and the increasing clarity and intensity of the X-ray diffraction pattern as sorption occurs indicates that sorption at sites within the granule substance is a reasonable hypothesis.

The form of the Cassie equation is analogous to the BET equation and thus fits the water sorption data equally well. For its proper use, the Cassie analysis requires knowledge of swelling pressures and deformations not available for starch; however, employing the Cassie sorption equation without correcting for swelling effects yields a minimum value of 0.7 to 1.0 sorbing site per glucose unit. The logical sites for sorption would be the hydroxyl groups of the molecule, and hence of the three sites per glucose unit theoretically available one-third to one-fourth are active. Such a high proportion of the hydroxyl groups active in sorption means a relatively great exposure of all of the starch molecules and hence renders the expression of a calculated surface area for water sorption of doubtful meaning.

Acknowledgment.—The authors are grateful to Dr. Majel M. MacMasters for supplying the starch samples used and to Mrs. Thelma Feger Boesch for assistance in the photomicrographic work and determination of water-sorption isotherms.

Summary

The surface area of dasheen, corn, tapioca and potato starches available to nitrogen at −195° was found to be 2.62, 0.70, 0.28 and 0.11 sq. m./g., respectively. Interpretation of the water-sorption isotherms of these four starches by the BET equation requires surface areas of 330, 334, 348 and 422 sq. m./g., respectively. It was concluded that the nitrogen-available surface area of starch does not give a quantitative explanation of the water-sorptive capacity of starches of different botanical origin.

The apparent external surface area of these starches was also determined photomicrographically and found to be in close agreement with the nitrogen-available surface area.

PEORIA 5, ILL. RECEIVED DECEMBER 30, 1949

[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

A Simplified Procedure for the Characterization of Starch Fractions by Viscosity and Iodine Sorption

BY IVAN A. WOLFF, LAETTA J. GUNDRUM AND C. E. RIST

Increased attention has been devoted in recent years to study of the component polysaccharides of common starches—the linear or amylose fraction and the branched or amylopectin fraction. Primary characterization of these fractions includes (a) information on the relative average molecular size and (b) measurements of the proportion of linear material contained.

The intrinsic viscosity[2] is commonly considered to be a function of the molecular weight in a homologous polymer series under given conditions in the same solvent. Iodine affinity is accepted as an indication of the amount of amylose present. For this reason a study of these methods for the characterization of amyloses and amylopectins was made.

This paper reports a method for estimating accurately the intrinsic viscosity in 1 N potassium hydroxide solution of each fraction, from a measurement made at a single concentration. The iodine affinity is determined by known methods on the same alkaline solution. Thus from a single sample, information on both relative molecular size and linear content of the starch fraction may be obtained.

Viscosity Studies.—Aqueous alkali has often been suggested[3] as a suitable solvent for determination of the viscosity of starch fractions. We found that a variety of amylose and amylopectin samples dissolved completely in 1 N potassium hydroxide solution at 0° in a period of twenty-four to forty-eight hours. Storage of the dispersions at 0° for an interval of twenty-four hours after solution was complete caused a maximum decrease in the intrinsic viscosity (measured at 25°) of 0.01 unit. All values reported in this paper were obtained within such a time period. Use of a nitrogen atmosphere as recommended by Whistler and Johnson[3] was not found beneficial, although our studies included no amylose fractions with a viscosity as low as that used for illustration by those authors.

The results of viscosity determinations at 25° on a series of amyloses and amylopectins of varying molecular sizes are shown graphically in Figs. 1 and 2, where the inherent viscosity is plotted against the concentration. The viscosity of each sample was determined at six different concentrations varying from 0.1 to 0.5%.

A linear relation between inherent viscosity and concentration, with an increasing negative slope for the materials of higher molecular weight,

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[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946. Article not copyrighted.

[2] The terminology recommended by L. H. Cragg, *J. Colloid Sci.*, **1**, 261 (1946), is followed in this paper.

