to rise during a run, adsorbed gases are not released and the high vacuum may be maintained with ease. Otherwise the rotor temperature will rise to undesirable values.

The optical system consists of a General Electric 85-watt high pressure mercury vapor lamp operating on 500 volts a. c. Light from this source is condensed, filtered for the green line (5461 Å.) and rendered parallel, then passed through a photographic enlargement of a Leitz contrast step eyepiece micrometer and through the cell to the camera. The latter is fitted with a 55-cm. aplanatic glass lens, usually operated at an aperture of about f : 150.

Operation.—In operation the cell is filled with the solution of suitable concentration—usually 0.5–1.0%—and aligned in the rotor. The rotor is placed on the shaft, the vacuum chamber warmed to a few degrees above room temperature, placed in position, the vacuum drawn and the chamber allowed to cool. When the pressure has been reduced to 10^{-5} mm., the shield is placed in position and air at 55–60 lb./sq. inch (4 atm.) is delivered to the manifold. Under these conditions about two minutes are required to reach a speed of 1000 r. p. s.; about three minutes to reach 1400 r. p. s. When the desired speed is attained, as roughly checked by an electronic audio oscillator, the manifold pressure is reduced to that which will just maintain it; from 8 to 12 lb./sq. in. (0.53 to 0.80 atm.).

(11) O. Lamm, *Z. physik. Chem.*, **A138**, 313 (1928); **A143**, 177 (1929).

(12) J. W. Beams and E. G. Pickels, *Rev. Sci. Instruments*, **6**, 299 (1935).

(13) T. Svedberg and J. B. Nichols, THIS JOURNAL, **48**, 3081 (1926).

(14) J. Biscoe, E. G. Pickels and R. W. Wyckoff, *Rev. Sci. Instruments*, **7**, 246 (1936).

(15) J. H. Bauer and E. G. Pickels, *J. Exptl. Med.*, **65**, 565 (1937).

Photographic exposures of duration five to ten seconds are made immediately upon attaining full speed and at definite periods thereafter. The speed is checked frequently by a mechanical stroboscope.

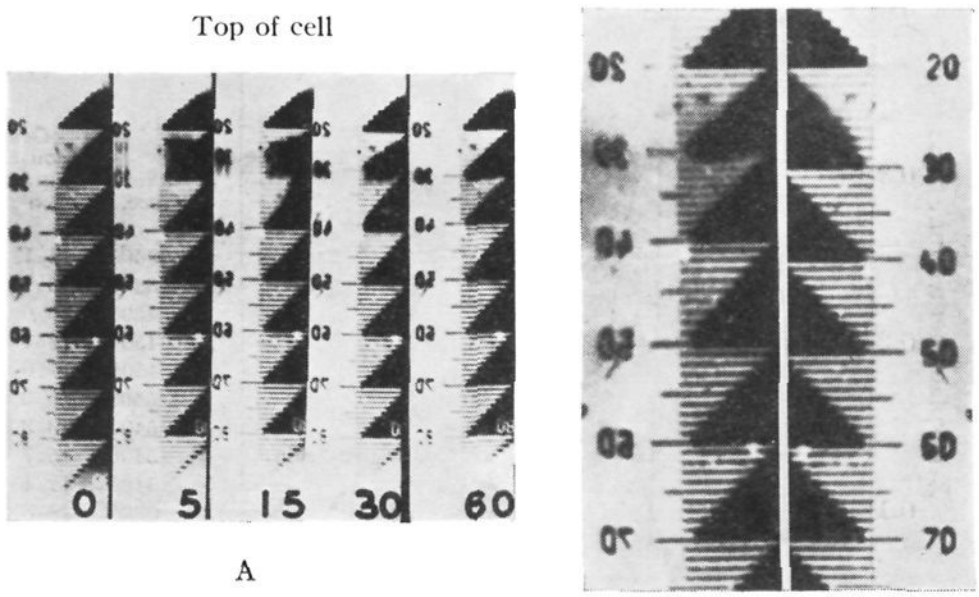
After development, the plate is placed in a comparator and the original position and displacement of the lines due to the refractive index gradient in the solution obtained. The distances of the original positions from the edge of the minor axis of the rotor are obtained so that the relative position of scale and cell may be determined. The displacements are then plotted against the positions. As the boundary moves in the centrifugal field, the concentration gradient and the maximum displacement of the lines travel away from the meniscus (Fig. 2). From the position of the peaks in successive exposures the sedimentation constant can be calculated, and from the spreading of the boundary as reflected in the shape of the curve, the diffusion constant may be calculated if the material is monodisperse.[11]

Calibration.—Svedberg and Sjögren[16] reported Nichols' value for the sedimentation constant of crystallizable egg albumin to be $3.32 \cdot 10^{-13}$ at 20°. Later work by Nichols[17] showed a range of values between 3 and 3.5 depending somewhat on the ionic environment. Figure 3 and Table I show the results of the analysis of a plate taken with this substance (prepared by Sörenson's[18] method and twice recrystallized) in the cell.

