

INTRINSIC VISCOSITY OF DIALDEHYDE STARCH IN ALKALINE UREA AFTER REDUCTION WITH BOROHYDRIDE¹

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ABSTRACT

Periodate-oxidized starch, commonly called dialdehyde starch (DAS), was reduced with borohydride at 1.5°C. to minimize alkaline degradation, and dispersed in an aqueous alkaline solvent system. The final solvent system consisted of 0.5*N* potassium hydroxide containing 25 g. urea per 100 ml. solution. Absence of nitrogen from reduced DAS isolated from dispersion in this system indicated that the pretreatment left no aldehyde groups available for reaction with urea.

Intrinsic viscosities of reduced DAS dispersed in this system were significantly higher and were more stable than those obtained without prior reduction. Comparable values were obtained in both systems with less-sensitive starch derivatives, but again, the reduction system gave improved stability.

Aqueous alkali has been widely used for many years as a solvent in determining viscosity of starchy substances (14). However, it cannot be applied to dialdehyde starches because of their sensitivity to chain scission by alkali.

Literature reports (7,13) have shown that alkaline degradation of periodate-oxidized polysaccharides is mainly a result of cleavage of carbon-oxygen linkages that are *beta* to carbonyl groups. The method described herein stabilizes the polymers by quantitatively converting carbonyl groups to alcohol groups with borohydride (9). Meller (6) has shown the effectiveness of borohydride in increasing the resistance of oxycelluloses toward boiling alkali. Whelan and Morgan (12) have demonstrated the absence of side reactions in the conversion of reducing sugars to the corresponding alcohols with borohydride. Varshney and Luner (11) recently reviewed the contribution of borohydride reduction to pulp and paper processes.

Preliminary work indicated that the polyol derived by reduction of completely oxidized dialdehyde starch was not soluble in alkali under the conditions used. This shortcoming was obviated by adding urea to the solvent mixture. Urea also shortened the time required for viscosity to reach a constant value.

This paper describes a solvent system for starch and certain of its derivatives based on the principles outlined above. Pertinent variable

¹Manuscript received September 8, 1961. Contribution from the Northern Regional Research Laboratory, Peoria, Illinois. This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

factors are discussed, and application of the solvent to measurement of intrinsic viscosities is described.

Materials and Apparatus

Materials. Dialdehyde starches were prepared by the two-stage electrolytic process described by Pfeifer and co-workers (8). In this paper, various dialdehyde starches are designated by the abbreviation DAS followed by the numerical value of extent of oxidation. Thus, DAS-10 denotes dialdehyde starch in which 10% of the anhydroglucose units of starch were oxidized to the dialdehyde structure. Pearl starch, thin-boiling starch, white dextrin, and hypochlorite-oxidized starches were obtained from commercial producers of starch products. Potassium borohydride, 97% purity, lot 158-1, was obtained from Metal Hydrides, Inc.²; it was stored over calcium chloride. Urea, U.S.P., and potassium hydroxide, "Baker Analyzed" Reagent, were from J. T. Baker Co. Use of cation potassium gave values of no significant difference from sodium borohydride and sodium hydroxide used in preliminary experiments. Distilled water was purged with nitrogen for 30 minutes while boiling (2).

Apparatus. Wrist-action shakers, adjusted to give minimum agitation, were used to keep the starchy substrates suspended. Centrifugation by an International Model U centrifuge using No. 833 head and No. 340, 100-ml. tubes at a speed of 3,500 r.p.m. gave the desired force of $2,500 \times g$. Intrinsic viscosities were determined at $25.00 \pm 0.01^\circ\text{C}$. with Ostwald-Cannon-Fenske No. 100 viscometers (3). Kinetic energy corrections were not applied. Since varying concentrations of potassium borohydride were used, curves of solvent flow time vs. borohydride concentration were made for each viscometer tube. Solvent flow times were read from the curves.

Method

The method for preparing dispersions, hereinafter called "the present method," was as follows: In a 50-ml. volumetric flask, approximately 0.25 g. of starch derivative, accurately weighed and moisture-corrected, was suspended in 20 ml. (pipetted) of water containing an eightfold excess of potassium borohydride. This excess requires 2 mols. of borohydride per mol. of aldehyde group, since 1 mol. of borohydride supplies 4 mols. of available hydrogen.

