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Determination of the degree of substitution of acetylated starch by hydrolysis, ^{1}H NMR and TGA/IR

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Abstract

The suitability of TGA/IR method for the analysis of the degree of substitution (DS) of acetylated starch (SA) was assessed. Ten standard samples were analysed for their DS by hydrolysis method and, those of the standards which could be properly dissolved, also by ^{1}H NMR. NMR analysis shows as well that the standards had other structural differences than DS. By TGA using derivatives of thermograms (DTG) it was possible to predict the DS with percentual coefficient of determination of 98. Somewhat more accurate results ($R2 = 98.7$) were received when the evolving acetic acid was analysed by IR. The result is very good because it is at the same precision level as the results of the hydrolysis and ^{1}H NMR methods.

Keywords: Acetylated starch; Degree of substitution; TGA/IR; NMR

1. Introduction

The acetylation of starch is a chemical modification, known for more than a century. In the modified starch, part of the hydroxyl groups of the α-D-glucopyranose units have been converted by esterification to acetyl groups. Highly acetylated starch with a degree of substitution (DS) of 2–3 were of research interest from 1950 through 1980 for their solubility into acetone and chloroform and for their thermo plasticity. Starch of high DS has not been produced commercially as a plastic because of a preference for cellulose acetate. The main concern has centred to the strength and cost of starch acetate. Starches having a DS of 0.01–0.2 are of commercial interest because of their usage based on properties with respect to film forming, binding, adhesion, thickening, stabilizing and texturing (Boutboul, Giampaoli, Feigenbaum, & Ducruet, 2002; Jarowenko, 1986). Acetylated starch (SA) with low DS is commonly obtained by esterification of native starch with acetic anhydride in aqueous medium in the presence of alkaline catalyst. Sodium hydroxide (NaOH) and other alkali metal and alkaline-earth metal hydroxides have also been used as catalyst. (Wang & Wang, 2002).

As a biodegradable material acetylated starch also has many potential uses in pharmaceutical applications (Paronen, Peltonen, Urtti, & Nakari, 1997). In all the applications, it is important to know the DS of acetylated starch. Accurate determinations of the degrees of substitution of starch acetates are necessary if meaningful structure–property relationships are to be established for this class of polymers. For instance, the solubility of substituted starch depends on the extent of substitution. The introduction of acetyl groups interrupts the ordered structure of native starch and interferes with the re-association of amylose and amylopectin in gelatinized starch, leading to decreased gelatinization temperature, increased swelling and solubility, and improved storage stability.

There are several methods to determine the DS. The most common one, the hydrolysis method, is based on the hydrolysis of the ester bonds in sodium hydroxide solution, and back titration to determine the amount of acid formed

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(ISI 14-2e, 1999). The method is quite reliable but demands usually a relatively large amount of sample (approximately 1.0 g). However, when for instance the biodegradation of SA is examined, there is a need for an easy analysis of DS from samples of only a few milligrams. Another method that has been proposed for DS analysis is FTIR (Ogawa et al., 1999). Unfortunately, it has not been proven to be practical so far. ^{1}H NMR is in principle a versatile method for DS analysis and gives at the same time structural information that cannot be gained by hydrolysis. (Laignel, Bliard, Massiot, & Nuzillard, 1996, Heins, Kulicke, Kauper, & Thielking, 1998). The problem with NMR is, however, that at low degrees of substitution there is no practical solvent for SA. The solid state NMR is out of question for the analysis for purposes which demand milligram level sample size and, furthermore, is not commonly available.

The aim of this study was to examine the suitability of Thermal Gravimetric Analysis/Infrared Spectroscopy (TGA/IR) for the analysis of the DS of acetylated starch. The TGA/IR analysis of the DS of starch was based on set of standard samples. A set of SA standards was available the DS of which was analyzed by the hydrolysis method. To get an idea about the precision of the by hydrolysis determined DS of the standards, ^{1}H NMR analysis was also performed. That was possible for the samples, which could be properly dissolved. In addition, the results of ^{1}H NMR analysis demonstrate some structural differences (other than DS) between the standards. The calibration was made, at first, based on the DTG-curves only and, secondly, based on the evolution curves of acetic acid, one of the major degradation products of acetylated starch. Both the by hydrolysis method and the by NMR determined DS-values were used in the calibration models.