[3] (a) Speiser and Whittenberger, *J. Chem. Phys.*, **13**, 349 (1945); (b) Whistler and Johnson, *Cereal Chem.*, **25**, 418 (1948); (c) Potter and Hassid, THIS JOURNAL, **70**, 3774 (1948); (d) Lansky, Kooi and Schoch, *ibid.*, **71**, 4066 (1949).

is evident in the amylose series (Fig. 1). No maxima in the curves, such as were obtained for amylose solutions in ethylenediamine,[4] were observed. The general similarity of this family of curves to that from a group of polyvinyl acetates similarly plotted suggested the application of Wagner's equation[5] to the amylose series. A

$$/\eta/ = \frac{\ln \eta_{\text{rel.}}}{c} + k\left(\frac{\ln \eta_{\text{rel.}}}{c}\right)^2$$

(c = the finite concentration in g. per 100 ml. of solution at which the inherent viscosity was determined; k is an empirically determined constant)

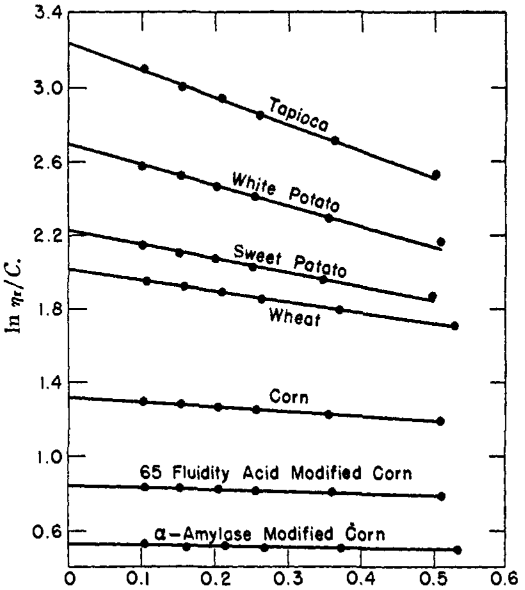
concentration of 0.2% was selected for trial and at this concentration the average value of k was found to be 0.032. The excellent agreement between the intrinsic viscosity found by extrapolation and that obtained by calculation from equation 1 is shown in Table I.

TABLE I

PROPERTIES OF THE STARCH FRACTIONS

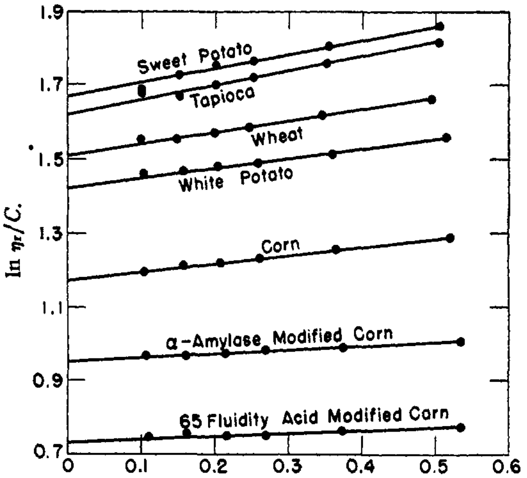
Material	Intrinsic viscosity at 25° Extrapol. from graph	Calcd. from 0.2% concn.[a]	Iodine sorption, mg. I_2 per g.[b] starch frac.
	AMYLOSES		
Tapioca	3.22	3.22, 3.24	199
White potato	2.69	2.66, 2.67	198
Sweet potato	2.22	2.22, 2.23	198
Wheat	2.01	2.01, 2.03	200
Corn	1.32	1.32, 1.34	197
65-Fluidity acid-modified corn	0.84	0.85, 0.84	186
α-Amylase-modified corn	0.52	0.52, 0.53	202
Corn[c]		1.41	186
Acid-modified corn[c]		0.57	156
	AMYLOPECTINS		
Sweet potato	1.67	1.67, 1.67	14.6
Tapioca	1.62	1.62, 1.62	3.2
Wheat	1.50	1.50, 1.49	8.8
White potato	1.42	1.42, 1.43	4.4
Corn	1.17	1.18, 1.19	17.5
α-Amylase-modified corn	0.95	0.95, 0.95	4.0
65-Fluidity acid-modified corn	0.73	0.73, 0.75	15.1

[a] The first figure shown is that calculated from the appropriate point in Figs. 1 and 2; the second is from an independent determination made at 0.2% concentration and gives some idea of the reproducibility of the determination. [b] All weights are calculated to the dry weight of the starch fraction. [c] These fractions, prepared by T. J. Schoch, were kindly furnished by W. Z. Hassid, who gives their molecular weights as 130,000 and 64,000, respectively. These values are derived from osmotic pressure measurements on the acetates.