After 2 days of mechanical shaking at 1.5°C ., the mixture was ad-

²The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

justed to approximately 0.2N with respect to potassium hydroxide by adding 0.370 ± 0.001 ml. of $11.0 \pm 0.1N$ reagent. After 2 more days, 25 ml. of $50.00 \pm 0.01\%$ (w/v) aqueous urea were pipetted into the mixture and shaking was resumed for another 2 days. The alkalinity was then raised to approximately 0.5N by adding 1.63 ± 0.01 ml. of $11.0N \pm 0.1$ potassium hydroxide.

After 2 days the sample was removed from the shaker, warmed to room temperature, and diluted to volume with $1.00 \pm 0.01N$ potassium hydroxide. Total shaking time was 8 days at $1.5^\circ C$. The final mixture consisted of reduced starch product and a small amount of boron salts in a solvent mixture of 0.5N potassium hydroxide containing 25 g. urea per 100 ml.

Analysis of completely oxidized DAS isolated by alcohol precipitation from dispersion revealed no nitrogen. The absence of any reaction with urea is evidence that treatment with borohydride completely reduced aldehyde groups (10).

All dispersing operations were carried out at $1.5^\circ C$., primarily because trials at room temperature resulted in viscosity values that were initially lower and then dropped upon standing. In addition, low temperature minimizes foaming due to evolution of hydrogen from borohydride.

Insoluble material was removed by centrifuging for 30 minutes at $2,500 \times g$. The concentration of starchy substrate in the centrifugate was calculated by subtracting the weight of insoluble material in the centrifuge residue from the initial weight.

The weight of insolubles was determined gravimetrically by drying the centrifuge residue to constant weight in a forced-air oven at $105^\circ C$. after removing soluble material in the occluded solvent. Soluble material was removed in four washes by suspension and centrifugation with 10 volumes of solvent based on the volume of compacted centrifuge residue. The first wash was with 25% urea in 0.5N potassium hydroxide, and the last three were with distilled water.

Development of the Present Method

Pretreatment with Borohydride. Reaction of carbohydrates with aqueous borohydride proceeds best without the addition of either acid or base (5,6). Preliminary trials indicated that an 8 to 1 ratio of available hydrogen to aldehyde gave maximum solubility and viscosity for starchy substrates.

Treatment with 0.2N Potassium Hydroxide. This step was introduced with the expectation that an intermediate amount of alkali

would have sufficient solvent action to enhance the reaction between partially dispersed material and borohydride, without completely inhibiting the reductive reaction. The last part of this presumption was based on the report of Abdel-Akher and co-workers (1) that sodium borohydride completely reduced amylose in the presence of 0.2*N* sodium hydroxide.

To test the efficacy of the treatment, two identical samples of pearl starch were treated with potassium borohydride for 2 days at 1.5°C. One of them was then made up to 1*N* with respect to potassium hydroxide. The other was made up to 0.2*N* and allowed to shake for 2 more days before adjustment to 1*N*. The following intrinsic viscosities (dl./g.) were obtained at intervals after initiation of treatment with borohydride:

	10 days	15 days
Without 0.2 <i>N</i> treatment	1.25	1.11
With 0.2 <i>N</i> treatment	1.23	1.31

The decrease in viscosity of the untreated sample suggests that its aldehyde groups were not completely reduced and it was, therefore, attacked by alkali. The rise in viscosity of the treated sample is probably due to continued solvation and extension of the polymer chains, a process which is accelerated in the presence of urea, as explained in the following section.

Order of Adding Urea and Potassium Hydroxide. Reversing the order of adding urea and the final portion of potassium hydroxide had

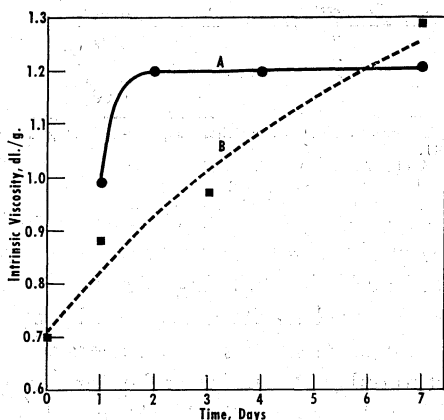


Fig. 1. Influence of the order of adding urea and potassium hydroxide on the viscosity of dialdehyde starch, DAS-5. Adding urea between the 0.2*N* and the 0.5*N* steps gave curve A; adding urea last gave curve B.

no effect on the viscosity of DAS-93. At low levels of oxidation, however, the order of addition influenced viscosity, as shown in Fig. 1. Adding urea before the potassium hydroxide resulted in a constant value for viscosity within 2 days after the last component was added to the solvent system. With the reverse order of addition, however, a constant value was not obtained within 7 days. Furthermore, the centrifuge residues obtained in the first case were well compacted and easily washed, in contrast to those obtained with the reverse order, which were less dense and therefore difficult to centrifuge down. Another advantage of the usual order was that the amount of insolubles decreased from approximately 21 to 13%. Unmodified pearl starch exhibited behavior similar to that of DAS-5. DAS-5 used in this study was a different preparation from that in Table II. The difference in solubility is being investigated, but cannot presently be explained.