2. Experimental

2.1. Materials

The potato starch used in acetylating was provided by Periva Oy (Kokemäki, Finland). Amylose was supplied by Sigma Aldrich (Amylose content 90%, Type II, potato).

Molecular weight of potato starch was reduced by enzymatic hydrolysis in aqueous solution at elevated temperature (60–95 °C). Various amounts of α-amylase (e.g. 50–200 g Ecostone A/1000 kg starch) was used in order to adjust the molecular weight to the desired level. After hydrolysis reaction (30–45 min) the enzyme was inactivated (95 °C) and the reaction mixture was dried and analysed. Molecular weight was determined with size exclusion chromatography. Three μHydrogel colons and one corresponding pre-colon was supplied by Waters Co. Aqueous sodium hydroxide (50 mM) was used as an eluent and a set of pullulan samples from Shodex were used as molecular weight standards.

Starch was acetylated using the method of Mehltretter and Mark (1974). Very highly acetylated starch can be made by reacting starch with excess of acetic anhydride using sodium hydroxide as the catalyst. The preferred catalyst, sodium hydroxide, is used in 50% aqueous solution at only 11% of the dry weight of the starch. The preferred temperature of reaction is about 125 °C with a time of acetylating of about 5 h to achieve maximum esterification. Reaction time varied from 10 min to 5 h with varying DS, e.g. DS 0.5, 10 min; DS 1, 2 h; DS 1.7, 2.5 h and DS 2.8, 5 h. The starch acetate product is recovered by precipitation with water in which it is completely insoluble. Starch acetate was washed with excess of water until the pH of filtrate was more than five. (Mehltretter & Mark, 1974).

A set of SA samples used in this work is presented in Table 1. The samples were synthesized by VTT Processes, Rajamäki, Finland.

2.2. Degree of substitution by the hydrolysis method

Since the repeating unit α-D-glucopyranose of starch has three hydroxyl groups, the maximum possible DS is 3.0. However, it should be noted that DS can slightly exceed 3.0 if the end units have four acetyl groups. Determination of DS by titration involved complete basic hydrolysis of the ester

Table 1
Standard samples

Standard	DS[a]	Sample description	Obs.
ST 0	0	Hydrolysed potato starch	Mw of used starch: 538 000 Bohlin viscosity: 66 mPa s
ST 1	3	Acetylated hydrolysed potato starch	Mw of used starch: 859 000 Bohlin viscosity: 160 mPa s
ST 2	2.8	Acetylated potato starch	
ST 3	2.8	Acetylated hydrolysed potato starch	Mw of used starch: 22 000 Bohlin viscosity: 2.3 mPa s
ST 4	2.6	Acetylated potato starch	
ST 5	1.7	Acetylated potato starch	Catalyst: Na-acetate
ST 6	1.7	Acetylated potato starch	Catalyst: NaOH
ST 7	1	Acetylated potato starch	
ST 8	0.5	Acetylated potato starch	
ST 9	3	Acetylated potato amylose	

[a] Determined by the hydrolysis method.

Table 2
Samples for the NMR studies

Standard	DS/hydrolysis	Concentration (mg/ml)	Solvent
ST1	3	50	DMSO-d6
ST2	2.8	25	DMSO-d6:CDCl3 (4:1)
ST3	2.8	50	DMSO-d6
ST4	2.6	50	DMSO-d6:CDCl3:D2O (8:1:1)
ST6	1.7	25	DMSO-d6:CDCl3 (7:5)
ST9	3.0	29	DMSO-d6:CDCl3 (5:2)

linkages and titration of the excess alkali. (Rudolph & Glowaky, 1978). DS was determined using the method of Wurzburg (1964). 1 g of grounded sample accurately weighted was added to the aqueous solution of ethanol (75%). Slurry was kept in the water bath (50 °C) for 30 min. After the slurry was cooled down an exact amount of aqueous solution of potassium hydroxide (0.5N, 30 ml) was added and solution was stirred for 72 h at room temperature. After indicator (phenolaphtalen) was added the excess of alkali was titrated with 0.5N hydrochloride acid. Reference sample and duplicate sample were treated similar way.

