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New ^{1}H NMR Procedure for the Characterization of Native and Modified Food-Grade Starches

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ABSTRACT: A novel, fast, and straightforward procedure is presented for the characterization of starch (the largest energy component in food) and modified starches (such as octenyl succinic anhydride (OSA)-modified starches used as a dispersing agent in the food industry). The method uses ^{1}H NMR to measure the degree of branching and also, for modified starches, the degree of chemical substitution. The substrate is dissolved in dimethyl-d_6 sulfoxide; addition of a very low amount of deuterated trifluoroacetic acid (d_1-TFA) to the medium gives rise to a shift to high frequency of the exchangeable protons of the starch hydroxyl groups, leading to a clear and well-defined ^{1}H NMR spectrum, which provides an improved way to determine the degrees of both branching and chemical substitution. Measurements of the size and molecular weight distributions by multiple-detector size exclusion chromatography show that degradation by TFA does not affect the accuracy of the method.

KEYWORDS: starch, NMR characterization, branching degree, degree of substitution

INTRODUCTION

Starch is the largest contributor to energy intake in human food and a major energy component of animal feed. Modified starches, such as those with octenyl succinic anhydride (OSA)[1] or glycopolymer derivatives,[2] have technical applications such as emulsifiers; for example, OSA-modified starches are used as dispersing agents for β-carotene in nutritional products. A structural parameter of interest in starch and modified starches is the degree of branching (DB, the number of branching points as a percentage of the total number of glucosidic linkages), whereas for chemically modified starches the degree of substitution (DS, the average number of substituted units per glucose monomer) is also useful. These structural parameters influence food properties such as digestibility of starch-containing foods[3,4] and emulsifier efficacy.[5,6]

DB can be measured by nuclear magnetic resonance (NMR);[7] although the original technique is effective, improvements are desirable for greater accuracy in the determination of this parameter. Two different techniques are typically used to measure the DS: alkaline titration or ^{1}H NMR spectroscopy. Some authors have reported a lack of accuracy and reproducibility in the titration method due to difficulties in end-point detection.[8−10] On the other hand, NMR is a powerful and reliable characterization technique, but only when it is performed under conditions in which there is complete and homogeneous dissolution of the sample. Several procedures differing in experimental parameters such as the solvent, the temperature, or elements of sample preparation have been reported (enzymatic digestion, predissolution, or pre-freeze-drying)[8,10−14] and will be discussed later in this paper.

Because of the presence of many different hydroxyl groups, the ^{1}H NMR spectra of polysaccharides can sometimes be complicated to interpret. Labile protons from OH or any other group containing exchangeable protons generally exhibit broad signals that can hide other peaks of interest. This is the case of starch in d_6-dimethyl sulfoxide (d_6-DMSO), for which the signals of the three OH protons and the anomeric H-1 are in the same region, vitiating the integration of the latter and consequently the calculation of the DB and DS. In a previous study, our group reported a procedure involving the dissolution of starch in an 80:20 d_6-DMSO/D_2O mixture to ensure a complete solubilization of the sample and an efficient removal of the hydroxyl signals.[15] However, solubility issues and insufficient spectrum clarification were seen if D_2O was not added in this exact ratio, resulting in a restricted method. Nilsson et al. reported a method of starch predeuteration, involving solubilization of OSA-modified starch in boiling D_2O followed by freeze-drying of the system prior to dissolution in d_6-DMSO.[13] Albeit efficient, this technique is time-consuming and has to be repeated several times to obtain a high level of deuteration of the starch.[11]

Ross and Lowe have shown that using deuterated trifluoroacetic acid (d_1-TFA) in ^{1}H NMR in d_6-DMSO is a very efficient way to shift all of the signals due to exchangeable protons to high frequency, leading to clear and simplified spectra.[16] The present work develops for the first time the use of d_1-TFA for an improved ^{1}H NMR characterization of starch and starch derivatives in d_6-DMSO. The new technique is tested on a native corn starch, giving spectra free of OH signals, which leads to an accurate measure of the DB. Extended to the corresponding OSA-modified product, this method gives accurate results for both the DS and DB.

To check for effects of any degradation of starch during any of the procedures, which if significant would imply that the characterization was not reliable, the size distributions were obtained

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using size exclusion chromatography (SEC, also termed gel permeation chromatography (GPC)).