Concentration, g./100 ml. of solution.

Fig. 1.—Viscosities of amyloses in 1 N KOH.



Concentration, g./100 ml. of solution.

Fig. 2.—Viscosities of amylopectins in 1 N KOH.

From the amylopectin samples also, a series of straight lines with increasing slope—this time positive in sign—for the higher molecular weight fractions was obtained (Fig. 2). Wagner's equation in the form

$$/\eta/ = \left(\frac{\ln \eta_{\text{rel.}}}{c}\right)_{0.2} - 0.028\left(\frac{\ln \eta_{\text{rel.}}}{c}\right)^2_{0.2}$$

where the subscript denotes the concentration at which the inherent viscosity was taken, was found applicable with good accuracy to the amylopectin viscosities (Table I). The numerical spread between the intrinsic viscosities of the highest and lowest molecular weight amylopectins was considerably less than in the amylose series.

Estimation of Iodine Affinity.—Iodine sorption of the starch fractions was measured by the potentiometric method of Bates, French and

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(4) Foster and Hixon, THIS JOURNAL, 65, 618 (1943).

(5) Wagner, *J. Polymer Sci.*, 2, 21 (1947).

Rundle[6] as modified by Wilson, Schoch and Hudson.[7]

In this determination a 0.2% solution of amylose or a 1% solution of amylopectin is normally prepared in 0.5 *N* potassium hydroxide. Neutralization of a 10-ml. aliquot with hydrochloric acid, addition of potassium iodide and dilution to 100 ml. then gives a solution 0.05 *N* with respect to both potassium chloride and potassium iodide. Since the solutions employed for viscosity are 1 *N* in potassium hydroxide concentration, the effect of doubling the initial potassium chloride concentration in the test solution was studied. The potassium iodide concentration was kept at 0.05 *N* and the iodine reagent was 0.05 *N* with respect to both potassium chloride and potassium iodide so there was no change in concentration of the latter salt during the titration but the potassium chloride concentration did change.

No difference in the shape or position of the electrometric titration curves, or in the iodine sorption of the samples as calculated therefrom, was detected with either starch fraction, despite the higher initial potassium chloride concentration. Appropriate blanks with 0.1 *N* potassium chloride likewise gave curves indistinguishable from those of normal blanks.

In practice, therefore, the same amylose solution can be used for estimating both iodine sorption and intrinsic viscosity. A 1% amylopectin solution in 1 *N* potassium hydroxide is prepared for determination of iodine affinity and a dilution of 0.2% concentration used for the viscosity determination.

Iodine sorption values of the materials used in this work are found in Table I.

Experimental

Materials.—The starch fractions were separated by the butanol precipitation procedure of Schoch,[8] somewhat modified at this Laboratory, and the amylose fractions were recrystallized one to three times. With the exception of the corn and white potato fractions, the amylose and amylopectin samples described were obtained from the same fractionation experiment.

The 65-fluidity acid-modified cornstarch was a commercial product. α-Amylase modified cornstarch was prepared by treating a 10% starch paste at 80° with 0.24 S.K.B. unit[9] of α-amylase per gram of starch for twenty minutes prior to fractionation with butanol.

Viscosity and Iodine Sorption of Amylose.—Between 0.0950 and 0.1050 g., dry weight, of amylose of known moisture content was weighed into a 50-ml. volumetric flask. Twenty-five ml. of potassium hydroxide solution, between 0.99 and 1.01 *N*, was added, and the stoppered flask stored at 0° until the amylose was completely dispersed. Dispersion time varied with the type and physical form of the amylose. The solution was then allowed to warm to room temperature, immediately made up to volume with the 1 *N* potassium hydroxide and filtered through a coarse porosity sintered glass funnel. Ten ml. of this 0.2% solution was pipetted into a calibrated No. 100 Ostwald–Cannon–Fenske viscometer maintained at 25.10 ± 0.03° in a thermostated bath. Flow times were determined to the nearest 0.05 second, with three to five measurements for each filling. The viscosities of both solvent and solution were usually determined by the equation η = density $(At - B/t)$ where A and B are viscometer constants. The density of the solution and solvent being the same within the required limits of accuracy, the relative viscosity may be calculated directly without measurement of the density.

