A Rapid Nuclear Magnetic Resonance Method for Determining Hydroxypropyl Group in Modified Starch[1]

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ABSTRACT

Classic chemical methods for determining the hydroxypropyl content of modified starches are tedious, time-consuming, and unsuitable as control procedures for the analysis of large numbers of samples. In this paper, nuclear magnetic resonance (NMR) spectroscopy was evaluated as a rapid means of measuring the hydroxypropyl content. The terminal methyl of the hydroxypropyl group appears as a distinct doublet in the NMR spectrum and was utilized as a basis of quantitation. The method was found suitable for the analysis of commercial modified starches and useful for detecting a lower limit of 0.5% hydroxypropyl content.

A number of analytical methods for determining hydroxyalkyl groups in starch can be found in the literature. The methods of Lortz (1) and Morgan (2) are modifications of the classic Zeisel method in which hydriodic acid is used to decompose the ether into an alkyl iodide and alkene. These methods fail to give qualitative information on the type of ether species present and are tedious. They are also sensitive to alcohols, glycols, esters, and other impurities present in the starch.

Recently gas chromatography has been used for this determination. The method of Tai et al. (3) is based on measuring the pyrolysis products of modified starches. This method is rapid and has been applied to hydroxyethyl starch but not hydroxypropyl starch, where quantitation may be more complicated. Lott and Brobst (4) studied the trimethylsilyl derivatives of hydrolyzed hydroxyethyl amylose. The procedure gives detailed qualitative information but is very time-consuming. The analysis of hydrolyzed unfractionated starch would be more complicated because of branching in the amylopectin fraction. A method described by Van der Bij (5) utilizes hydrolysis by hydriodic acid, followed by gas chromatographic analysis for alkyl iodide.

Johnson (6) recently reported a spectrophotometric method for determining the hydroxypropyl group in starch. The method involves hydrolysis of the hydroxypropyl group to propylene glycol and its dehydration to propionaldehyde and the enolic form of allyl alcohol. These products form a purple complex with ninhydrin, which is then measured spectrophotometrically. Two procedures are given for high and low levels of hydroxypropyl group.

In recent years, high-resolution nuclear magnetic resonance (NMR) has been used extensively in structure elucidation of carbohydrates. Casu et al. (7) used NMR to determine the degree of substitution in methylated amylose and cyclodextrin. In brief, the NMR provides a "fingerprint" pattern which gives information concerning the chemical nature, spatial position, and number of each type of hydrogen present in a molecule. The major advantages as an analytical tool are speed, simple sample preparation, and insensitivity to minor impurities.

In our study, high-resolution NMR was evaluated as a rapid means for

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determining the hydroxypropyl content of modified starches. The fact that evidence could be established for the presence of a hydroxypropyl group rather than other alkyl groups was considered a major asset. An initial problem in the application of this method to modified starches was the need to use high concentrations of starch to detect the hydroxypropyl peak in the spectrum. Hydrolysis of the sample with acid permitted the achievement of these high concentrations and a reduction of viscosity. Reduction of viscosity is important for proper resolution of the spectrum.

The NMR spectra of hydrolyzed unmodified and modified corn starch are shown in Figs. 1 and 2. The spectra show similarities except for the doublet at ~1.5 p.p.m. (relative to external tetramethylsilane at 0.0 p.p.m.) for the modified sample. This peak is due to the methyl group of the hydroxypropyl ether. It is the area of this doublet which is used to determine the degree of modification of the starch.

MATERIAL AND METHODS

The basic procedure for the preparation of starch samples for NMR analysis is as follows:

Twelve grams (dry basis) of starch is added to a 25-ml. volumetric flask. Ten milliliters of 10% hydrochloric acid is added to the sample which is then placed in a boiling-water bath for 30 min. The sample is allowed to cool and 2.5 ml. of a freshly prepared 10% acetic acid solution (v./v.) is added as an internal standard and made to volume with distilled water.

Approximately 0.5 ml. of the solution is added to a standard 5-mm. NMR tube and placed in a Varian A-60 spectrometer. The instrumental gain, R_f field, and sweep rate are adjusted to give a suitable spectrum and integral measurement. The only peaks that need to be evaluated are the methyl peaks of the acetic acid (internal standard) and the hydroxypropyl group (unknown). These peaks appear