MATERIALS AND METHODS

Corn starch (batch 059K0191), OSA (97% mixture of *cis* and *trans*), lithium bromide (LiBr; Reagent Plus, ≥ 99%), d_6-DMSO (99.5% atom D), and d_1-TFA (99% atom D) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia) and used as received. Water was ultrapure (Milli-Q, Millipore), and DMSO and ethanol (EtOH) were of analytical grade and purchased from Merck.

Preparation of OSA-Modified Corn Starch. The OSA-modified starch used here was prepared from pure corn starch as a starting material. Extraneous substances such as proteins, lipids, and nonstarch polysaccharides that could be found in commercial material could lead to the presence of unwanted signals in the resulting ^{1}H NMR spectra. Pure corn starch (12.5 g, dry weight) was suspended in 37.5 g of water (25 wt % solid content), and the slurry was adjusted to pH 8.5 by dropwise addition of a 3 wt % NaOH aqueous solution. The starch slurry was continuously gently mixed with a magnetic stirrer for 15 min at 35 °C, and 2.25 g of an OSA/EtOH mixture (1:2 w/w) was added dropwise over 2 h. The system was allowed to react for 3 h at 35 °C, maintaining the pH at 8.5 using the NaOH solution. After this time, the reaction mixture was adjusted to pH 6.0 with 1.0 M HCl and transferred into 50 mL Falcon tubes. The solid was collected by centrifugation (4000 rpm, 20 °C, 10 min) and then allowed to soak in 100 mL of ethanol over 24 h to remove unreacted OSA. The whole purification process (centrifugation plus ethanol wash) was repeated three times, and the modified starch was dried in an oven at 60 °C for 24 h. ^{1}H NMR (d_6-DMSO) δ 5.49 (bm, 1, CH_2—CH═C$\underline{H}$—CH—C_5H_{11}), 5.36 (bm, 1, CH_2—C$\underline{H}$═CH—C_5H_{11}), 5.11 (bm, 1, H-1 α-1,4 internal), 4.75 (bm, 1, H-1 α-1,6 internal), 4.00−3.20 (m, 6, $\underline{H}$-2, $\underline{H}$-3, $\underline{H}$-4, $\underline{H}$-5, $\underline{H}$-6), 2.59 (bm, 5, CO—$C\underline{H}_2$—C$\underline{H}$COONa—$C\underline{H}_2$—CH_2), 1.99 (bm, 2, CH═CH—$C\underline{H}_2$—C_4H_9), 1.42−1.31 (bm, 6, CH═CH—CH_2−$C_3\underline{H}_6$—CH_3), 0.89 (bm, 3, CH═CH—C_4H_9—C$\underline{H}_3$).

^{1}H NMR Spectroscopy. Samples were prepared and NMR spectra were measured as described previously.[17] d_1-TFA was added directly to the medium just before the NMR measurement, and the whole mixture was transferred to a 5 mm NMR tube using a Pasteur glass pipet. The tube was sealed and wrapped with Parafilm before an NMR spectrum was recorded. Every experiment was repeated three times to check for repeatability. NMR spectra were measured on a Bruker Avance NMR spectrometer operating at a Larmor frequency of 500.13 MHz for ^{1}H, equipped with a TXISz probe (Bruker Biospin) (8 μs 90° pulse, a repetition time of 15.07 s composed of an acquisition time of 3.07 s and a relaxation delay of 12 s, 128 scans, d_6-DMSO containing 0.5 wt % LiBr). Acquisitions were started after ensuring a complete dissolution of the product; the temperature dependence of both the starch backbone and the OSA side chains solubilities are given in Figure 1. A temperature calibration of the NMR spectrometer and probe was carried out with a pure ethylene glycol standard (distilled, in a sealed tube) following the procedure recommended by Ammann et al.[18] The chemical shift scale was calibrated using the residual nondeuterated DMSO signal at 2.549 ppm.[17] Care was taken to ensure that the magnetization was fully recovered between pulses for both OSA-modified starch and DMSO signals to determine true relative signal integrals. The longitudinal relaxation times T_1 for starch anomeric and CH_3 (OSA) protons were determined using the inversion−recovery technique (T = 343 K, 56 scans, d_6-DMSO/d_1-TFA, 14 recovery delays ranging from 0.15 to 22 s). The T_1 values obtained were around 1.9 s for both CH_3 (OSA) and α-1,6 anomeric protons and 2.2 s for α-1,4 anomeric protons. To quantify the integral of the signal of interest, the relaxation delay must be at least 5 times the value of T_1, and hence a relaxation delay of 12 s was used for both the DB and DS quantification.