Acetyl content (%A) was calculated according to following Eq. (1)

$$\%A = [(V_0 - V_n) \times N \times 0,043 \times 100]/M \quad (1)$$

where:

V_0 = ml of 0.5N HCl used to titrate blank
V_n = ml of 0.5N HCl used to titrate sample
N = normality of used HCl
M = sample amount as dry substance, g
43 = weight of acetyl group

Acetyl content (%A) was used to calculate the degree of substitution, DS, according to following Eq. (2)

$$DS = 162 \times \%A/[4300 - (42 \times \%A)] \quad (2)$$

Reported DS value was the mean value of these replicate samples. The deviation from the mean was less than 0.1 DS-units.

2.3. NMR

Samples for the NMR-spectroscopy were prepared according to Table 2. Dissolving was done stirring the solvent and acetylated starch 24 h at 315 K. After the dissolving solutions were filtered and transferred to 5 mm NMR tubes. All the 1D ^{1}H NMR measurements were performed with Bruker AVANCE DRX 500 spectrometer operating at 500.13 MHz. 1D ^{1}H NMR spectra were measured at 329 K with 64 scans, a spectral width of 5252 Hz, a relaxation delay of 5 s between scans and an acquisition time of 6.24 s. 2D TOCSY spectra for selected samples were measured at 329 K with 1024 × 256 data matrix, 16 scans per increment, a spectral width of 2790 Hz in both dimensions, a relaxation delay of 2 s and a mixing time of 160 ms. All of the spectra were processed by using a cosine bell filtering function in both dimensions. The size of 2D FT matrix was 1024 × 1024.

2.3.1. Analysis of the 1D spectra

Assignments for the signals were taken from publication of Laignel, Bliard, Massiot, and Nuzillard (1996). Determination of the DS was based on total-line-shape (TLS) fitting of the spectra (Fig. 1) (Laatikainen, Niemitz, Malaisse, & Willem, 1996) using the PERCH software (PERCH, 2002). The TLS fitting method is not sensitive for base-line artefacts and allows also quantification of overlapping signals. The quantification of the DS was based on the calculation of areas of the methyl proton signals of the acetyl groups and those of the polymers' α-D-glucopyranose units (except protons of hydroxyl groups the signals of which were not measured by this method). Because each α-D-glucopyranose unit has seven protons (excluding the OH-protons) and each acetyl group three

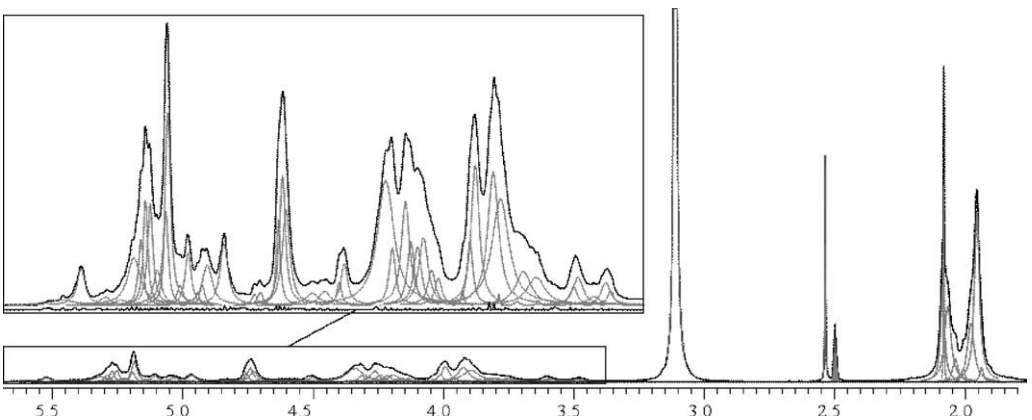

Fig. 1. TLS-fitting of partially acetylated starch. DS of 2.79 ± 0.04 was determined by NMR. Black line is the observed 500 MHz ^{1}H NMR spectrum of ST2 and the grey lines represent the fitted lines. DS can be determined comparing the backbone signal areas (3.3–5.8 ppm) to methyl proton signals' area (1.8–2.2 ppm). Below the spectrum in the insert also the difference between the observed and fitted spectrum is shown.

