A quantitative $^1$H-NMR method for the determination of the Molar Substitution (MS) of acetylated and hydroxypropylated starches was developed and tested for MS ranging from 0.09 to 0.5. Results were checked using the Johnson method and a titration method for hydroxypropylated and acetylated starch, respectively. Hydroxypropylated starch was produced using both a static mixer reactor (SMX type) and a co-rotating twin screw extruder. Acetylated starch was produced using a counter-rotating twin screw extruder. Quantitative analysis results of the $^1$H-NMR method were in good agreement with traditional analysis methods for all samples tested. Main advantage of the $^1$H-NMR method is the considerable time saving as compared to the traditional analysis methods.

1 Introduction

Nowadays, large quantities of chemically modified starches are used in industrial processes. Two important product groups are the starch ethers and the starch esters. In this study hydroxypropylated and acetylated starches were used.

Quantitative Analysis of Chemically Modified Starches by $^1$H-NMR Spectroscopy

R.A. de Graaf, G. Lammers, L.P.B.M. Janssen, and A.A.C.M. Beenackers, Groningen (The Netherlands)
nylacetate (Lammers et al.) [3]. This way, hydroxypropylated and acetylated starches are produced, respectively (Figure 1). The average number of substituents per D-glucose unit is given by the Molar Substitutions (MS). Thus, an MS of 1.0 means that on average one substituent is present per D-glucose unit (Figure 1). Since three reactive hydroxyl groups are present in a D-glucose unit, the maximum MS is 3.0. In case of hydroxypropyl starch the MS can be higher because the hydroxypropyl group can react with another propylene oxide, thus forming poly hydroxypropyl groups.

Various processes for the production of starch derivatives exist (Ruitenberg and Solarek) [4], each with their own advantages. The samples analysed here resulted from processing of concentrated starch solutions in extruders and static mixer reactors (Lammers et al. [5] and de Graaf et al. [6]). As part of a larger research program to determine the feasibility of different reactor types for starch derivatization, a direct method for the analysis of modified starches was developed.

2 Experimental

2.1 Materials and Methods.

Hydroxypropyl starch was produced both in a static mixer reactor (Lammers et al. [5]) and in a co-rotating twin screw extruder (de Graaf et al. [6]). Acetylated starch was produced in a counter rotating closely intermeshing twin screw extruder. The static mixer reactor was fed with an aqueous slurry of native potato starch (AVEBE, food grade), the extruder was fed with native potato starch with a moisture content of 15% by weight. Vinylacetate and propylene oxide were a pro analysis grade from Merck, Germany. Sodium hydroxide solutions were used as a catalyst. Analysis of the starches were carried out using a 200MHz Varian Gemini NMR spectrometer.

2.2 Sample preparation

Techniques used for preparing the samples were partly taken from Marsman et al. [7]. Samples from the static mixer reactor

<table>
<thead>
<tr>
<th>Peak (ppm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3 - 4.2</td>
<td>Protons of the anhydroglucose units of starch</td>
</tr>
<tr>
<td>4.5 - 4.7</td>
<td>Water</td>
</tr>
<tr>
<td>5.1 - 5.5</td>
<td>Equatorial proton of the anhydroglucose unit of starch</td>
</tr>
</tbody>
</table>

Figure 1. Chemical structures of acetylated and hydroxypropylated starch.

Figure 2. 1H-NMR spectra of gelatinised starch.
Peak listing hydroxypropylated starch:

<table>
<thead>
<tr>
<th>Peak (ppm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 - 1.2</td>
<td>Doublet of hydroxypropyl group on starch</td>
</tr>
<tr>
<td>3.3 - 3.4</td>
<td>Methanol</td>
</tr>
<tr>
<td>3.3 - 4.2</td>
<td>Protons of the anhydroglucose units of starch</td>
</tr>
<tr>
<td>4.5 - 4.7</td>
<td>Water</td>
</tr>
<tr>
<td>5.3 - 5.5</td>
<td>Equatorial proton of the anhydroglucose unit of starch</td>
</tr>
</tbody>
</table>

Figure 3. $^1$H-NMR spectra of hydroxypropyl starch.

Peak listing acetylated starch:

<table>
<thead>
<tr>
<th>Peak (ppm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 - 2.1</td>
<td>Methyl protons of the acetyl group on starch</td>
</tr>
<tr>
<td>3.2</td>
<td>Methanol</td>
</tr>
<tr>
<td>3.3 - 4.2</td>
<td>Protons of the anhydroglucose units of starch</td>
</tr>
<tr>
<td>4.5 - 4.7</td>
<td>Water</td>
</tr>
<tr>
<td>5.1 - 5.5</td>
<td>Equatorial proton of the anhydroglucose unit of starch</td>
</tr>
</tbody>
</table>

Figure 4. $^1$H-NMR spectra of acetylated starch.
were quenched in cold water, neutralized with hydrochloric acid and then precipitated in cold \((-10^\circ C)\) acetone. The precipitated starch was washed thrice with cold acetone and then dried in a vacuum stove set at 80°C. Samples from the extruder were quenched in liquid nitrogen. The frozen sample (ca. 15g) was grinded in a mill (Janke & Kunkel IKA-A10; F.R.G.) resulting in a powder. The by-products and reactants were extracted with 100 cm³ methanol in which 10 cm³ titrisol buffer (pH 7) was dissolved. The resulting mixture was neutralised with hydrochloric acid. The precipitated starch was filtered over a Büchner funnel with a Schleicher & Schüll Filter N° 589 and washed with 50cm³ methanol and then vacuum dried (12h, 60°C).

