PHYSICOCHEMICAL STUDIES OF THE ACID HYDROLYSIS OF CORN STARCH[1]

W. G. HUNT,[2] F. T. HENZLER,[2] AND E. A. SOWELL[2]

ABSTRACT

In the production of thin-boiling starches, the physical properties of starch pastes are modified by acid treatment in the granule state so as to obtain certain desired characteristics; this degree of modification is followed by viscometric measurement of an alkali fluidity type. With measurements of this sort, little information is obtained concerning molecular dimensions or concentrations of the various components in heterogeneous pastes.

Sedimentation patterns at various solute concentrations of a series of thin-boiling starches were made, using a Spinco Model E ultracentrifuge. From these patterns estimations of concentration, sedimentation, heterogeneity, and concentration dependence of sedimentation were obtained for amylose and amylopectin fractions of these fluidity starches. Diffusion constants were measured at a 1% solute concentration on the A and B fractions of these starches using a Tiselius electrophoresis cell.

The principal uses of starch involve the properties of pastes made by heating aqueous suspensions of the starch granules. To obtain certain desired paste characteristics, starch slurries are treated with dilute mineral acid at temperatures below the gelatinization temperature of the starch. The degree of modification is usually followed by some kind of viscometric measurement of pastes made from small samples taken from the acidified slurry. When the desired degree of modi-

[1]Manuscript received June 1, 1962.
[2]Anheuser-Busch, Inc., St. Louis, Mo.

fication has been attained, the acidified slurry is neutralized and the starch granules are collected by filtration and dried.

These acid-modified starches are graded into a series of fluidity starches which are characterized by means of flow through a glass funnel having a standardized tip. The funnel and tip are adjusted to allow 100 ml. of water at 25°C. to pass from the funnel in 70 sec. The number of ml. of 5% starch dispersion (in 1% sodium hydroxide) that flows through the funnel in 70 sec. is taken as the fluidity. Under these conditions water has a fluidity of 100, a starch dispersion fluid enough for 40 ml. to pass has a fluidity of 40, and an unmodified starch which hardly flows through the funnel will have a fluidity of almost 0 (1).

Results obtained from the above fluidity test often vary from operator to operator. Starches deviate from true Newtonian properties of viscous flow, and the viscosity is usually complicated by plasticity and by elastic effects. Viscosities of starch pastes are also dependent upon the extent of gelatinization, dispersion, pH, and temperature of measurement. These factors appear to be reduced to a minimum when starch is pasted at low concentrations using a standard set of conditions.

To minimize the variance of samples, a series of acid-modified thin-boiling starches of known production history were prepared by a member company of the Corn Industries Research Foundation. These samples were distributed to members of the Analytical Procedures Subcommittee of the Foundation for collaborative studies on a method for measuring fluidities at low starch concentrations.

With viscosity measurements of this kind, little information is obtained concerning the molecular dimensions or concentrations of the various components in such heterogeneous pastes. Kerr (2) has separated the A- and B-fractions of a series of thin-boiling starches by the general method outlined by Lansky, Kooi, and Schoch (3). These fractions were then acetylated to their corresponding triacetates and the number average degree of polymerization of these fractions was determined by osmotic pressure measurements of a dilution series in chloroform.

It was thought that similar information could be obtained from sedimentation patterns and if so, it might be of some help in characterizing this series of acid-modified thin-boiling starches.

Material and Methods

Dispersion of Starch Samples. Samples were prepared by suspending the starch in 150 ml. of water. Sodium hydroxide, 2*M* (150 ml.),

was then added with stirring at 200–225 r.p.m. and stirred for 20 min. at 25°C.

Measurement of Sedimentation Velocity. Rates of sedimentation of 1.5, 1.0, 0.75, and 0.5% samples of a 0- (unmodified), 20-, 24-, 35-, 43-, 47-, 58-, 70-, and 80-fluidity series of starches were determined (Spinco Model E ultracentrifuge). Measurements were made in a 12-mm. cell incorporating a Kel-F centerpiece. All runs were made at 59,780 r.p.m. and were completed in less than 1 hr. The centrifuge rotor and cell were precooled to 20°C. The pressure in the vacuum chamber was less than 1 μ mercury, and the temperature rise of the rotor was less than 0.5°C. during the run. Procedures were standardized so that there was a 20-min. interval from the time the dispersions were prepared until the start of rotor acceleration. The rotor was then accelerated at its maximum rate to 59,780 r.p.m., when the first exposure was made. Exposures were then made at 8-min. intervals.