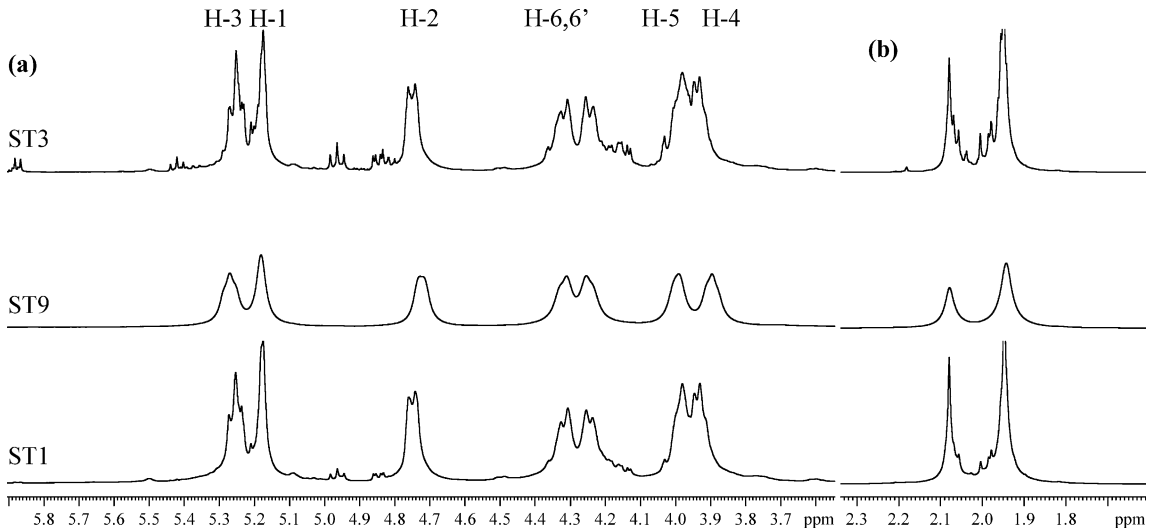

Fig. 2. 500 MHz ^{1}H-spectrum of the (a) the starch acetate backbone protons (as multiplied by factor 6 as compared to (b)) and (b) methyl protons. At the top ST3, hydrolysed acetylated potato starch, at the middle ST9, acetylated potato amylose and at the bottom ST1, hydrolysed acetylated potato starch. The backbone proton signal assignments are shown at the top of the spectra.

protons, the DS can be calculated by the equation:

$$DS = 7A_{ace}/3A_{aqu}, \quad (3)$$

where A_{ace} is the area of the methyl signals and A_{aqu} is the area of the proton signals of the α-D-glucopyranose unit. The DS analyses were performed as triplicates.

2.4. TGA/IR

The thermogravimetric analyser used in this study was a Seiko Instruments EXSTAR 6200 TGA/DTA system The thermogravimetric analyser was interfaced to a Nicolet SXCE FTIR-spectrometer (Nicolet Nexus Series TGA/IR interface).

The samples sizes ranged from 8 to 11 mg (open Al crucibles) and the heating rate was 5 °C/min. (50–400 °C) and 10 °C/min (400–600 °C). The purge gas was N_2 (60 ml/min.). Gases evolved from the heated sample were transferred to the IR gas cell via a 1/16 in. diameter SUS tubing (1.5 m) supplied by Seiko. Both the transfer line and gas cell was maintained at 225 °C in order to eliminate condensation. The spectra were obtained at 4 cm^{-1} resolution with a time resolution of 14 s.