The vertical bisector of the area under the curve should theoretically be taken as the position of the boundary but here the vertical bisectors of three horizontal chords at $^1/_4$, $^1/_2$ and $^3/_4$ of the height of the curve have been used. They nearly coincide in most cases. The extrapolated value for the position of the meniscus, line 24.2 compares favorably with the observed value, 24.5. The observed position of each boundary must be corrected for the effect of concentration gradient by subtracting half the height of the peak, $Z_m/2$, and reducing to cell distances. Svedberg's sedimentation constant then becomes for this case

$$S_{20} = \frac{(x - x_0 - Z_m/2)}{x_m \Delta t \omega^2} \frac{\eta_{27}}{\eta_{20}}$$

where x is the position of the boundary as taken from the curve, x_0 is the position of the meniscus, x_m, the average distance from the axis of rotation, all expressed in consistent units. Z_m is the maximum height of the curve in cm., l is the distance from the lens to the scale, b is the distance from the scale to the center of the cell, G is the photographic enlargement of the scale, t is the time in seconds, η, the viscosity of the solution, and ω, the angular velocity. In subsequent tables $x_c = (x - x_0 - Z_m/2)$. If u = the half breadth of the curve at the points of inflection, taken at a height $Z = Z_m/\sqrt{e}$ in subsequent calculations, the diffusion constant D is given by the equation $2Dt = u^2$. Correcting plate distances to cell distances this becomes $D = 7.7 \times 10^{-5}\, u^2/\Delta t$ for the geometry of this apparatus.



A b B a

Fig. 2.—A, Appearance of the scale on the plate at successive time intervals (minutes) showing excessive distortion in the early stages; B, scale displacement during sedimentation; a, photograph taken at start of centrifuging; b, appearance of scale after 60 min. The lines are crowded together between 25 and 35 and spread apart between 40 and 50. The position of the maximum is between 35 and 40.

Since the base line is not well defined in this case the diffusion constant is determined as follows.[11] Two arbitrary horizontal chords are drawn, of half breadth Δx_1 and Δx_2 such that $\Delta x_1 = \sqrt{2}\Delta x_2$. If the vertical distance between Δx_1 and Δx_2 be denoted by α and the vertical distance from Δx_2 to the peak by h, then the diffusion constant is given by

$$Dt = \frac{(\Delta x_2)^2}{4 \ln(h/\alpha)}$$

It is necessary to reduce Δx_2 to the dimensions in the cell. In this case the factor is 1.65×10^{-5} when Δx is expressed in line numbers, whence

$$D = \frac{(\Delta x_2)^2 (1.67 \times 10^{-5})}{\Delta t \log (h/\alpha)}$$

(16) T. Svedberg and B. Sjögren, THIS JOURNAL, **51**, 3594 (1929).
(17) J. B. Nichols, *ibid.*, **52**, 5176 (1930).
(18) S. P. L. Sörenson and M. Höyrup, *Compt. rend. trav. lab. Carlsberg*, **12**, 164 (1917).

The diffusion constant is also subject to a correction because of the effect of higher order terms in the complete solution of the differential equation for simultaneous sedimentation and diffusion in a sector-shaped cell[19]

$$D_{corr.} = D\,(1 - \omega^2 st)$$

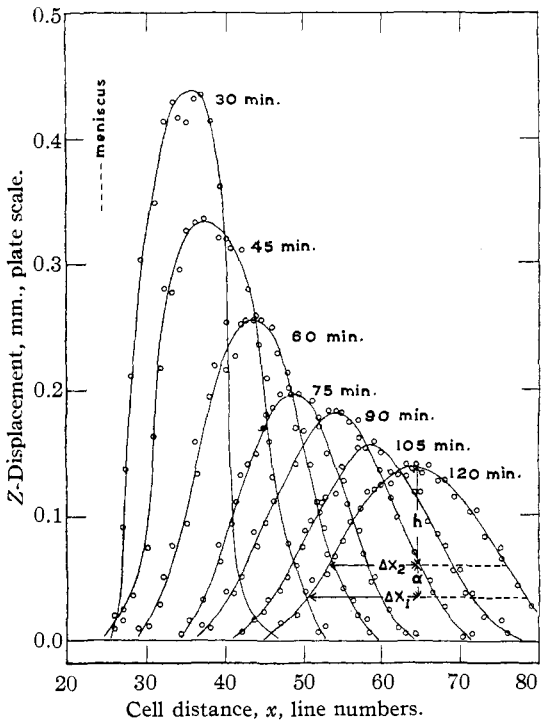


Fig. 3.—Analysis of data for egg albumin: concn. = 0.75%; *p*H 5.1; temp. 26–28°; speed 1308 r. p. s.; $\omega^2 = 6.75 \times 10^7$; $l = 97.8$ cm.; $b = 12.0$ cm.; $(l - b)/l = 0.878$; $G = 1.57$; plate scale = 0.0222 cm. per line; $\eta_{27}/\eta_{20} = 0.846$.