The sedimentation coefficient, s, was calculated by the equation:

$$d \log X/dt = \omega^2 s/2.3$$

where X is the distance of the boundary in cm. from the axis of rotation at time, t, and ω the angular velocity in radians per sec. The slope (d log X/dt) was determined from a plot of log X *vs.* t which is a linear function.

Measurement of Concentration. Areas under the curves were determined by enlarging the pattern 4.2 times with a photographic enlarger, superposing the curve over an enlarged tracing of the solvent base line, and measuring the area under the curve with a planimeter. The concentration of the sedimenting material in percent, C_o, is obtained from the equation:

$$C_o = (A \tan \Theta X^2)/(abm_x m_c \Delta n E^2 X_o^2)$$

where A is the measured area, a is the cell thickness in cm. along the optical path, b is the optical lever arm in cm., Θ is the angle of the schlieren diaphragm, m_x and m_c are the magnification factors of the camera and cylindrical lenses, and E the magnification of the enlarger. X_o is the position of the boundary at zero time and X is the position of the boundary on the pattern being measured. Δn is equal to the difference in refractive index between solvent and solution at a concentration of 1%.

Fractionation of Starches. The series of fluidity starches were fractionated by the procedure outlined by Lansky, Kooi, and Schoch (3), using Pentasol to precipitate the linear or A-fractions. The A-fractions were twice recrystallized from hot aqueous Pentasol solutions. The

branched or B-fraction was isolated by treating the primary Pentasol supernatant with one-third volume of methanol while stirring vigorously. The iodine affinities of these fractions were determined by the procedure described in *Chemistry and Industry of Starch* (4).

Measurement of Free Diffusion. The free diffusion experiments were determined; a Tiselius electrophoresis cell and the procedure described by Lundgren and Ward were used (5). Diffusion constants at a 1% solute concentration in 1*N* sodium hydroxide of the above fractions were determined at 20°C. The square of the second moment, σ^2, of these fractions was determined from photographic records of the concentration gradients taken at different time intervals; a Philpot-Svensson cylindrical lens was used. Allowance for initial boundary disturbance during its formation and movement to the center of the cell was made by plotting ½ the square of the second moment, σ^2, *vs.* time. The slope of this line equals D.

Discussion

Svedberg and Pedersen (6) have shown that the sedimentation coefficient, s, of the main component in a sample for which the sedimentation coefficients are independent of concentration is readily evaluated if the observed refractive index gradient curve resolves into two separate peaks corresponding to the two different solutes. If the peak corresponding to an impurity does not resolve entirely from that of the main component, but its tail does not overlap the maximum of the main peak, s may still be determined directly, provided again that the sedimentation coefficients are independent of concentration.

As shown in Figs. 1 and 2, the sedimentation constant, s, varies with concentration. This concentration dependence is eliminated by determining s at different concentrations and extrapolating to zero a plot of s *vs.* concentration. The lack of a linear relationship between s and concentration makes accurate extrapolations of the B-fractions of 24 fluidity and lower to zero concentrations extremely difficult; however, in the higher-fluidity ranges the degree of interaction among molecules is considerably reduced, allowing more reliable extrapolations.

Sedimentation patterns on 0-fluidity (unmodified) starch showed two main peaks and a small, very fast-moving peak to be present. Stacy and Foster (7) found two peaks with amylopectin preparations, and have shown that amylopectin is an extremely heterogeneous branched polymer with sedimentation coefficients ranging from 0 to 400 S units (sedimentation constant, the Svedberg, 10^{-13} sec.).