3. Results and discussion

3.1. NMR

3.1.1. NMR Spectroscopy-molecular properties

Since the solubility of the starch acetates is poor, we tested different solvents. The best solubility was achieved

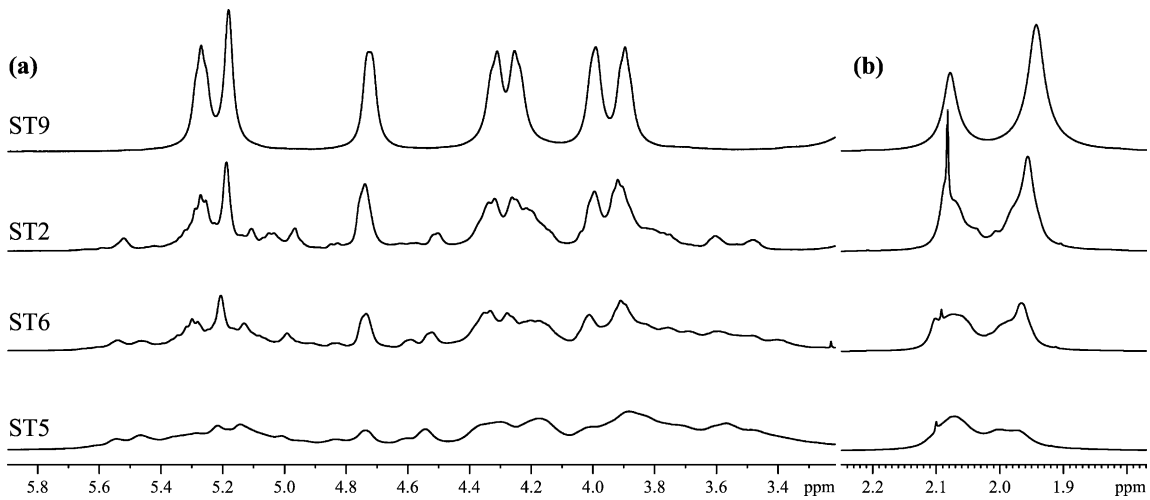

Fig. 3. 500 MHz ^{1}H-spectrum of (a) the starch acetate backbone protons (as multiplied by factor 6 as compared to (b)) and (b) methyl protons. From the top: ST9 DS3, acetylated potato amylose, ST2 DS2.8 acetylated potato starch, ST6 DS1.9, acetylated potato starch and ST5 DS1.7, acetylated potato starch.

Table 3
Degrees of substitution by NMR

Standard	DS/ by hydrolysis	DS/NMR
ST 1	3	3.03 ± 0.03
ST 2	2.8	2.79 ± 0.04
ST 3	2.8	3.08 ± 0.04
ST 4	2.6	2.85 ± 0.02
ST 6	1.7	1.9 ± 0.2
ST 9	3	3.02 ± 0.04

with DMSO-chloroform mixture (1:1). When using the mixture, the solution becomes also less hygroscopic and less viscous than using pure DMSO.

The lines of the polymer ^{1}H NMR spectra are broad, due to overlap of signals from slightly different chemical environments and also due to the viscosity of the samples. In most cases, the starch backbone signals at 3.9–5.5 ppm could be assigned (Fig. 2a). The most intense signals arise from the protons of trisubstituted backbone (2,3,6-tri-*O*-acetyl-(1 → 4)-α-D-glucopyranose). At the spectrum there are also a few well-resolved minor signals, for example at 4.85 ppm (dd) and 4.96 ppm (t). According to Laignel et al. (1996) these signals belong to terminal glucopyranose units. However, when the DS is low, backbone signals get broader and the assignment is more difficult (Fig. 3a). The methyl protons of acetate are found at 1.9–2.1 ppm (Figs. 2b and 3b).

The degrees of substitution by NMR are presented in Table 3. The results indicate that the accuracy of the ^{1}H NMR methods drops below DS of 2.0. The DS values exceeding 3.0 arise from end units having four acetyl groups.