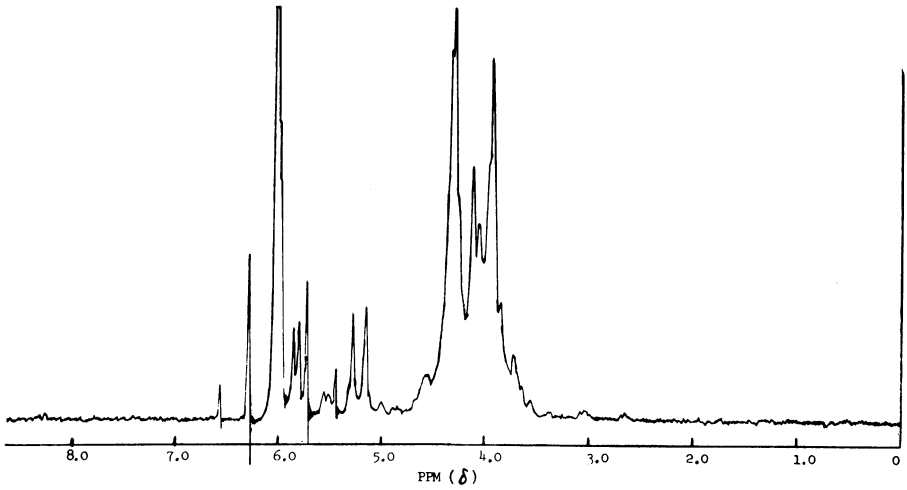

Fig. 1. NMR spectrum of unmodified corn starch (hydrolyzed).

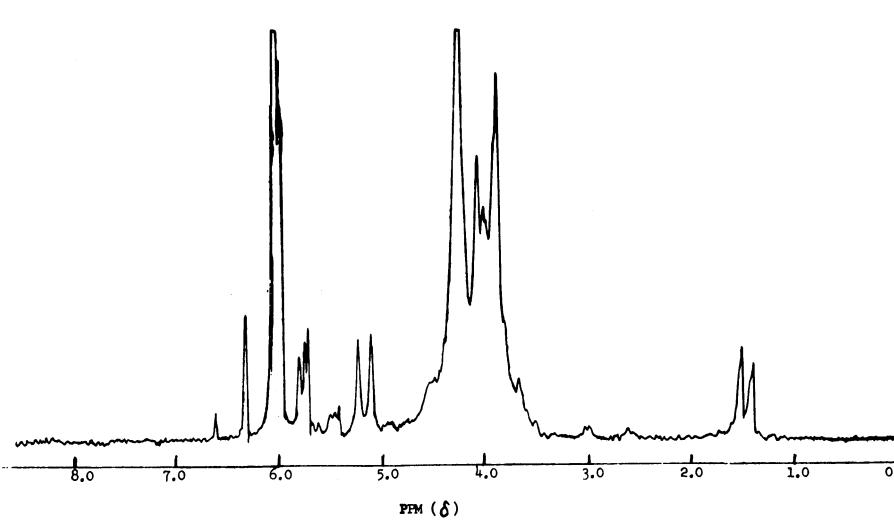

Fig. 2. NMR spectrum of modified corn starch (hydrolyzed).

The spectra in Figs. 1 and 2 were obtained with 10% deuterium chloride in deuterium oxide used for the hydrolysis procedure in place of 10% HCl. Since deuterium compounds do not produce a NMR signal, a more detailed figure is obtained for illustration purposes. Peak assignments other than the hydroxypropyl doublet are: large singlet at 6.0 p.p.m. is due to hydroxyl absorption of moisture and hydrolyzed starch; 5.0 to 6.0 p.p.m., the anomeric proton (H_1); and 3.5 to 4.5, remaining hydrogens of the starch hydrolysate. Symmetrical peaks to the left and right of hydroxyl peak are due to spinning sidebands from the strong hydroxyl absorption.

sufficiently upfield from the large water and sugar hydrogen peaks to permit quantitative determination. These peaks are integrated several times and the average value taken (Fig. 3). The area of the acetic acid peak is proportional to the number of absorbing protons if saturation effects are negligible. This area can be used to calculate the weight of hydroxypropyl group present. The percent hydroxypropyl groups in the starch may be calculated from the following formula:

$$\%\ \text{Hydroxypropyl group in modified starch} = \frac{0.2623\ \text{g.}}{} \cdot \frac{\frac{59.08\ \text{integral (hydroxypropyl-doublet)}}{60.05\ \text{integral (acetic acid-singlet)}}}{12.00\ \text{g. starch}} \times 100$$

where 0.2623 = wt. of acetic acid in sample; 59.08 = mol. wt. of hydroxypropyl group; and 60.05 = mol. wt. of acetic acid.

Simplified, the relationship becomes:

$$\%\ \text{Hydroxypropyl group} = 2.151 \times \frac{\text{integral (hydroxypropyl-doublet)}}{\text{integral (acetic acid-singlet)}}$$

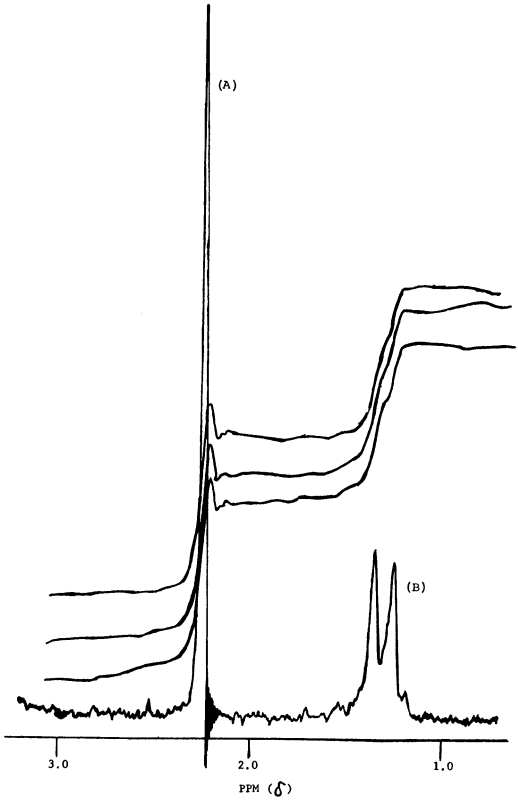

Fig. 3. NMR spectrum and integrals of the methyl groups of acetic acid (A) and modified starch (B).