Sedimentation patterns for amylose were well defined at all concentrations; however, the peak corresponding to the amylopectin fraction

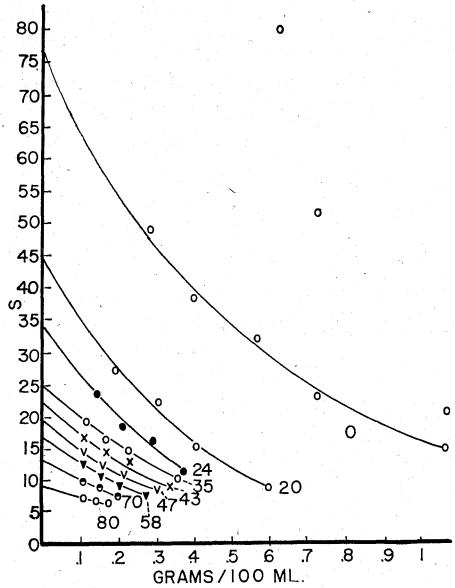

Fig. 1. Effect of concentration on the sedimentation of amylopectin.

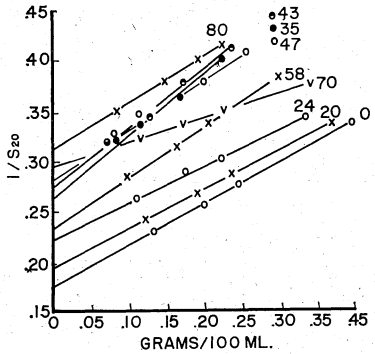

Fig. 2. Effect of concentration on the sedimentation of amylose.

spread as it crossed the cell. The spreading became more severe in the more dilute solutions, making it difficult to determine accurately the maximum of the peak as the boundary progressed.

No attempt was made to estimate S for the fast-moving amylopectin peak of unmodified starch represented by the three dots on Fig. 1; however, it can be seen easily that this fraction would have an S of well over 300 S units.

If the sedimentation constant of amylose (A-fraction), Fig. 2, is expressed as a reciprocal, then a linear relationship of 1/S *vs.* concen-

tration is obtained (8). The degradation of amylose appears to follow that of amylopectin, with S decreasing as the fluidity increases. At the 58-fluidity level S has increased, and it appears that the average molecular size of the A-fraction has increased. Amylose is largely responsible for the retrogradation of starch pastes.

If a material is homogeneous and at high dilution to eliminate artificial boundary sharpening and concentration dependence, an apparent diffusion constant can be calculated from the sedimentation diagram by the expression:

$$D_{app} = (1/4\pi T)\ (\text{area/height})^2\ (1 - \omega^2 st)$$

If a material contains molecules of various sedimentation coefficients, then D_{app} will increase in time (9). D_{app} was evaluated from 0.5% sedimentation diagrams. Since both fractions are extremely heterogeneous, apparent diffusion constants obtained in this manner are of little value for molecular-weight studies. The slope of a plot of D_{app} *vs.* t is an indication of the homogeneity of the peak. Van Holde (10), modifying Fujita's method, was able to estimate the diffusion constant, sedimentation coefficient, and concentration dependence of S from a single sedimentation experiment of a homogeneous bovine serum preparation by a graphical treatment of his data. He obtained a linear relationship at various concentrations, and the slopes of these plots were related to the influence of concentration on S.

Thus $\Delta D/\Delta t$ is a composite of heterogeneity and concentration dependence. The slopes ($\Delta D/\Delta t$) of the plots of D_{app} *vs.* t for the starches of various fluidities yield a very low slope for the 0-fluidity amylose fraction to progressively increasing slopes as the fluidity increases, whereas the slopes obtained for the amylopectin fraction decrease with increasing fluidity. The A-fraction of 0-fluidity starch has a very low slope, indicating that the material is homogeneous and S independent of concentration; however, Fig. 2 shows S to be influenced by concentration. If the amylose or A-fraction is homogeneous, then the weight-average-molecular-weight determinations obtained by sedimentation studies should be the same as the number-average figures obtained by osmotic pressure and viscosity measurements. Values of $317{,}000 M_{SD}$ and $180{,}000 M_N$ were obtained by Greenwood and co-workers (8), indicating that it is not homogeneous. Beckman and Landis (11) in their studies have shown that corn amylose was heterogeneous, with S values ranging from 2 to 12 and about 50% of the material having an S of 4 at concentrations ranging from 0.75 to 1.4%. Calculating diffusion constants from their sedimentation patterns gave a value of 4.7×10^{-7} cm.^{2}/sec. A diffusion coefficient of 1×10^{-7} cm.^{2}/

sec., obtained on a 0.2% solution with a Jamin interferometer, was also reported by Greenwood (8). Extrapolating the plot of D_{app} *vs.* t to zero yields a value of 1.1×10^{-7} when corrected to the viscosity of water at 20°C.