In 500 MHz 2D TOCSY spectrum more detailed structural properties can be seen (Fig. 4). Besides of starch backbone glucopyranose unit (2,3,6-tri-*O*-acetyl-(1 → 4)-α-D-glucopyranose) signals terminal glucopyranose unit (2,3,4,6-tetra-*O*-acetyl-(1 → 4)-α-D-glucopyranose) signals can be found easily at 4.85 ppm (dd), 4.96 ppm (t), 4.14 ppm (td), 4.00 ppm (t). These signals are not as well resolved in 1D ^{1}H NMR. In addition, there are also two other spin-systems in the TOCSY spectrum. These arise obviously from branching or partially substituted glucopyranose units. The ^{1}H NMR spectra allow thus to estimate how much of the starch is branched. For example when

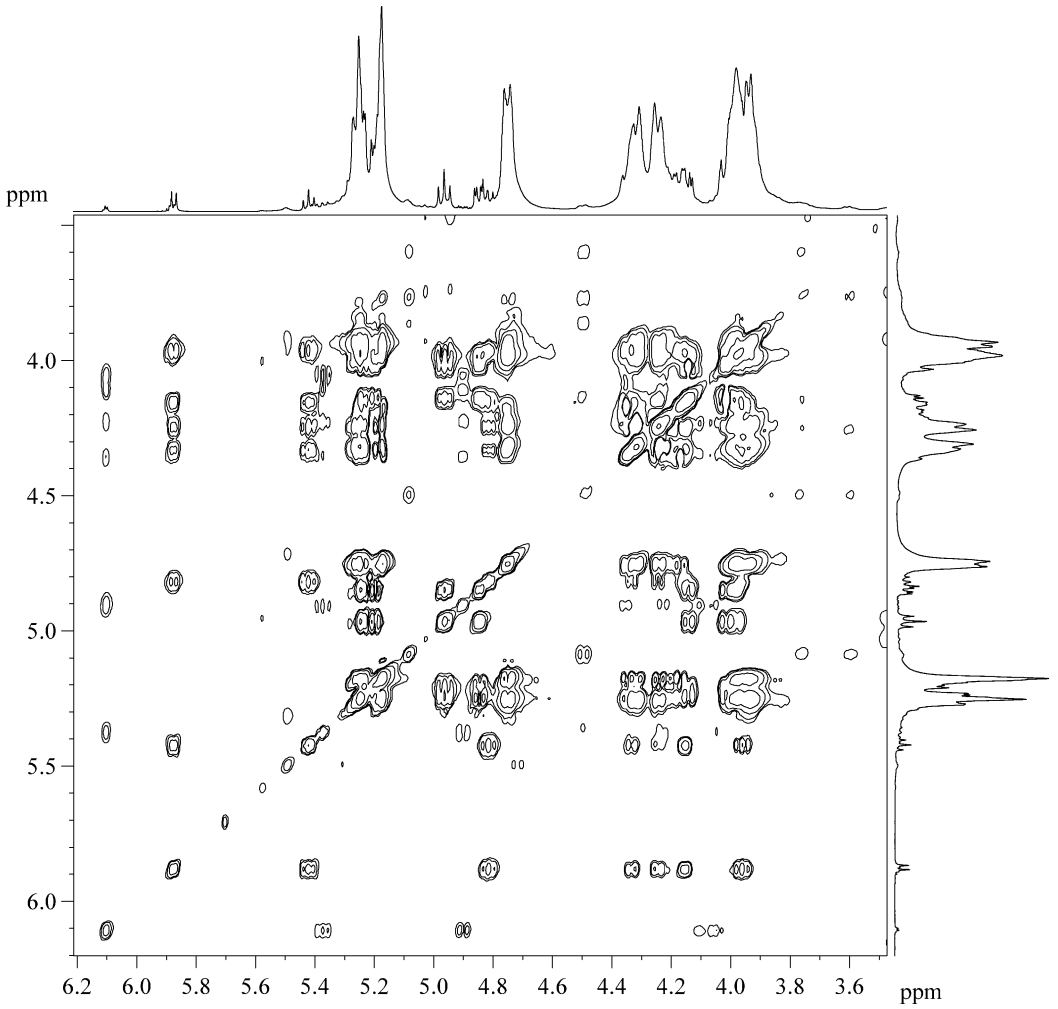

Fig. 4. 500 MHz 2D TOCSY of acetylated starch, DS/hydrolysis: 2.8.