2.3 Measuring the MS of acetylated starch by titration

Deacetylation of acetylated starch will go to completion in diluted aqueous sodium hydroxide solutions. This property can be used to measure the amount of acetyl groups in acetylated starch. About 0.7 g dry acetylated starch was weighted accurately and added to 25 cm³ demineralised water. The pH of the obtained solution was measured and 25 cm³ 0.1 N sodium hydroxide solution was added. After 12h the solution was titrated back with 0.1 N HCl down to its original pH prior to the NaOH addition. The MS followed from an iterative procedure:

\[
M_{AGU} = \frac{m_{\text{dried acetylated starch}}}{(M_{WAGU} + MS M_{WAcetyl})} \quad (1)
\]

\[
MS = \frac{M_{\text{Acetyl groups}}}{M_{AGU}}
\]

\[
= \frac{(m/M)_{\text{NaOH, added}} - (m/M)_{\text{HCl, added}}}{m_{\text{starch}}/M_{WAGU}}
\]

With \(M_{WAcetyl}\), the molecular mass of the acetylated group, \(M_{AGU}\), the amount of starch moles present in the dried acetylated starch, \((m/M)\) is the number of moles of a component, \(M_{WAGU}\) is the molecular mass of one anhydroglucose unit and \(m_{\text{starch}}\), the mass of anhydroglucose units in the final solution. Iterative solving of eqns. 1–2 stopped when \(MS_{\text{new}} - MS_{\text{old}} \leq 0.001\).

2.4 Measuring of the MS of hydroxypropyl starch by the Johnson method

The MS of hydroxypropylated starch was determined using the Johnson method [8]. The hydroxypropyl group of starch was hydrolysed to propylene glycol which in turn was dehydrated to propionaldehyde and the enolic form of allyl alcohol. The products were reacted with ninhydrin (1,2,3-triketo-
hydrindene monohydrate) forming a purple coloured complex. The amount of propylene glycol could be determined spectrophotometrically.

2.5 Measuring of the MS of acetylated or hydroxypropylated starch by ¹H-NMR

Depending on expected MS 0.01 to 0.05 g. of dry modified starch (acetylated as well as hydroxypropylated) was dissolved in 2 cm³ D₂O. Two analysing techniques were used to obtain the MS. The first technique uses acetic acid and t-butanol as an internal standard, for hydroxypropylated starch and acetylated starch, respectively. To these samples the appropriate internal standard was added until 10 mg internal standard/g D₂O was reached. The amount of internal standard was adjusted in such a way that the NMR peak ratio of the signals of the internal standard and the acetyl or hydroxypropyl group was equal to one. Alternatively, the equatorial proton of starch (Fig. 2) was used as an internal standard. This peak can be found at 5.4ppm and depends linearly on the amount of anhydroglucose units present in the sample. Then 0.01 g of dry modified starch was dissolved in 2 cm³ D₂O. Vigorous shaking resulted in a clear solution, which was transferred to an NMR-tube. Good spectra were obtained from 32 pulses with a delay of 5s between each pulse.

3 Results and Discussion

Figures 2, 3 and 4 show spectra for native gelatinised, acetylated and hydroxypropylated starch, respectively. For the chemically modified starches, the respective internal standards were added. Acetic acid and tert-butanol have characteristic proton signals at 2.0ppm and 1.2ppm, respectively. Hydroxypropyl starch and acetylated starch have characteristic proton signals at 1.2ppm (a doublet) and 2.1ppm, respectively. The surface area of the characteristic proton of the substituent group and of the internal standard was calculated by numerical integration. The moles of tested hydroxypropyl starch was determined from:

\[ M_{\text{HPS}} = \frac{m_{\text{starch}}}{(M_{\text{w,AGU}} + (M_{\text{assumed}} \cdot M_{\text{w, PO}}))} \]  

with \( m_{\text{starch}} \): amount of hydroxypropyl starch in test sample,
\( M_{\text{w,AGU}} \): molecular weight of one anhydroglucose unit,
\( M_{\text{assumed}} \): assumed MS,
\( M_{\text{w,PO}} \): molecular weight of the attached hydroxypropyl group,
\( M_{\text{HPS}} \): amount of moles of hydroxypropyl starch.