Figure 1 shows that the concentration dependence of S of the B-fraction (amylopectin) is rapidly reduced during the early stages of acid degradation, yielding lower $\Delta D/\Delta t$ values. By hydrolysis of the extremely large amylopectin molecules the B-fraction could become more homogeneous, thereby yielding lower $\Delta D/\Delta t$ values. As the fluidity increases, concentration dependence on S also decreases, and heterogeneity becomes the dominant factor in determining $\Delta D/\Delta t$. From the data obtained it appears that the A-fraction is becoming more heterogeneous as the fluidity increases.

A plot of $\sigma^2/2$ versus t is shown in Figs. 3 and 4. These plots do not intercept the X-axis at zero time, since there is some boundary disturbance during boundary formation and movement to the center of the

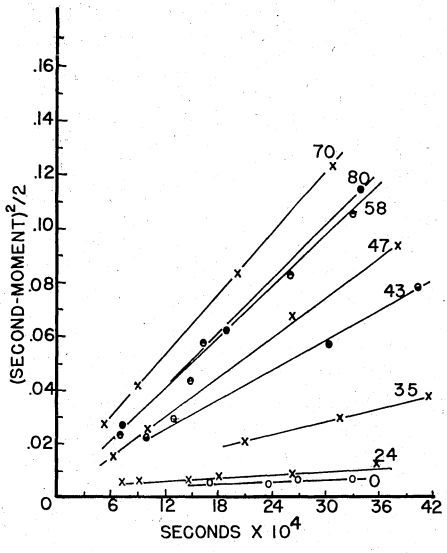

Fig. 3. Effect of time on the moment of amylopectin.

cell. An allowance for this disturbance on diffusion rates is made by using the slope which is equal to D. The slopes of both the A- and B-fractions increase with fluidity, with the exception of the 20- and 24-fluidity A-fractions which were contaminated with B-fraction fragments, as indicated by their lowered iodine affinities.

The results of sedimentation studies, diffusion studies, and other

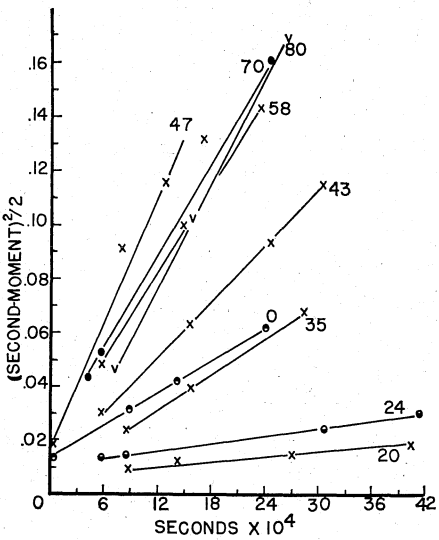

Fig. 4. Effect of time on the moment of amylose.

TABLE I

CHARACTERISTICS OF AMYLOSE AND AMYLOPECTIN OF CORN AND THIN-BOILING ACID-MODIFIED STARCHES

FLUIDITY	VISCOSITY [a]	WEIGHT PERCENT	$(S_{20})_0$ [b]	$\Delta D_{app}/\Delta t$	IODINE AFFINITY	D [c]
	cp.					
		Amylopectin (B-Fraction)				
0	4.028	70	96	1,600	0.31	0.17
20	1.929	38	53	1,000	.37	...
24	1.771	28	41	...	.40	0.24
35	1.632	22	30	340	.33	1.1
43	1.552	19	27	100	.29	2.3
47	1.389	20	23	280	.27	3.1
58	1.265	19	20	104	.26	3.2
70	1.119	12	17	...	.25	5.0
80	0.974	11	11	390	0.22	4.3
		Amylose (A-Fraction)				
0	4.028	24	6.6	0.42	16.1	2.5
20	1.929	23	6.2	9.2	...	0.38
24	1.771	22	5.4	11.7	14	0.59
35	1.632	15	4.4	14.5	14.5	2.96
43	1.552	17	4.2	15.0	16	6.5
47	1.389	16	4.3	17.5	16	5.35
58	1.265	20	5.1	17.8	16	5.4
70	1.119	22	4.0	22.9	18	6.4
80	0.974	18	3.8	25.4	17	8.2