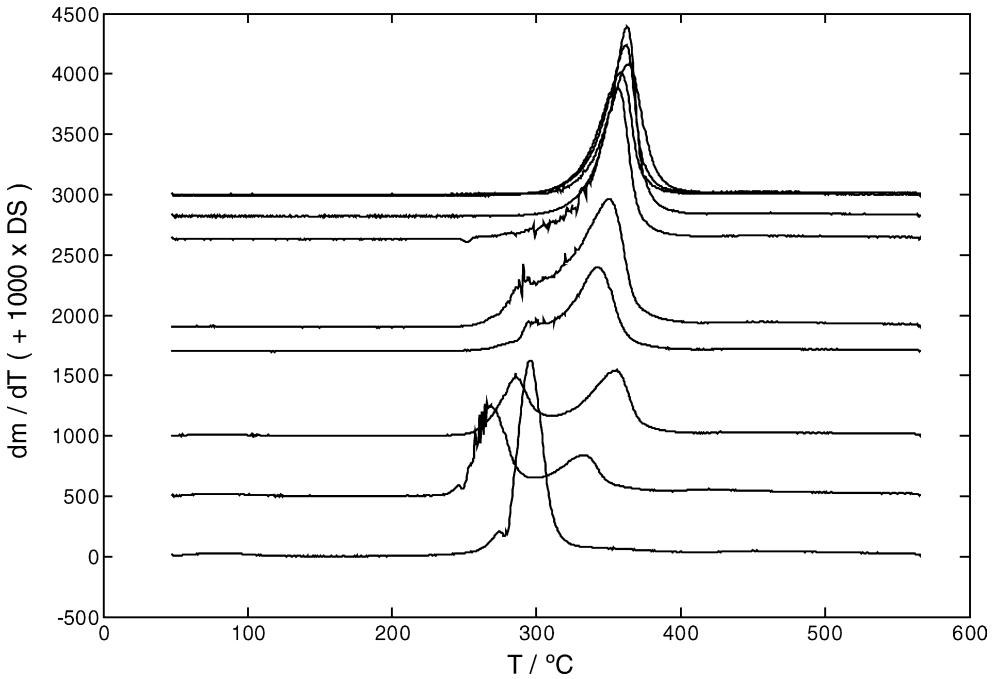

Fig. 5. DTG curves of the standards. Baselines of the curves have been moved up by DS × 1000 × dm/dT.

comparing the TOCSY of ST3 (Fig. 4) to the TOCSY of ST9 (not shown), there are only main backbone and terminal unit correlations in the TOCSY of ST9.

3.2. TGA/IR

Derivates of TGA signals of the standards are presented in Fig. 5. Note that the vertical position of the baseline of each curve indicates the DS (× 1000) of the respective sample. Thermal stability of per-acetylated starch is much better than that of the original starch. The decomposition temperature of the upmost curves is over 300 °C and the decomposition of starch, the undermost curve, starts at approximately 250 °C in these experimental conditions. Thermal stability of per-acetylated starch is much better than that of the original starch. However, partially substituted samples decompose under heating in two stages, the first of which occurs at lower temperature as compared to starch (Fig. 5).

DTG curves of the polymers were used in a PLS-model (independent or *x*-variables) to predict the DS (dependent or *y*-variables). As *y*-variables both the values by hydrolysis (Table 1) and the values available by NMR (Table 3) were used. The PLS-models were calculated using Data analysis toolbox published by Haario and Taavitsainen (1996) for MATLAB®. The best dimension (the number of latent variables used) for the PLS-model was determined by PLSTEST and was found to be five. PLS models had percentual coefficients of determination of 98.1 and 97.9 without and with the NMR-results, respectively.