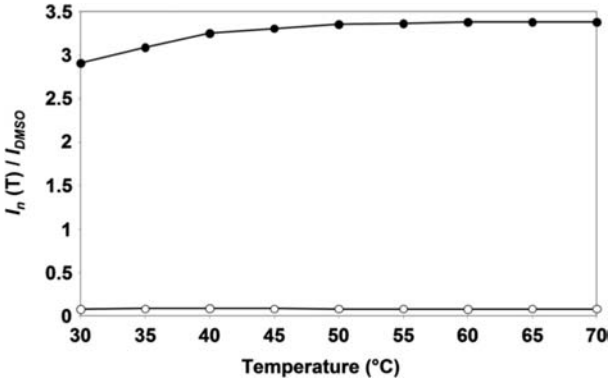

Figure 1. Temperature dependence of the integrated ^{1}H NMR signals (as the ratio of integrated signal of solution to that of pure solvent) during the dissolution of OSA-modified corn starch (2.9 mg) in d_6-DMSO (0.7 mL) + d_1-TFA (18 mg) (●, starch backbone; ○, OSA side chains).

The residual DMSO signal was taken as an internal reference in experiments shown in Figure 1 (dissolution as a function of the temperature). The T_1 of DMSO at 70 °C was determined according to the method previously described (12.2 s). Thus, the relaxation delay in this set of experiments was fixed to 5 × 12.2 s = 61 s to ensure a full magnetization recovery and quantitative ^{1}H NMR spectra. All OSA-starch signals exhibit T_1 significantly shorter than 12.2 s.

Data were processed using TOPSPIN software. All spectra were manually phased and baseline-corrected. A Lorentzian fit was used for spectral deconvolution.

SEC. To obtain information on how much the TFA treatment resulted in degradation of the substrate, thereby possibly vitiating subsequent NMR results, characterization of the molecular size distribution was performed on treated and untreated samples. Cleavage of α-1,4 and/or α-1,6 bonds would result in a decrease in the size distribution of the substrate, enabling some quantification of this effect. SEC was performed on a PSS (Mainz, Germany) Agilent 1100 series with differential refractive index (DRI), multiple-angle laser light scattering (MALLS), and viscometric detection. SEC experimental conditions have been chosen to minimize starch degradation of larger molecules due to shear scission during SEC characterization, as follows. Most of polymer in the samples used here was contained in a range of hydrodynamic radius below 10^2 nm. Flow conditions, etc., were chosen corresponding to optimal conditions as shown in Figures 2 and 3 of Cave et al.,[19] which indicate minimal shear scission below this size. Details of the sample preparation, SEC equipment, and data processing are given elsewhere.[20,21]

SEC separates by size, not molecular weight, and the size separation parameter is the hydrodynamic volume, V_h, or equivalently the corresponding hydrodynamic radius R_h, with $V_h = {}^4/_3\pi {R_h}^3$. V_h is a complex quantity, proportional to the product of the weight average of the intrinsic viscosity and the number average of the molecular weight $\overline{M}_n$.[22] For starch, there is evidence[23] that R_h is close to the radius of gyration, R_g. It is important to be aware that for a branched polymer such as starch, there is in general no unique relationship between size and molecular weight: two branched molecules with different branching structures and also different molecular weights can have the same size (R_h or R_g). However, it is meaningful to report average quantities such as $\overline{M}_n$ in an eluent slice as a function of R_h. Multiple-detector SEC data are presented here as the so-called SEC weight distribution, $w(\log V_h)$, which is the (relative) weight of chains in an increment of hydrodynamic volume d log V_h, and $\overline{M}_n(V_h)$.

In using SEC to characterize the samples, it is important[24] to know if "recovery" is quantitative, that is, that no significant amount of analyte is lost during the size characterization process, resulting in loss of analyte