[a] Viscosity of a 1% solution in 1*N* sodium hydroxide at 25°C.
[b] $(S_{20})_0$ is the sedimentation constant, $\times 10^{+13}$ c.g.s. units corrected to water at 20°C. at infinite dilution.
[c] Diffusion coefficient of 1% solution $\times 10^{+7}$ cm.^{2}/sec. Corrected to the viscosity of water at 20°C.

analyses carried out on the fluidity starches and their A and B fractions are summarized in Table I.

Ideally diffusing substances yield curves having the normal probability curve, and materials diffusing from a boundary not initially sharp approach the same shape as the changes due to diffusion become more important than the initial disturbance. The curves obtained during diffusion of the A- and B-fractions of these fluidity starches were more pointed than the normal curve because of their heterogeneous nature and were somewhat skewed on the solution side of the peak. The skewing was probably due to interaction among solute molecules and differences in viscosity of the solvent and solution. The skewing was less pronounced in the higher-fluidity ranges.

Since sedimentation and diffusion are dependent upon the same factors, such as molecular shape, extent of hydration, and interaction among solute molecules, it can be assumed that concentration would also influence D in much the same manner as S; hence, the ratio of S/D is almost concentration-independent. If S and D were determined at the same concentration in dilute solutions, then zero concentration values for molecular-weight calculations would not be required in a study of this kind. As shown in Figs. 1 and 2, the influence of concentration on S is considerable, and diffusion studies at more dilute solute concentrations are required to match S concentrations or for extrapolation to infinite dilution for molecular-weight studies. As the fluidity increases, the concentration dependence of S is considerably reduced, and the diffusion coefficients at 1% of the 80-fluidity starch fractions probably closely approach the values obtained at infinite dilution. Table II shows the concentrations and molecular weights of the A- and B-fractions of a 0- and an 80-fluidity starch.

TABLE II

WEIGHT PERCENT AND WEIGHT AVERAGE MOLECULAR WEIGHTS OF THE A- AND B-FRACTIONS OF 0- AND 80-FLUIDITY STARCH

FLUIDITY	AMYLOPECTIN		AMYLOSE	
	Weight Percent	M_{SD}	Weight Percent	M_{SD}
0	70	8,800,000[a]	24	317,000[b]
80	11	166,000	18	30,000

[a] Sedimentation and diffusion of 1% solute concentration used for this calculation.
[b] See ref. 8.

Approximately 94% of the total solids in native corn starch is accounted for by measuring the area under the amylose and amylopectin peaks. The material which did not appear to be completely dispersed, and sedimented very rapidly, could account for the other 6%. As the

degree of acid degradation increases there is a large decrease in area under the B peak. At the 80-fluidity level only 29% of the original material sediments as these two fractions. Only the areas under the A and B peaks were measured; other deviations from the solvent base line which did not form a peak were not measured and could account for the balance of the material. No yields were determined from the Pentasol fractionation procedure. Kerr (2) reports a yield of 23% for the A-fraction of an 80-fluidity starch by a similar fractionation procedure, as compared with a yield of 18% obtained by measuring area of the refractive index gradient curve used in this study.