In the FTIR-analysis of evolving gases the relative amount of acetic acid was measured. Curves of evolving acetic acid are shown in Fig. 6. Using the evolving curves as independent variables similarly as with the DTG-curves,

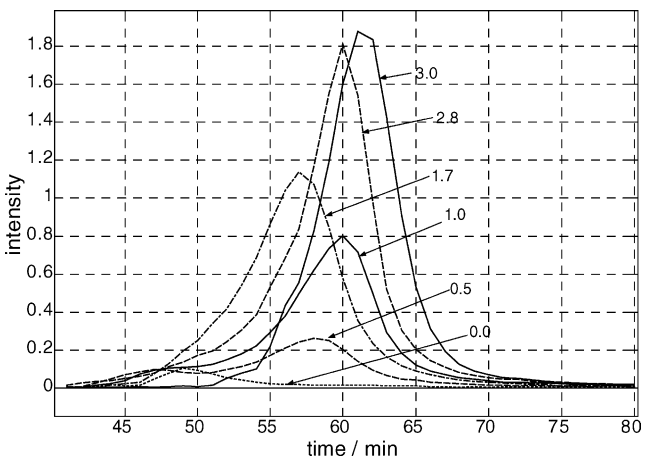

Fig. 6. Evolving curves of acetic acid. Peak intensity integrated over 1120–1251 cm^{-1}. DS values marked on the curves.

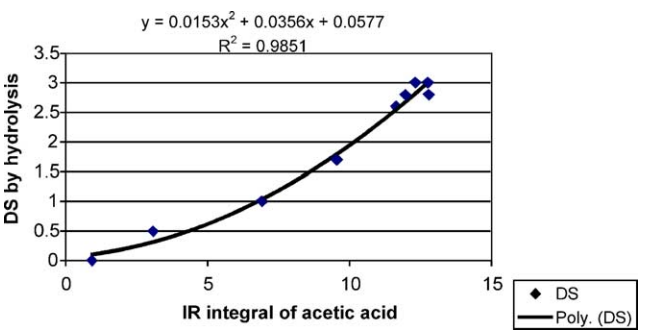

Fig. 7. DS by hydrolysis versus IR integral of acetic acid. The solid line presents polynomial fit of second degree.

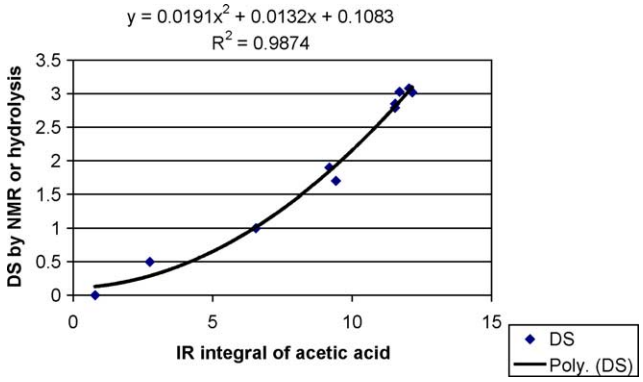

Fig. 8. DS by NMR (DS > 1.5) and by hydrolysis (DS < 1.5) versus IR integral of acetic acid. The solid line presents polynomial fit of the second degree.

PLS-models had degrees of determination of 98.57 and 98.74 without and with the NMR-results respectively. Essentially the same result could be gained by a simple regression model in which the integrated areas of acetic acid evolving curves (examples of which are shown in Fig. 6.) were used as the independent variables (Figs. 7 and 8).

4. Conclusions

The titration method and ^{1}H NMR are absolute methods for the determination DS of acetylated starch They gave comparable results to degree of determination of 0.98 (if one of them is considered to be correct and the other as an estimate). The ^{1}H NMR method works very well only for DS of larger than ca. 2 but, on the other hand, gives also invaluable information about the structure of the starch. Against this comparison TGA/IR provides a versatile tool for measurement of the DS of acetylated starch in whole the DS range with a same level of precision as the other methods. Small sample size and no need of preparation together with no need of dissolubility of the samples are important benefits of this method. The calibration model based on the evolving acetic acid is concluded to be good enough for practical use. The method has potential for analysis of other substituted polysaccharides as well but needs another method to produce appropriate standards.

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