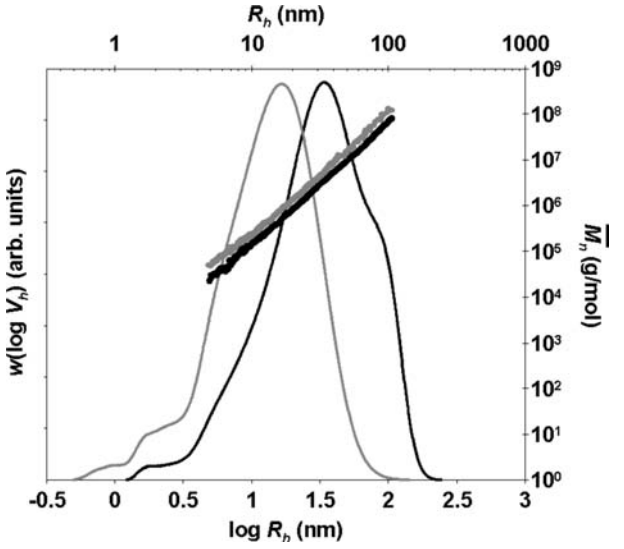

Figure 2. Weight- and number-average molecular weight distributions of corn starch obtained from SEC measurements before (black; mass recovery after analysis = 109 ± 14%) and after (gray; mass recovery after analysis = 97 ± 12%) NMR characterization.

in the SEC columns or "plumbing" (frits, etc.). Mass recoveries after analysis can be calculated from the DRI detector signal, together with various instrument constants and the samples' specific refractive index increment dn/dc, which is the change in refractive index with polymer concentration. This was implemented here assuming that dn/dc values for the starch samples, which contain both amylopectin and amylose, lie between the values of pure amylopectin and pure amylose, dn/dc = 0.0717 and 0.0929 mL g^{-1}, respectively in the eluent used here. These dn/dc values were measured by Michael Krämer and Peter Kilz of PSS, Mainz, Germany. The quantity dn/dc is governed by the polarizability and electron density of the molecule, and although amylose and amylopectin have similar structures, the latter has a branch point about every ~17 monomer units compared to much greater distances between branches in amylose, and it is not surprising that this structural difference is sufficient to account for the ~20% difference in dn/dc. The SEC data shown in Figure 2 give the size distributions as a factor of the hydrodynamic radius R_h, with $V_h = {}^4/_3 R_h{}^3$. The DRI data give upper and lower estimates for mass recovery with these two values of dn/dc, defining the range of possible values and the experimental error. These give mass recovery for the SEC analysis of the native nonmodified corn starch sample as 109 ± 14 and 97 ± 12% for the same sample after ^{1}H NMR characterization using d_1-TFA. It is thus apparent that recovery is essentially 100% within experimental error.

Figure 2 shows that there is a large decrease in size after addition of TFA (the peak in $w(\log V_h)$ going from ~30 to ~15 nm); that is, this reagent causes significant degradation of the starch. The overall $\overline{M}_n$ values similarly change from ~4 × 10^6 to ~1 × 10^6 g/mol. One objective of the characterization tests used here is to find the degree of branching, and so it must be determined if this degradation will affect the accuracy of this measurement. (It is noted that this is an apparently low molecular weight, because that of native amylopectin is typically ~10^8; however, the sample is a commercial corn starch, the preparation of which invariably involves degradation.)

The change in $\overline{M}_n$ shows that the addition of d_1-TFA in the conditions of dilution used leads to the cleavage of 4 × 10^6/1 × 10^6 = 4 α-1,4 linkages per starch macromolecule. It is therefore likely that mainly α-1,4 linkages have been cleaved during the NMR experiment as the removal of side chains should not lead to such a marked loss in molecular weight. The number of cleaved α-1,6 (branching) linkages

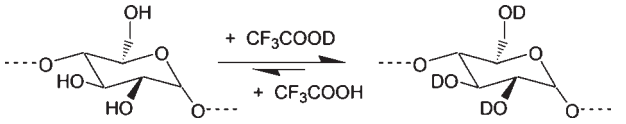

Figure 3. Schematic representation of the deuteration of starch with d_1-trifluoroacetic acid (d_1-TFA).

is negligible compared to the total number of such linkages, as shown by the following simple estimate. For $\overline{M}_n$ = 4 × 10^6 g/mol, the number-average degree of polymerization is ~2.5 × 10^4. The number-average degree of polymerization of amylopectin branched chains, the dominant component of this starch, is typically ~17. Hence, there are ~2.5 × 10^4/17 ≈ 1500 branches per molecule in the samples used here, which is very much greater than the number of bonds cleaved by TFA. The ratio of α-1,4 to α-1,6 linkages thus remains almost unchanged during the NMR experiment (indeed, such a slight degradation of starch chains is not detectable by ^{1}H NMR). Further support for this inference comes from the dependence of $\overline{M}_n$ on hydrodynamic volume shown in Figure 2. This dependence is almost unchanged with TFA treatment. Because, for a given polymer, this dependence is dictated by the branching structure, this again shows that the branching structure is almost unchanged by TFA treatment. It thus appears that the procedure described in this study is valid for a quantitative measurement of the DB of starch.