It appears from the results of these studies that there is no preferential hydrolysis on either the A- or B-fraction of these fluidity starches. As shown in Table I, there is a considerable reduction in concentration and sedimentation of the B-fraction, with a slight decrease in the A-fraction during the early stages of acid hydrolysis. Since the molecular weight of amylopectin is considerably larger than that of amylose, the sedimentation should decrease faster. The increased slope of $\Delta D/\Delta t$ shows that the A-fraction becomes more heterogeneous as fluidity increases. This increase in heterogeneity is probably due to degraded B-fraction fragments which have been sufficiently hydrolyzed to fall into this sedimentation range. Iodine affinities of the A-fractions precipitated with Pentasol indicate that these fractions are still substantially linear; however, Pentasol complexes predominantly with linear fractions, and iodine affinities of these selectively precipitated linear A-fractions would be high; whereas $\Delta D/\Delta t$ values were obtained from sedimentation patterns of the unfractionated material. The decrease in diffusion of the 20- and 24-fluidity A-fractions is likewise due to contamination by B-fraction fragments. At the 60-fluidity level there is an increase in sedimentation and concentration of the A-fraction without a corresponding decrease in B-fraction concentration. These increases must be due to retrogradation, with the A-fraction reassociating with smaller degraded linear fractions not having sufficient molecular size to sediment with the A-fraction.

In an attempt to form a more complete dispersion of native starch, the dispersion was allowed to stand for 24 hr. at 25°C. Sedimentation patterns of this dispersion still showed approximately the same amount of sediment described previously. Longer solution times did not appear to lower the amount of sediment; however, the area under the curve corresponding to the fast-moving amylopectin peak increased. The boundary spread so rapidly that it was not possible to measure the area under the curve; however, there was a decrease in area under the slower-sedimenting amylopectin peak. Viscosity measurements of

this aged dispersion showed a slight increase when compared to solutions prepared under the shorter dispersion times.

Conclusions

The preparation of fluidity starches by acid hydrolysis of corn starch in the granule state does not appear to involve a preferential hydrolysis of either the A- or the B-fraction. At the 60-fluidity level the change in molecular size and concentration of the A-fraction is probably due to retrogradation or reassociation of the A-fraction and smaller linear polymers.

Low-fluidity starches would not completely disperse when this solvent was used. Observations made during sedimentation of unmodified starch dispersions prepared by exposing the solute to the solvent for longer periods of time indicate that the solvent (1*N* sodium hydroxide) under certain conditions induces some kind of reassociation of amylopectin molecules to form complexes of extremely high molecular weights.

The viscosity of low-fluidity starches appears to be dominantly influenced by the size and concentration of the A- (amylopectin) fraction.

Literature Cited

1. BUEL, H. A. A study of some of the physical properties of starches. Com. 8th Intern. Congr. Appl. Chem. **13:** 63–76 (1912).
2. KERR, R. W. Degradation of corn starch in the granule state by acid. Stärke **3:** 39–41 (1952).
3. LANSKY, S., KOOI, M., and SCHOCH, T. J. Properties of the fractions and linear subfractions from various starches. J. Am. Chem. Soc. **71:** 4066–4075 (1949).
4. KERR, R. W. Chemistry and industry of starch, chap. XXIV. Academic Press: New York (1950).
5. LUNDGREN, H. P., and WARD, W. H. Amino acids and proteins (Greenburg, ed.), chap. 6. Charles C. Thomas: Springfield, Ill. (1951).
6. SVEDBERG, T., and PEDERSEN, K. O. The ultracentrifuge, pp. 295–300. Oxford Univ. Press: London (1940).
7. STACY, C. J., and FOSTER, J. F. Molecular weight heterogeneity in starch and amylopectins. J. Polymer Sci. **25:** 39–42 (1957).
8. GREENWOOD, G. T., and DAS GUPTA, P. C. Physico-chemical studies on starch. Part XI. The granular starch of sweet corn, *Zea mays*. J. Chem. Soc. **1958:** 707–710.
9. SCHACHMAN, H. K. Ultracentrifugation, diffusion, and viscometry. Methods in Enzymol. **4:** 58–59 (1957).
10. VAN HOLDE, K. E. A modification of Fujita's method for the calculation of diffusion coefficients from boundary spreading in the ultracentrifuge. J. Phys. Chem. **64:** 1582–1583 (1960).
11. BECKMANN, C. O., and LANDIS, Q. The molecular weight of beta-amylose from corn starch by means of the ultracentrifuge. J. Am. Chem. Soc. **61:** 1495–1503 (1939).