A new MS was calculated from:

\[ M_{\text{S,new}} = \frac{M_{\text{HAc}} \cdot I_{\text{PO, HPS}}}{M_{\text{HPS}} \cdot I_{\text{HAc}}} \]

with \( M_{\text{HAc}} \): amount of moles of acetic acid in the sample,
\( I_{\text{PO, HPS}} \): integrated signal of the NMR peak from the hydroxypropyl group (Figure 3),

- NMR, with equatorial anhydroglucose proton as internal standard, (Extruder) / \( r = 0.990 \)
- NMR, with t-butanol as internal standard, (Extruder) / \( r = 0.997 \)

![Figure 6. Parity plot of MS analysis of acetylated starch according to the ¹H-NMR and the titration method.](image-url)
Table 1. MS Values Obtained from H-NMR and from Johnson’s Method.
a) Samples produced by the static mixer. b) and c) Samples produced by the extrusion process.

<table>
<thead>
<tr>
<th>Static Mixer</th>
<th>Co rotating twin screw extruder</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydroxypropylated starch</strong></td>
<td><strong>Acetylated starch</strong></td>
</tr>
<tr>
<td><strong>a)</strong></td>
<td><strong>Co rotating twin screw extruder</strong></td>
</tr>
<tr>
<td><strong>MS</strong></td>
<td><strong>MS</strong></td>
</tr>
<tr>
<td>(NMR with acetic acid)</td>
<td>(NMR with equatorial AGU proton)</td>
</tr>
<tr>
<td>(as internal standard)</td>
<td>(as internal standard)</td>
</tr>
<tr>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>0.166</td>
<td>0.146</td>
</tr>
<tr>
<td>0.19</td>
<td>0.172</td>
</tr>
<tr>
<td>0.455</td>
<td>0.135</td>
</tr>
<tr>
<td>0.47</td>
<td>0.151</td>
</tr>
<tr>
<td>0.294</td>
<td>0.105</td>
</tr>
<tr>
<td>0.31</td>
<td>0.12</td>
</tr>
<tr>
<td>0.488</td>
<td>0.118</td>
</tr>
<tr>
<td>0.5</td>
<td>0.113</td>
</tr>
</tbody>
</table>

| **Hydroxypropylated starch** | **Acetylated starch** |
| **b)** | **Co rotating twin screw extruder** |
| **MS** | **MS** |
| (NMR with acetic acid) | (NMR with t-butanol) |
| (as internal standard) | (as internal standard) |
| (-) | (-) |
| 0.146 | 0.045 |
| 0.135 | 0.130 |
| 0.105 | 0.145 |
| 0.118 | 0.167 |
| 0.108 | 0.213 |
| 0.102 | 0.196 |

Comparing the MS (Fig. 5) of acetylated starch measured by proton NMR and by titration, shows a slight deviation. This difference can be attributed to the determination of the exact end point in the titration technique. Because starch acts as a weak polyacid [9, 10] it buffers the sample solution. Determining the exact end point becomes more difficult this way. The results of proton NMR using acetic acid or t-butanol as internal standard do not differ significantly from the results obtained from proton NMR using the equatorial proton of the starch anhydroglucose unit as an internal standard.

4 Conclusions

$^1$H-NMR spectroscopy can be used as an easy and accurate tool for analysis of the MS of acetylated and hydroxypropylated starch. For hydroxypropyl starch, MS results were in good agreement with results obtained from the Johnson method. This technique was tested for $0.04<MS<0.5$. For acetylated starch, the technique was tested for $0.03<MS<0.25$. Titration was used as a reference. The equatorial proton of the anhydroglucose unit of the starch can be used as an internal standard for the MS analysis of hydroxypropylated as well as acetylated starch. Results from this method are comparable to analyses with acetic acid or t-butanol added as an internal standard.

Nomenclature

- **MS**: Molecular Substitution (-)
- **M**: concentration (mole/m³)
- **I**: area under the NMR peak (-)
- **m**: mass (g)
- **Mw**: molecular mass (mole/g)

Acknowledgement

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Abbaubare Polymerwerkstoffe auf der Basis nachwachsender Rohstoffe — Möglichkeiten und Grenzen

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Biodegradable Polymer Systems Based on Renewable Raw Materials — Chances and Limitations. This contribution describes formulation and preparation concepts for the generation of biodegradable polymer systems under the inclusion of growing-again raw materials. The incorporation of native starch into aliphatic polyurethanes as well as the degradation behaviour of such filled systems will be discussed. In a further chapter, concepts for the generation of thermoplastic starch using a twin-screw kneader, will be presented. These destructurized polysaccharides can form the polymer matrix of flax fibre reinforced composites. The preparation of polymer blends, composed of thermoplastic starch and biodegradable synthetic thermoplastics leads to competitive two-phase polymers including a remarkable potential of development. As a result of close meshed structure/property interrelationships, the morphology formation during the compound process is of utmost importance. Dimensionless characteristics will be presented that can be used to estimate and to assess the resulting phase structures.

1 Einführung


Im folgenden soll anhand neuer Werkstoffentwicklungen aufgezeigt werden, wie realistisch derartige Vorstellungen sind [1].

2 Ausgangssituation

1990 wurden in der Bundesrepublik Deutschland (alte Bundesländer) 10,2 Mio Mpz polymerwerkstoffe produziert (Abbil-