As pointed out by an anonymous reviewer, there is a shoulder at about R_h ~ 2 nm on the SEC distribution in Figure 2, corresponding to oligosaccharides. The fact that one sees this as a distinct feature (a shoulder) shows that this is not simply due to random cleavage during the TFA-induced starch hydrolysis. This could perhaps be due to the cleavage of linkages close to a nonreducing end and/or of small clusters on the outer part of the molecule. If this were exclusively of α-1,6 linkages, then NMR analysis might give an underestimate of the DB; if only α-1,4 linkages were cleaved, an overestimate of DB would result. The area under this shoulder corresponds to 3.6 wt % of the sample, suggesting that, at most, this would be the maximum error induced in DB inferred by NMR.

RESULTS AND DISCUSSION

Characterization of Native Corn Starch. The labile deuteron of the carboxylic acid group of d_1-TFA can exchange very quickly with the protons of starch hydroxyl groups (Figure 3), increasing the mobility of the latter, which has a strong impact on their chemical shift. Figure 4 shows the ^{1}H NMR spectra in d_6-DMSO of a native corn starch before and after direct addition of d_1-TFA to the NMR tube. Although the resonances of the starch chain protons denoted 2−6 can be readily identified in the region of 3.21−3.89 ppm,[10] the anomeric protons denoted 1 and 1′, corresponding to the internal α-1,4 and α-1,6 linkages, respectively, are poorly defined because of the presence of many other peaks from starch OH groups in this region (4.19−5.61 ppm). Moreover, the signal of the residual water peak is present at 3.14 ppm, despite using a freshly opened bottle of d_6-DMSO and drying the starch for 24 h at 60 °C prior to analysis. This signal is present because of the hygroscopic nature of both DMSO and starch and is difficult to avoid. However, after the addition of a small amount of d_1-TFA, protons belonging to water, the OH groups, and TFA undergo rapid exchange, giving rise to only a single peak at 6.78 ppm. The anomeric signals 1 and 1′ are now clearly visible at 5.11 and 4.75 ppm, respectively, and the signals of the other protons 2−6 are better defined, which could indicate that some exchangeable protons are present in this region (3.21−3.89 ppm). As in a previous study by Ross and Lowe,[16] the chemical shift of the final signal of exchangeable protons can

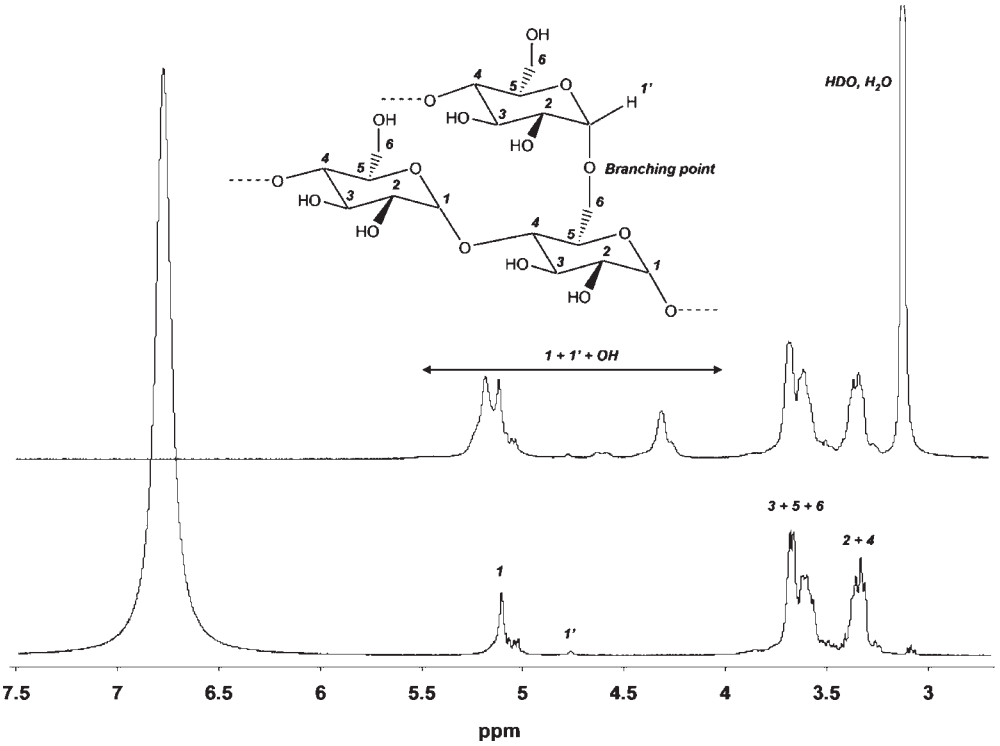

Figure 4. ^{1}H NMR spectrum of corn starch in d_6-DMSO at 70 °C prior to (upper panel) and after (lower panel) addition of d_1-TFA.