For the data represented in Fig. 1, 0.5% solutions of amylose were prepared as described, at 0°, and the lower concentrations obtained by dilution. Calculation of the intrinsic viscosity was by the equation of Wagner described above, with $k = 0.032$.

Iodine sorption of a 10-ml. aliquot of the 0.2% amylose solution was measured potentiometrically.[6,7] The volume of iodine solution added was plotted *vs.* e.m.f. and the ml. of iodine at the inflection point, corrected for the amount utilized in a "blank" at that same e.m.f., was used in calculating the iodine affinity of the sample in mg. I_2/g. of sample.

Viscosity and Iodine Sorption of Amylopectin.—The determinations were carried out as for the amylose except that 50 ml. of a 1.00 ± 0.05% solution was prepared and utilized for iodine sorption measurement and the 0.2% concentration for measurement of viscosity was obtained from the more concentrated solution by dilution with 1 *N* KOH solution.

Discussion

In the evaluation of methods for fractionating starch, the efficiency of the separation and utility of the products are indicated by the extent to which linear and branched materials are freed from each other, and also by the amount of degradation occurring in the process. A saving in time and effort is achieved by using a single sample for the determination of intrinsic viscosity and by using the same sample for estimation of amylose content.

Replicate determinations have indicated that a precision of 1 to 2% in the determinations of the intrinsic viscosity can be expected. In the absence of molecular weights determined by other physical methods on sub-fractionated materials, the intrinsic viscosity provides an excellent evaluation of the molecular size of an amylose or an amylopectin, relative to other samples of the same fraction.

In the experiments made during the establishment of the method, it was observed that a different type of breakdown was apparently involved in the acid modification of starch granules to the 65-fluidity range, as compared with the action of α-amylase on starch paste in the fashion described. The former treatment affects the linear fraction to a lesser extent than the branched fraction as compared with the enzymatic degradation (*cf.* Figs. 1 and 2 and Table I). Further studies on the fractions obtained from modified starches are planned.

Acknowledgment.—The coöperation of J. E. Hodge and R. J. Dimler in furnishing certain of the starch fractions used in this study is gratefully acknowledged.

Summary

A simplified procedure is presented for determining the intrinsic viscosity and iodine affinity

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[6] Bates, French and Rundle, THIS JOURNAL, 65, 142 (1943).

[7] Wilson, Schoch and Hudson, *ibid.*, 65, 1380 (1943).

[8] Schoch, *ibid.*, 64, 2957 (1942).

[9] Sandstedt, Kneen and Blish, *Cereal Chem.*, 16, 712 (1939).

of a starch fraction. Equations are given for estimating accurately the intrinsic viscosities of amyloses and amylopectins in 1 N KOH solution from relative viscosities taken at 0.2% concentration. Iodine affinities of these same alkaline solutions are determined potentiometrically, to measure the proportion of linear material in the starch fractions. Experimental data are presented illustrating the validity of the procedure.