If Δx_1 is chosen too small, log (h/α) becomes very small and magnifies the errors. Hence an arbitrary limit of log $(h/\alpha) = 0.25$ has been set in this work. Moreover, when back diffusion from the bottom of the cell makes the curve unsymmetrical, as at one hundred and twenty minutes in this case, Δx_1 cannot be taken too large or this effect will modify the result. The concentration gradients in the early curves (thirty and forty-five minutes) were so large that no attempt was made to evaluate diffusion constants from them.

The value of $3.22 \pm 0.08 \times 10^{-13}$ obtained for s_{20} is in satisfactory agreement with the reported values[7] (p. 164). The diffusion constant evaluated from these curves is $7.44 \pm 0.34 \times 10^{-7}$ at 27°. Svedberg's value of 9.58×10^{-7} at 20° given in various tables of such data apparently is a calculated and not a measured value.[7] Egg albumin is one of a number of proteins whose molecular weight by the sedimentation equilibrium method does not coincide with that by the sedimentation velocity method. In a later report Nichols[17] gives no data but states that he found experimentally a value of 7.6×10^{-7} at 30° (6.08 at 20°) with which our value checks satisfactorily. As this investigation is concerned with inhomogeneous materials, the true diffusion constant has little if any significance and has been calculated primarily to serve as a rough index of heterogeneity. Plotting the reciprocal of the apparent diffusion constant against the time of sedimentation often results in a straight line, the slope of which multiplied by D^2 and recorded as $D^2 \mathrm{d}(1/D)/\mathrm{d}t$ is a relative empirical measure of the degree of heterogeneity.

(19) O. Lamm, *Arkiv. Mat., Astron., Fysik*, **21B**, No. 2 (1929); H. Faxen, *ibid.*, **21B**, No. 3 (1929); *cf.* W. J. Archibald, *Phys. Rev.*, **53**, 746 (1938); **54**, 371 (1938).

TABLE I

EGG ALBUMIN

SEDIMENTATION

Time, sec.	Curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{27} \times 10^{13}$
1800	0.441	1.98	44.87	3.63
2700	.335	3.13	45.44	3.78
3600	.257	4.27	46.01	3.82
4500	.196	5.42	46.58	3.84
5400	.183	6.59	47.17	3.54
6300	.157	7.72	47.73	3.81
7200	.139	9.01	48.38	3.84

mean s_{27} $3.75 \pm 0.09 \times 10^{-13}$

s_{20} $3.22 \pm .08 \times 10^{-13}$

DIFFUSION

Time, sec.	Graphical construction, Line numbers Δx_1	Δx_2	α, mm.	h, mm.	Log (h/α)	$\omega^2 st$	$D \times 10^7$
3600	14.1	9.98	0.098	0.384	0.586	0.08	7.25
3600	16.1	11.4	.0885	.425	.682	.08	8.11
4500	15.0	10.6	.094	.299	.502	.10	7.50
4500	13.1	9.26	.110	.251	.358	.10	8.01
4500	14.0	9.90	.1025	.274	.427	.10	7.67
5400	18.3	13.3	.0665	.300	.654	.12	7.35
5400	13.3	9.41	.095	.205	.334	.12	7.21
5400	15.7	11.1	.0815	.253	.492	.12	6.84
5400	12.1	8.55	.0990	.177	.252	.12	7.90
6300	18.8	13.3	.0625	.248	.698	.14	6.76
6300	12.3	8.70	.083	.147	.248	.14	6.96
6300	15.5	11.0	.076	.202	.425	.14	6.50
7200	16.7	11.8	.077	.170	.344	.16	7.87
7200	17.5	12.4	.076	.180	.374	.16	7.96
7200	15.7	11.1	.078	.156	.301	.16	7.33

Mean $D_{27} = 7.44 \pm 0.34$

$D_{20} = 6.30 \pm .29$

Calculation of Molecular Weights

The calculation of molecular weights from the data obtained by the sedimentation velocity is

attended by some uncertainty whose cause is not understood completely at the present time. In spite of the general applicability of the laws of hydrodynamics to colloidal solutions, one might expect some molecules to behave differently in the diffusion process and in the sedimentation process so that the same frictional coefficient cannot be used to describe both phenomena. Since diffusion is the resultant of Brownian motion, the frictional coefficient in the direction of the maximum concentration gradient measured by this process will be an average of the coefficients for all possible orientations and deformations of the molecule. On the other hand, sedimentation is due to the superposition of an external field on Brownian motion and can produce a preferred orientation[20] and deformation of the molecule giving rise to a different coefficient. This orientation, in turn may result in some change in the apparent diffusion constant.