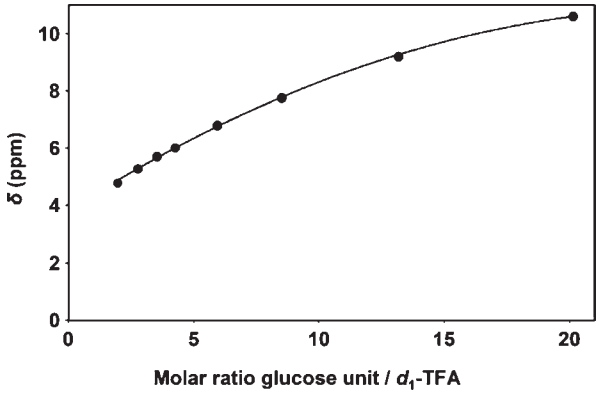

Figure 5. Variation of the chemical shift of the exchangeable protons NMR signal of corn starch (3.5 mg) in d_6-DMSO (0.7 mL) with amount of d_1-TFA.

be easily tuned to an appropriate position in the spectrum by adjusting the amount of d_1-TFA. As illustrated by Figure 5, this shift is progressively moved to high frequencies with increasing amounts of d_1-TFA. One can see that the addition of very small quantities of TFA is sufficient to displace the signal to 5.5 ppm (1.42 wt % of d_1-TFA with respect to d_6-DMSO), a region which is free of other signals for native starch. Therefore, the versatility of this procedure is of particular interest when characterizing chemically modified starches that may contain groups with specific chemical shifts above 5.5 ppm and consequently may require a precise adjustment of the position of this signal.

The addition of d_1-TFA did not induce any detectable appearance of α and β reducing-end signals (at 4.91 and 4.28 ppm, respectively), as shown by the corn starch spectrum in Figure 4 after addition of d_1-TFA. Furthermore, no noticeable variation of the relative intensities of 1 and 1′ signals can be observed 24 h after the addition of d_1-TFA at the NMR detection limit, indicating that starch chains are quite stable in diluted TFA in DSMO solutions. One can conclude that the addition of a small amount of d_1-TFA is an almost nondegradative method to give improved ^{1}H NMR spectra of starches. This is especially useful for finding the DB of starch and modified starches, as this requires well-resolved 1 and 1′ anomeric NMR signals using eq 1:[7,25]

$$\mathrm{DB}\ (\%) = 100\frac{I_{\alpha\text{-}1,6}}{I_{\alpha\text{-}1,6} + I_{\alpha\text{-}1,4}} \qquad (1)$$

Here, $I_{\alpha\text{-}1,4}$ and $I_{\alpha\text{-}1,6}$ are the ^{1}H NMR integrals of internal α-1,4 and α-1,6 linkages, respectively. Generally, the main source of error for NMR calculation originates from the inability to truly integrate the signals of anomeric protons 1 and 1′ because of the presence of OH protons in the same area of the spectrum. Owing to the very good resolution of these signals obtained here after the addition of d_1-TFA, the choice of an integral region both starting and ending at the baseline is straightforward and allows a reliable and reproducible calculation of DB. The value found for the corn starch used in this study is DB = 3.34 ± 0.02% (n = 3), which is consistent with what is generally observed for starches (DB generally lies between 1 and 5% depending on the botanical source and/or the amylose/amylopectin ratio).[7,25]

Characterization of OSA-Modified Corn Starch. Following the same procedure, that is, addition of a small amount of d_1-TFA to the NMR tube, the new method was utilized for the ^{1}H NMR characterization in d_6-DMSO of OSA-modified corn starch. As observed for the corresponding native corn starch shown in Figure 4, the spectrum of the OSA starch derivative is better with d_1-TFA due to the fast exchange of labile protons of residual