RESULTS AND DISCUSSION

Five commercial starch samples were analyzed in the first phase of this study. Each sample was prepared and analyzed five times in random fashion over a period of 3 days. A control starch containing a known amount of propylene glycol was analyzed each day as a check of instrument sensitivity. This standardization is now replaced by the use of acetic acid as an internal standard. Averaged results and standard deviations were calculated on the basis of the five analyses and are shown in Table I.

These five samples and available control samples were submitted to an outside laboratory to be analyzed by the Lortz modification of the Zeisel method. The samples were analyzed in our laboratory by the photometric method of Johnson (6). A comparison of the three methods is given in Table II.

Results show that the Lortz method gives consistently higher values than the NMR and photometric methods. The corrected values in the Lortz method are

TABLE I. NMR ANALYSIS OF MODIFIED STARCHES

Sample No.	Type of Starch	OC_3H_7 %	Standard Deviation
1	Corn	4.6	±0.2
2	Corn	0.9	±0.04
3	Corn	1.7	±0.1
4	Waxy maize	2.2	±0.06
5	Tapioca	1.2	±0.05

TABLE II. HYDROXYPROPYL MODIFICATION BY LORTZ, NMR, AND PHOTOMETRIC METHODS

Sample	Lortz Method		NMR Method	Photometric Method
	Determined %	Corrected %	%	%
1	8.4 (6.7)	6.9	4.6	4.1
2	1.6	0.1	0.9	0.5
3	3.3	1.8	1.7	1.7
4	5.1	...	2.2	2.5
5	2.7	1.7	1.2	1.2
Corn blank	1.5 (0.7)	...	...	...
Tapioca blank	1.0	...	...	...

obtained by subtracting the results of the blank determinations (unmodified starches). The photometric procedure provides for a blank starch correction. No NMR signal was detected for the above control samples[2]. To check the reproducibility of the Lortz method, sample 1 and a corn starch blank were resubmitted for analysis. Results obtained are given in brackets in Table II and indicate poor reproducibility.

Correlation between the photometric (6) and NMR methods appears to be good, although only a single determination on each sample was made by this method. All samples were analyzed by the first method of the photometric procedure; sample 2 may have shown better correlation if analyzed by the second method for low levels of hydroxypropyl group. The advantage of the NMR method is that information can be gained as to the nature of derivatized samples, such as acetylated starches, and that it is more rapid and less tedious. The photometric method is subjected to time-dependent color development and the handling of relatively large quantities of concentrated sulfuric acid. The photometric method, however, uses an inexpensive, readily available spectrophotometer.

To resolve the discrepancies between the Lortz method and the NMR and photometric methods, a material balance study was performed. This was based on

[2]Some starches of high oil content, e.g., corn, showed a weak peak at 1.5 p.p.m. This peak could be removed by adding a small amount of benzene to the NMR tube and shaking; the oil dissolves in the benzene layer and the peak disappears from the spectrum. The error introduced by the presence of oil is very small and correctable.

TABLE III. RESULTS OF MATERIAL BALANCE EXPERIMENT

Sample No.	Weight of Propylene Oxide		Total Recovery Propylene Oxide	Added Propylene Oxide
	In Starch Cake	In Water Fraction		
	g.	g.	g.	g.
1	0.43	0.57	1.00	0.99
2	0.63	0.58	1.21	1.05
3	0.65	0.51	1.16	1.00

the method of Kesler and Hjermstad (8) for modifying starch with propylene oxide. In our case, 1 g. of propylene oxide was added to an alkaline slurry containing 1 g. of corn starch. The starch was allowed to react for 4 days, after which the slurry was acidified to convert any unreacted propylene oxide to glycol. The starch cake and water fraction were analyzed by the NMR method, and total propylene oxide in both phases was calculated. Table III shows the results of this experiment.

These recoveries indicate that the NMR method gives higher results. We feel this error might be due to experimental technique, such as loss of water. However, the error would be much greater if these samples were analyzed by the Lortz method.

SUMMARY AND CONCLUSIONS

Results indicate that NMR may be used as a rapid means of measuring percent hydroxypropyl group. A single determination can be performed within 1 hr., and multiple determinations significantly reduce the man-time required per sample. The method does not require the analysis of control starch; interference by small amounts of oil can be removed by shaking the NMR tube with benzene and reanalyzing.

Although our efforts were devoted to the analysis of hydroxypropyl-modified starches, the method should be equally applicable for quantitation of other starch derivatives.

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