In the present work, three molar frictional coefficients are defined and the results reported as molecular weights times the appropriate powers of the ratio of two of the coefficients. Hydrodynamically, the molar frictional coefficient, f, is the ratio of the force, F, acting on a mole of particles to the constant velocity, W, attained in the steady state of motion, *i. e.*

$$f = F/W \tag{1}$$

The molar frictional coefficient for the diffusion process, f_D, is given by the Sutherland–Einstein equation

$$f_D = RT/D \tag{2}$$

where R is the gas content, T, the absolute temperature and D, the diffusion constant. The molar frictional coefficient for the sedimentation process, f_s, is given by

$$f_s = (1 - V\rho)M/s \tag{3}$$

where V is the partial specific volume of the solute, M its molecular weight, s, the sedimentation constant and ρ, the density of the solvent.

If the molecule is a solid sphere, the molar frictional coefficient, f_0, is given by Stokes' law for a sedimenting particle

$$f_0 = 6\pi N\eta r = 6\pi N\eta \sqrt[3]{\frac{3VM}{4\pi N}} \tag{4}$$

where r is the radius of the sphere, N Avogadro's number and η the viscosity of the medium. Since f_s and f_0 are defined for the same process (sedimentation) their ratio, f_s/f_0, Svedberg's dissymmetry or form factor, serves as a measure of the deviation of the form of the molecule from that of a perfect solid sphere.

If, for the general case, we take the three coefficients unequal, *i. e.*

$$f_0 \neq f_s \neq f_D \tag{5}$$

it becomes impossible to determine the molecular weight, for the combination of any two of the equations (2), (3), (4) will always contain a ratio of f's which is not determinable from the sedimentation velocity method which gives only s and D. The combination of (2) and (3) gives

$$M\left(\frac{f_D}{f_s}\right) = \frac{RTs}{(1 - V\rho)D} \tag{6}$$

The combination of (3) and (4) gives

$$M\left(\frac{f_0}{f_s}\right)^{3/2} = \left(\frac{6\pi\eta}{1 - V\rho}\right)^{3/2}\left(\frac{3N^2}{4\pi}\right)^{1/2} V^{1/2}s^{3/2} \tag{7}$$

Finally, the combination of (2) and (4) gives

$$M\left(\frac{f_D}{f_0}\right)^3 = \left(\frac{RT}{6\pi\eta}\right)^3\left(\frac{4\pi}{3N^2}\right)\frac{1}{VD^3} \tag{8}$$

Equations (7) and (8) are valid only under the assumption (arising from Stokes' law) that the particles are spherical while equation (6), is independent of the shape of the particle and reduces to Svedberg's equation on setting $f_s = f_D$.

$$M = RTs/(1 - V\rho)D \tag{9}$$

The difference between the molecular weight from the sedimentation equilibrium method and that from the sedimentation velocity method often exceeds the experimental error. In no known case, however, has this difference exceeded 15%. One can, therefore, use equation (9) for the calculation of the molecular weight with reasonable certainty and with this value calculate the form factor from equation (7). Further, it is reasonable to assume that arguments based on differences in molecular weights or form factors exceeding 15% are sound.

For our results on egg albumin: taking the usual value of 0.746 for the partial specific volume of the proteins, $(1 - V\rho) = 0.254$ and $(s/(1 - V\rho)) = 1.27 \times 10^{-12}$ we have

$$M(f_0/f_s)^{3/2} = 30{,}100$$
$$M(f_D/f_s) = 49{,}100$$
$$M(f_D/f_0)^3 = 130{,}000$$

Recently reported results on egg albumin[21] indicate that it is not justified to take $f_D/f_s = 1$. While we have no equilibrium measurements on our sample of egg albumin, we may estimate

(20) W. Kuhn, *Z. physik. Chem.*, **A161**, 1 (1932).

(21) T. Svedberg, *Ind. Eng. Chem., Anal. Ed.*, **10**, 113 (1938).

the magnitude of the correction factors by taking 40,500 as the latest equilibrium determination for egg albumin, whence we obtain

$$(f_s/f_0) = 1.20$$
$$(f_s/f_D) = 1.21$$
$$(f_0/f_D) = 1.48$$

For the earlier value of 35,400 the ratios are, respectively, 1.11, 1.39 and 1.55.

The molecular weights reported subsequently should be interpreted with caution in the light of this discussion.