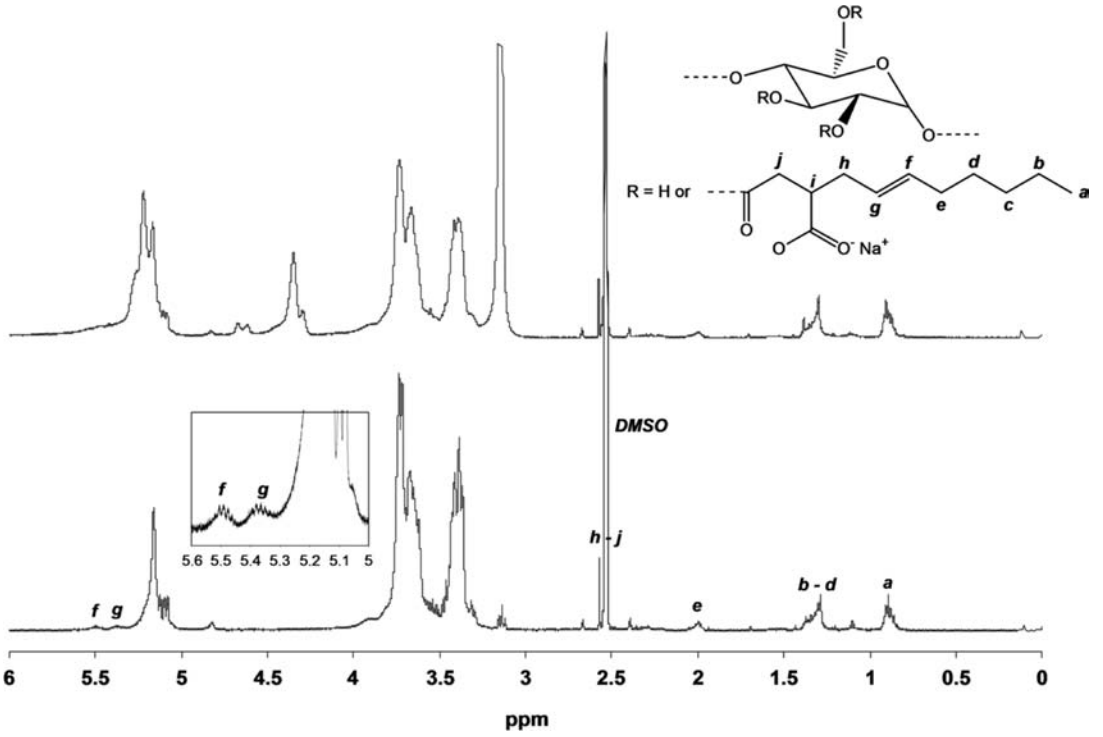

Figure 6. ^{1}H NMR spectrum of OSA modified corn starch in d_6-DMSO at 70 °C prior to (upper panel) and after (lower panel) addition of d_1-TFA.

water and of hydroxyl groups with the deuterium of d_1-TFA (Figure 6). Peaks arising from the OSA side chains were assigned according to the literature,[10] and most of them were found not to be affected by the presence of d_1-TFA, except those corresponding to the double bond, denoted "f" and "g" in Figure 6, at 5.49 and 5.36 ppm, respectively. These two signals are indistinguishable beneath the peaks of the starch OH groups, a situation that is only marginally improved even after the addition of d_1-TFA (inset in Figure 6). No residual signals of unreacted OSA were observed in the ^{1}H NMR spectrum around 3 ppm in d_6-DMSO, which is that for C**H**$_2$ protons of the anhydride cycle, suggesting a successful removal by the purification procedure. As d_1-TFA addition leads to a very good definition of signals of the anomeric protons 1 and 1′, it provides an improved means to measure the degree of substitution (DS) of such modified starches. This parameter is the average number of OSA side chains per glucose unit along the starch backbone and can be obtained via ^{1}H NMR experiments by eq 2:

$$\mathrm{DS} = \frac{I_{0.89}}{3(I_{\alpha\text{-}1,6} + I_{\alpha\text{-}1,4} + I_{\text{r-e}})} \qquad (2)$$

where $I_{0.89}$ is the ^{1}H NMR integral of the signal of the CH_3 group of OSA in d_6-DMSO and $I_{\text{r-e}}$ corresponds to the reducing chain ends. For the reason described above, DS can be so obtained with good accuracy. The value found for this OSA-modified corn starch is DS = 0.0326 ± 0.0006 (n = 3). Note that DB = 3.55 ± 0.06% (n = 3) for this sample, indicating that the OSA modification induces only a slight variation of the starch backbone DB. It is likely that TFA results in partial deesterification of OSA starch, but this would not affect the calculation of the DS. d_1-TFA is added directly to the NMR tube (which already contains the sample dissolved in d_6-DMSO). Thus, even if some OSA groups were removed from the starch backbone by acid hydrolysis, the molar ratio between starch anomeric protons 1 and 1′ and CH_3 of OSA would remain exactly the same, without affecting the DS calculation.