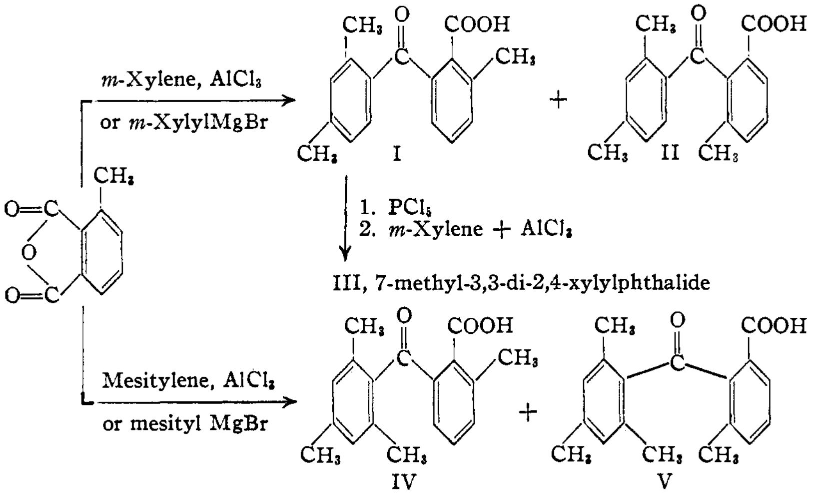
PEORIA, ILL. RECEIVED APRIL 21, 1950

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF THE OHIO STATE UNIVERSITY]

The Behavior of 3-Methylphthalic Anhydride in Friedel–Crafts and Grignard Condensations. II[1]

BY MELVIN S. NEWMAN AND CHESTER W. MUTH

In a previous publication the condensations of 3-methylphthalic anhydride with benzene and with phenylmagnesium bromide were described.[2] We report here on the condensations of this unsymmetrical anhydride with *m*-xylene, mesitylene, *m*-xylylmagnesium bromide and mesitylmagnesium bromide. These reactions are outlined in the chart and the results of this and previous[2] work are summarized in Table I.



Inspection of the results recorded in Table I reveals that the ratio of isomeric ketoacids formed is markedly affected by the reagent. The greater the number of methyl groups ortho to the position at which condensation with the anhydride occurs the greater the preference for reaction away from the methyl group in the anhydride. The Grignard condensations are more sensitive to this effect than the Friedel–Crafts condensations. The question as to how much of this effect is due to polar and how much to steric effects cannot be answered by these experiments but the results may be of use in predicting the ratio of products in other reactions of unsymmetrical anhydrides.

The methods used for separating I and II from their mixtures and for IV and V are of interest. Mixtures of I and II were esterified with diazomethane and the mixture of normal methyl esters of I and II thus obtained dissolved in concentrated sulfuric acid. After a short time the solution was poured on ice. The acid fraction proved to be almost pure I while the neutral fraction was the methyl ester of II. This finding may be explained by postulating a complex ionization in sulfuric acid of the methyl ester of I[3] whereas the methyl ester of II undergoes simple ionization.

The separation of IV and V was accomplished by taking advantage of the fact that IV esterifies much more slowly than V and that the methyl ester of IV saponifies under alkaline conditions much more slowly than the methyl ester of V.

Experimental

The values listed in Table I do not necessarily represent a single experiment. Rather we have tried to give a value which we believe to be most accurate. All reactions were run several times and the values indicated are taken from two or more checking experiments. Typical Friedel–Crafts and Grignard reactions are described below.

Reactions of 3-Methylphthalic Anhydride. (a) With Grignard Reagents.—To a well-stirred solution of 8 g. of 3-methylphthalic anhydride[2] in 80 ml. of benzene and 80 ml. of ether was added one equivalent of about 1.0 M Grignard reagent (titrated). After stirring at room temperature for fifteen minutes the mixture was hydrolyzed with 30 ml. of concentrated hydrochloric acid and separated into acidic and neutral fractions. The crude acids, I and II, thus obtained are listed in Table I. The neutral material was not examined as it amounted to less than 5% and was obviously a mixture. Analytical samples of I and II were obtained by recrystallization of portions of I and II isolated as described below. I melted at 118.4–119.5°, and II at 176.2–178.0°.

Anal.[4] Calcd. for $C_{17}H_{16}O_3$: C, 76.1; H, 6.0. Found: (I) C, 76.1; H, 5.7; (II) C, 75.9; H, 6.1.

In a similar way, analytical samples of IV and V were obtained from samples of IV and V isolated as described

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(1) Taken from the Ph.D. thesis of C. W. M., 1949. Chemistry Department, University of West Virginia, Morgantown, W. Va.

(2) M. S. Newman and C. D. McCleary, THIS JOURNAL, **63**, 1542 (1941).

(3) M. S. Newman, R. A. Craig and A. B. Garrett, *ibid.*, **71**, 869 (1949), equation 15.