Analysis of Corn β-Amylose.—A sample of corn starch whose granules were disrupted by dry grinding in a pebble mill for one hundred sixty-eight hours was dispersed in water and analyzed by the procedure outlined above. Figure 4 shows the results of an analysis with a water solution of this material in the cell. The substance obviously is heterogeneous, and the observable sedimentation constants range from 3.91 to 6.01 × 10^{-13}, with a mean value of 4.72 × 10^{-13}. In this experiment the α-amylose

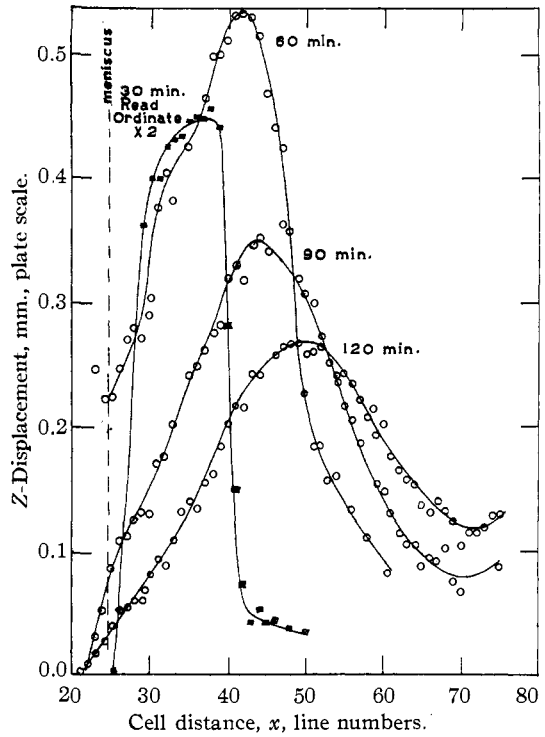


Fig. 4.—Crude β-amylose: concn. 1.4%; temp. 26–27°; speed, 937 r. p. s.; $\omega^2 = 3.47 \times 10^7$. Centrifuge geometry for this and subsequent curves as in Fig. 3.

Time, sec.	Curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{27} \times 10^{13}$	$s_{20} \times 10^{13}$
1800_1	0.900	1.54	45.27	5.45	4.61
1800_2	.900	2.03	45.57	7.10	6.01
3600_1	.536	2.94	46.02	5.10	4.32
3600_2	.536	3.41	46.25	5.90	4.99
5400_1	.360	4.34	46.72	4.96	4.20
7200_1	.268	5.40	47.25	4.63	3.91

Range s_{20}, 3.91–6.01 × 10^{-13}. Mean s_{20}, 4.72 × 10^{-13} at 20°. D_{20} extrapolated, 10.0 × 10^{-7}. $D^2(d(1/D)/dt) = -3200$. 1, mean value (bisector of area). 2, heavy component (peak value).

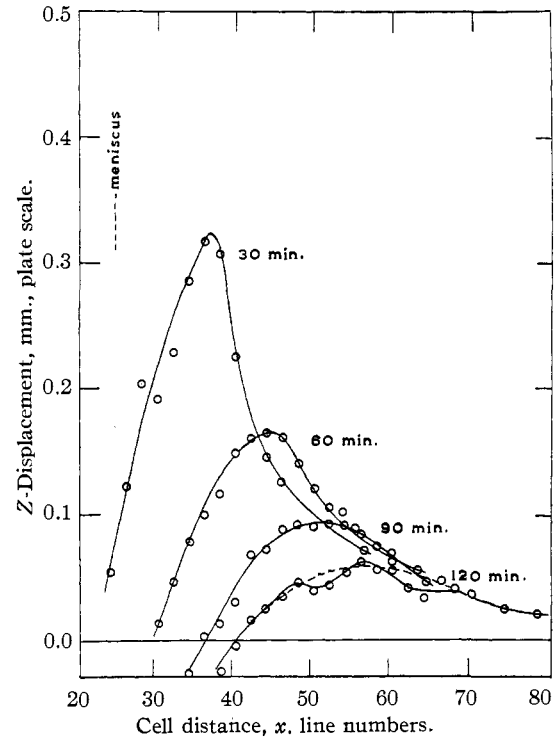


Fig. 5.—β-Amylose precipitated by 25–28.5% methyl alcohol, soluble portion (56% of the total fraction): concn. 0.75%; temp. 27°; speed, 1061 r. p. s.; $\omega^2 = 4.45 \times 10^7$.

SEDIMENTATION

Time, sec.	Curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{27} \times 10^{13}$	$s_{20} \times 10^{13}$
1800	0.314	2.47	45.20	7.54	6.37
1800[a]	.300	2.19	45.05	6.08	5.14
3600	.165	4.28	46.06	5.80	4.90
5400	.093	6.09	46.95	5.40	4.56
7200[a]	.055	7.46	47.63	4.90	4.14

Range s_{20} 4.14 − 6.37 × 10^{-13}. Mean s_{20} = 5.02 × 10^{-13}.