Viscosity values were not changed by increasing the concentration of either urea or potassium hydroxide.

Applications

Application of the present method to DAS and its comparison with two known procedures (4,14) are presented in Table I. The present

TABLE I
COMPARISON OF INTRINSIC VISCOSITIES DETERMINED BY THREE PROCEDURES
APPLIED TO TWO DIALDEHYDE STARCHES

SAMPLE	1N KOH		PRESENT METHOD
	Dispersed at Room Temperature ^a	Dispersed at 1.5C. ^b	
	dl/g	dl/g	
DAS-0.5	0.37	0.22	0.57 ^c
DAS-5	0.06	0.06	0.19 ^c

^a Method similar to that of Lansky *et al.* (4), except that samples were not gelatinized prior to dispersion.

^b Method of Wolff *et al.* (14).

^c Centrifugation removed 59% of reduced DAS-5. Loss of other samples was less than 1%.

method gave significantly higher values than either of the other two. The inapplicability of alkali without prior reduction is emphasized by the data for DAS-5. In addition to being low initially, viscosities of the samples that were dispersed in the absence of borohydride were also less stable. Their reduced viscosities fell an average of 0.014 of a unit per day in contrast to only 0.003 unit per day for the two borohydride-treated samples. More highly oxidized DAS could not be studied in alkali without prior reduction because of rapid degradation which resulted in discolored solutions of negligible viscosity.

More highly oxidized DAS has, however, been studied by the present method. Table II gives viscosity and solubility data for samples of

TABLE II
INTRINSIC VISCOSITY AND SOLUBILITY OF DAS AT DIFFERENT LEVELS OF OXIDATION

SAMPLE	INTRINSIC VISCOSITY	LOSS ON CENTRIFUGING
	<i>dl/g</i>	%
DAS-0.5	0.57	<1
DAS-3	0.31	16
DAS-5	0.19	59
DAS-10	0.17	42
DAS-31	...	84
DAS-65	...	79
DAS-93	0.72	38

DAS at varying levels of oxidation. Current studies are being directed toward explaining the low solubility of these materials.

Table III shows good agreement between the present method and

TABLE III
COMPARISON OF INTRINSIC VISCOSITIES DETERMINED BY TWO PROCEDURES APPLIED TO
COMMERCIAL STARCH MODIFICATIONS

SAMPLE	IN KOH, ROOM TEMPERATURE ^a	PRESENT METHOD
	<i>dl/g</i>	<i>dl/g</i>
Hypochlorite-oxidized starch A	0.40	0.40
Hypochlorite-oxidized starch B	0.30	0.31
Thin-boiling starch	0.35	0.33
White dextrin	0.25	0.25

^a Method similar to that of Lansky *et al.* (4), except that samples were not gelatinized prior to dispersion.

a known procedure (4) when applied to starch derivatives that are not so sensitive to alkali as DAS. The present method, as in the preceding examples, again gave better stability. When samples from both methods were stored under refrigeration, those from the present method exhibited an average decrease in reduced viscosity of less than 0.1% during a 3-day period, while those from the other procedure decreased an average of 4.0% in 24 hours.

Stability of viscosity in the present solvent system was evaluated by observing changes in the viscosity of untreated starch during a storage period of 1 month at 1.5°C. Untreated starch was chosen because its high viscosity made it a more sensitive indicator of alkali-induced reduction of viscosity than the other materials studied. The intrinsic viscosity after 30 days was 1.46 compared to 1.42 initially, indicating

minimum, if any, chain scission.

Varshney and Luner recently suggested (11) that a borohydride-alkali system might serve as a standard for the D.P. determination of cellulose. The present method shows the possibility of extending such a standard to include alkali-sensitive starch derivatives.

Acknowledgment

The authors are indebted to D. M. Schmidt for assistance during part of the investigation, to M. D. Glick for viscosities of some of the samples that were dispersed in aqueous potassium hydroxide solution at room temperature, and to H. F. Conway for supplying the samples of dialdehyde starches.

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