Optimal determination of the DS of modified starches by NMR has several requirements. First, the choice of the solvent is important. As OSA-modified starches are amphiphilic (the starch backbone being the hydrophilic part and OSA the hydrophobic one), the use of D_2O must be avoided. In a study on the rheological properties of OSA-modified starches in aqueous medium, Ortega-Ojeda et al. provided evidence that OSA side chains aggregate into hydrophobic domains in water even at quite low concentrations.[26] Evidence for the aggregation of OSA-modified starches into micelles in aqueous medium has also been put forward recently.[27] Hydrophobic interactions can induce a shift and significant signal reduction in ^{1}H NMR characterization of amphiphilic products,[28] leading to an incorrect estimation of the DS. This phenomenon is accentuated as the DS increases. In a recent study, Bai et al. reported the ^{1}H NMR characterization in D_2O of an OSA-modified starch after hydrolysis with α-amylase.[11] Although this hydrolysis will increase the water solubility of the final product, thereby aiding analysis, it does not prevent hydrophobic interactions from occurring. Indeed, Bai et al. have reported a shift and a broadening of the CH_3 signals of OSA side chains, suggesting their aggregation into hydrophobic domains and resulting in a low resolution of this signal. Similar results were also obtained here with our OSA starch when D_2O was used instead of d_6-DMSO. Spectra with low resolution were obtained even when the analysis was performed in D_2O at 70 °C, suggesting that hydrophobic interactions between OSA side chains are still present in water at 70 °C. Indeed, increasing the temperature is a common way to weaken such physical interactions.

On the basis of these observations, d_6-DMSO appears to be the most suitable solvent for the NMR characterization of OSA-modified starches, even though these modified starches appear to be quite water-soluble. However, the solubilities of starch and

OSA in DMSO are different, and care must be taken when such amphiphilic macromolecular compounds are characterized by NMR. To evaluate the impact of the temperature on the solubility of OSA-modified corn starch, various ^{1}H NMR spectra of OSA-modified starch in d_6-DMSO/LiBr in the presence of a small amount of d_1-TFA were recorded on a sample allowed to remain in the spectrometer at different temperatures, and acquisitions were started after a complete dissolution of the product had been ensured (Figure 1). It was found that the equilibration time ranged between 20 and 50 min depending on the temperature. The integrals under the starch signals (3.00−5.27 ppm) and the CH_3 signal of OSA (0.89 ppm) are directly proportional to the amount of dissolved product and were compared to the constant residual DMSO signal at 2.549 ppm.[29] Figure 1 shows that the area under the signal at 0.89 ppm is also essentially temperature-independent over the range examined, suggesting a complete dissolution of OSA side chains even at low temperature. Conversely, the starch backbone is not completely dissolved below 60 °C, the temperature at which a plateau is observed. Total dissolution of starch in DMSO requires disrupting the hydrogen bonding between the longer chains such as those in amylose, which can be achieved only at higher temperatures and is facilitated by the presence of inorganic salts such as LiBr.[17] This shows that NMR experiments performed in d_6-DMSO below ~60 °C are expected to underestimate the concentration of starch in the sample and consequently overestimate the DS.

In conclusion, we have developed a new procedure utilizing the fast exchange between the labile protons of hydroxyl groups and the labile deuterons of d_1-TFA for the ^{1}H NMR characterization of starch and OSA-modified starches in d_6-DMSO. Molecular size and weight distributions obtained with multiple-detector SEC shows that the accuracy of the method is unaffected by TFA-induced degradation of the substrate. The technique has been demonstrated to be suitable for the obtention of ^{1}H NMR spectra in which all of the resonances of the starch protons are well resolved. It provides an effective tool for determining both the DB in unmodified and modified starches and the DS in modified ones, with better accuracy than current techniques. Experimental parameters such as temperature and solvent nature must be taken into account for optimal conditions.

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