[a] Approximate vertical bisector of area under curve.

DIFFUSION

Time, sec.	$Z_m/\sqrt{t}$, mm.	Curve data line numbers u	u^2	$D \times 10^7$	$1/D \times 10^{-7}$
3600	0.099	8.6	74	15.8	0.063
5400	.058	9.8	96	13.7	.073
7200	.035	13.0	169	18.1	.055

$(1/D)$ at zero time; extrapolated 0.082. D_{27} = 12.2 × 10^{-7}. D_{20} = 10.3 × 10^{-7}. $D^2(d(1/D)/dt) = -3900$.

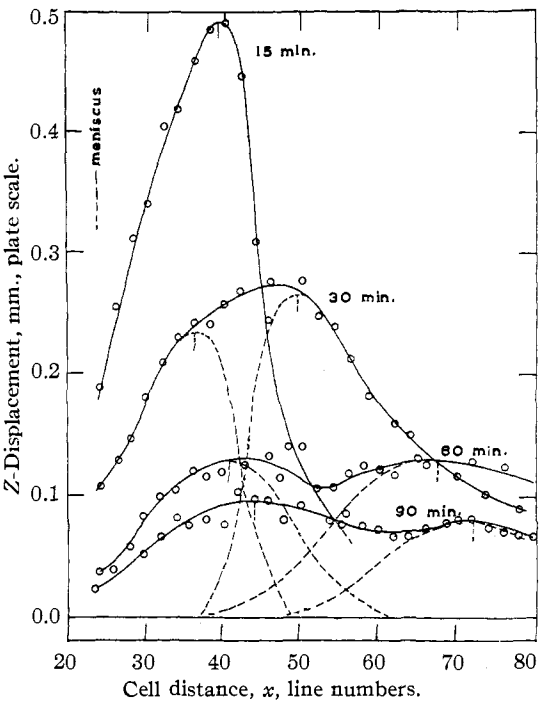


Fig. 6.—β-Amylose fraction precipitated by 28.5–35% MeOH: concn. 0.75%; temp. 26°; speed, 1085 r. p. s.; $\omega^2 = 4.64 \times 10^7$.

Time, sec.	Curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{26} \times 10^{13}$	$s_{20} \times 10^{13}$ I	II
1800	0.180	2.54	44.35	6.86	5.80	
3600	.100	3.08	44.62	4.14	3.50	
5400	.045	4.37	45.27	3.86	3.26	
1800	.210	5.74	45.95	15.00		12.7
3600	.105	9.99	48.00	12.47		10.5
5400	.032	10.72	48.44	8.85		7.5

Component 1, range 3.26–5.80, mean 4.19×10^{-13}.
Component 2, range 7.5–12.7, mean 10.2×10^{-13}.

was not previously separated and a rough estimate of its size was obtained by running the centrifuge at a speed of 250 r. p. s. for a short time. Most of the material was thrown out between five and ten minutes from which an approximate value of 6000×10^{-13} was deduced for s, agreeing fairly well with the 1000:1 ratio for particle size of α-amylose to β-amylose previously found by light scattering measurements.[22]

In order to obtain a better estimate of the range of sizes existing in the material a sample of β-amylose after separation from α-amylose by the electrophoretic procedure of Taylor and Iddles[23] was fractionated with acetone-free methyl alcohol as the precipitant. Typical sedimentation curves

(22) T. C. Taylor, C. O. Beckmann and Q. Landis, unpublished results.

(23) T. C. Taylor and H. A. Iddles, *Ind. Eng. Chem.*, **18**, 713 (1926).

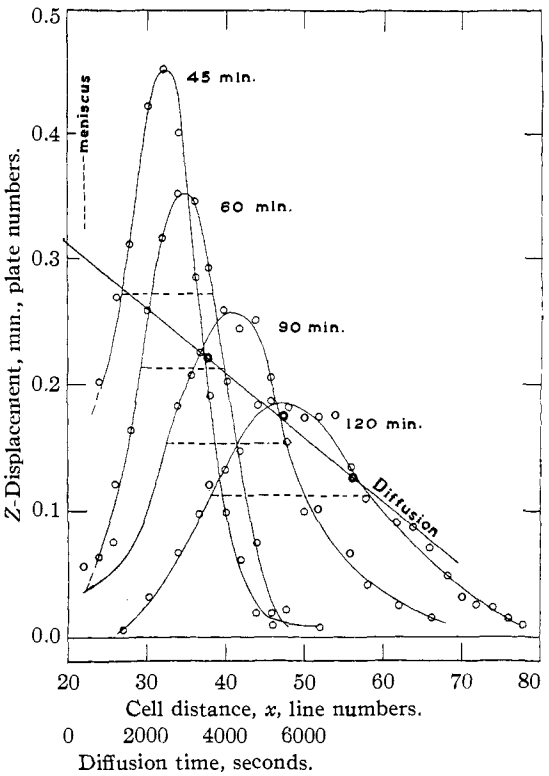


Fig. 7.—β-Amylose fraction precipitated by 50–65% MeOH: concn. 0.75%; temp. 26°; speed, 1241 r. p. s.; $\omega^2 = 6.08 \times 10^7$.

SEDIMENTATION

Time, sec.	Curve data, mm., plate scale Z_m	Δx_c	x_m	$s_{26} \times 10^{13}$	$s_{20} \times 10^{13}$
2700	0.454	1.98	44.61	3.06	2.59
3600	.354	2.55	44.89	2.69	2.28
5400	.258	3.87	45.55	2.69	2.28
7200	.185	5.46	46.34	2.82	2.39

Mean $s_{20} = 2.39 \pm 0.10 \times 10^{-13}$.

DIFFUSION

Time, sec.	$Z_m/\sqrt{t}$ mm.	Curve data line numbers u	u^2	$D \times 10^7$	$1/D \times 10^7$
3600	0.213	5.5	31.4	6.70	0.149
5400	.155	7.7	59.2	8.45	.118
7200	.111	10.0	100.0	10.70	.094

$(1/D)$ at zero time; extrapolated 0.195. $D_{26} = 5.12 \times 10^{-7}$. $D_{20} = 4.34 \times 10^{-7}$. $D^2(d(1/D)/dt) = -2630$.

are shown in Figs. 5, 6 and 7 and the data for all fractions are collected in Table II. In addition to the ultracentrifugal analysis, the "alkali-labile" value (A. L.) given in column 6 was determined on each sample by the method of Taylor, Fletcher and Adams.[6]

Discussion

The evidence presented proves beyond a doubt that the β-amylose from corn starch disrupted by

TABLE II

FRACTIONATION OF GROUND CORN β-AMYLASE BY METHYL ALCOHOL

Fraction	MeOH % by vol.	% of total	Mean s_{20}, 10^{13}	Extrap. $D_{20} \cdot 10^{7}$	A. L. value	$D^2\frac{(d1/D)}{dt}$	$M(f_D/f_s)$	f_s/f_0	Chains per mol. Haworth	Chains per mol. Hess	C_6 units per mol.
1—total	25-28.5	9.9	...	...	32.3	....	.....	..	...	...	..
sol.	.....	5.6	5.02	10.3	31.4	−3900	30,800	1.09	6.16	3.62	190
retrog.	.....	4.3	...	...	34.6	....	.....	..	...	...	..
2—total	28.5-35	19.9	...	...	28.0	....	.....	..	...	...	..
I	.....	..	10.20	...	..	....	225,000	2.00[a]	45.0	26.5	1400
II	.....	..	4.19	...	..	....	60,000	2.00[a]	12.0	7.06	370
3—total	35-50	45.5	3.42	4.92	37.2	−6650	43,000	1.99	8.6	5.06	266
I	.....	..	4.60	4.92[a]	..	....	58,000	1.80	11.6	6.84	366
II	.....	..	2.52	4.92[a]	..	....	31,600	2.22	6.32	3.72	195
4	50-65	18.7	2.39	4.34	46.4	−2630	34,100	2.43	6.85	4.02	210
5	65-90	3.9	1.30	4.70	50.8	−2650	17,100	2.76	3.42	2.01	105
Recovery		97.9									

[a] Assumed value.

dry grinding is heterogeneous with respect to particle size, the molecular weights ranging from 17,000 to 225,000. From the data of Table II an approximate particle size distribution curve for this sample of β-amylose has been constructed (Fig. 8). It shows a great preponderance

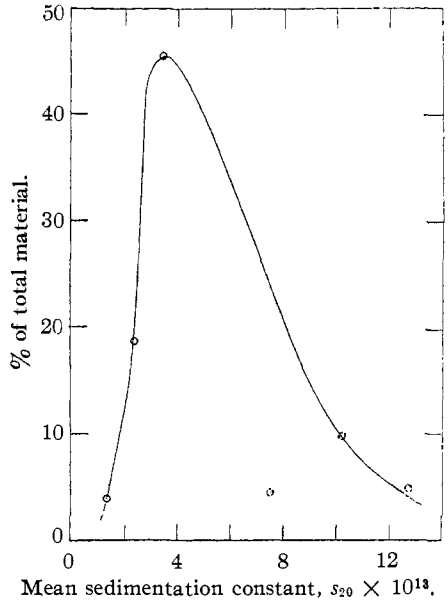


Fig. 8.—Particle size distribution curve for ground corn β-amylase.

of material, nearly 50%, with a sedimentation constant near 4.0×10^{-13} (mol. wts. 31,000–60,000) agreeing fairly well with the mean value of 4.72×10^{-13} found for the original unelectrophoresed sample. The agreement is still better when the unsymmetrical nature of the curve is taken into consideration, which would tend to shift the mean toward the heavier side of the peak.

The molecular weights (assuming $f_s = f_D$) reported in column 8 of Table II are very closely whole-numbered multiples of Haworth's chain of weight 5000, or, better, Hess' chain of weight 8500. We feel, however, in view of the uncertainties discussed above and the limited precision of calculations for heterogeneous substances, that to conclude that the molecules are composed either of the Haworth units or the Hess units would be entirely unjustified.

The degrees of polymerization in terms of glucopyranose units (column 12) are of the same order of magnitude as those reported by Staudinger and are considerably lower than those reported by Hess, both obtained by the use of Staudinger's viscosity formula.

With the exception of Fraction 1, one finds the expected solubility relationships, namely, that the larger particles are thrown out of solution by a lower alcohol concentration than the smaller particles. The anomalous behavior of Fraction 1 is discussed below and is accounted for satisfactorily. A comparison of the alkali labile values with the sedimentation constants in column 4 shows, as was predicted by Taylor, that the smaller particle has the higher A. L. value. Fractions 1 and 2 gave a deep blue color with iodine, the color progressively shifting toward the red in subsequent lighter fractions, the lightest giving a dark red hue.

The anomalous behavior of Fraction 1 becomes clear from a comparison of the molecular weights and form factors calculated by the methods outlined above. The partial specific volume, V, of β-amylose in 2% solution was found to be 0.606 in close agreement with Lamm's value of 0.6015 for Lintner soluble starch. With this value we calculate the following quantities

$$M(f_0/f_s)^{3/2} = 28{,}000$$
$$M(f_D/f_s) = 30{,}800$$
$$M(f_D/f_0)^3 = 36{,}700$$

If we assume with Svedberg that $f_D = f_s$, the form factor becomes

$$f_s/f_0 = f_D/f_0 = 1.09$$

This indicates even less departure from sphericity than for our sample of egg albumin. Fraction 3, precipitated by 35–50% methyl alcohol, has a lower sedimentation constant, 3.42×10^{-13} but is sufficiently close to that of Fraction 1 for the purpose of comparison. The extrapolated diffusion constant is 4.92×10^{-7}, whence

$$M(f_0/f_s)^{3/2} = 15{,}400$$
$$M(f_D/f_s) = 43{,}000$$
$$M(f_D/f_0)^3 = 307{,}000$$

On the same assumption, we find the form factor to be

$$f_s/f_0 = f_D/f_0 = 1.99$$

This factor, representative of the main portion of the β-amylose, is nearly twice that of Fraction 1, which indicates a great departure from sphericity. It was further observed that the more easily precipitable Fraction 1 retrograded much more readily than the other fractions. It precipitates from methanol solutions in flocculent or granular form, whereas the other portions form slimy and tenacious masses. It is suggested that, since the particles of Fraction 1 are almost spherical in shape and have, therefore, a minimum specific surface, they hydrate less completely and hence are more easily thrown out of solution. The fact that there is no departure from the sedimentation constant-alkali labile value relationship indicated in Table II and to be further developed in a later publication, however, does not allow the postulation of a unique chemical structure, *i. e.*, this fraction is still "isochemical" with the other fractions. Thus it appears evident that the shape as deduced from the form factor and the resulting effect on the degree of hydration of the particle is the important property which determines the tendency toward retrogradation rather than size alone.[24]

Summary

1. An air-driven ultracentrifuge of the Beams type with the optical arrangement of Lamm's refractive method of determining concentration gradients is described.

2. The limitations of the sedimentation velocity method are discussed briefly.

3. β-Amylose from corn starch disrupted by dry grinding for one hundred sixty-eight hours was found to be heterogeneous with respect to particle weight. About 50% of the material had an average sedimentation constant of 4.0×10^{-13} while the values for the whole material ranged from 1.30 to over 12×10^{-13}.

4. α-Amylose from corn starch has a sedimentation constant of the order 6000×10^{-13}.

5. The behavior of a light fraction of the β-amylose which precipitates and retrogrades readily is explained in terms of particle shape and hydration.

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(24) This is in accord with Brönsted's theory of the solubility of high molecular weight substances. As Brönsted points out, the potential energy of a particle in solution is proportional to the molecular weight (M) for thread-like molecules and to the two-thirds power of the molecular weight ($M^{2/3}$) for spherical molecules. From this it can be shown easily that of two isochemical substances with the same molecular weight, the one with spherical particles is always less soluble than the one with thread-like particles. J. N. Brönsted, *Compt. rend. trav. lab. Carlsberg*, Ser. chim., **22**, 99 (1938).