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TEMPO-mediated selective oxidation of substituted polysaccharides—an efficient approach for the determination of the degree of substitution at C-6

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ABSTRACT

2,2,6,6-Tetramethyl-1-piperidinyloxy radical (TEMPO)-mediated oxidations of substituted polysaccharides were studied at pH 10.2 and at a temperature of 0 °C with NaOCl as the oxidant. The reaction is highly selective, and it was shown that the oxidation can proceed to a yield of nearly 100%. The oxidation process was investigated for several substituted polysaccharides, especially for a series of hydroxypropyl guar gums with different molar degrees of substitution. It was shown that this oxidation can be used for the determination of the degree of substitution at C-6 of the polysaccharide by comparing the difference in oxidation yield between substituted and natural polysaccharides. Studies on several hydroxypropyl guar gums showed that the degrees of substitution at C-6—for MS of 0.08, 0.34, 0.62, and 1.08—are 0.06, 0.24, 0.40, and 0.44, respectively. The results were extended to other polysaccharides such as carboxymethyl cellulose, cationic guar gum, carboxymethyl pullulan, and methyl cellulose. It can be concluded that the TEMPO-mediated oxidation is a useful method for the determination of the DS at the substituted C-6 position for different kinds of modified polysaccharides.

The chemical modification of polysaccharides by introducing substituents on polysaccharidic chains can often improve and change their physical and chemical properties.[1,2] The degree of substitution (DS) is closely related to the polysaccharide derivative properties, and many methods have been established for the determination of DS using NMR and IR spectroscopies, potentiometric titration, etc.[3] For example, the degree of substitution was determined by potentiometric back-titration for the carboxymethylated cashew tree gum[4] and by complete degradation of the polymer followed by monomeric analysis.[5] NMR spectroscopy gives often a simple way for the determination of the molar degree of substitution (MS) of hydroxypropyl galactomannan and the degree of the substitution (DS) of guar gum methyl ethers.[6] Recently, capillary electrophoresis with UV-detection (CE/UV) was applied to the analysis of carboxymethyl starches (CMS).[7,8] The monomer composition of CMS can be determined by CE/UV after hydrolysis and reductive amination with aminobenzonitrile, which gives complete molar composition of monomers as well as the total DS and the amount of unsubstituted, mono-, di-, and trisubstituted glucose units. NMR spectroscopy is one of the most efficient techniques to characterize the structure of polymers, but its application to polysaccharides meets with some limitation because of the high viscosity of aqueous polysaccharide solutions and the heterogeneity of their structures. For carboxymethyl cellulose (CMC), this problem was solved by degrading the polymer to very short oligomers; ^{1}H and ^{13}C NMR techniques[9–13] were applied to CMC providing detailed structural information, including the distribution of the carboxymethyl substituent at OH-2, OH-3, and OH-6 of the glucose unit. However, in the case of hydroxypropyl-substituted polysaccharides, the NMR method cannot give any information on the degree of substitution of individual hydroxyls of the glucose units due to the low resolution and difficulties of NMR spectral resonances assignment.

In the early 1990, DeNooy, Besemer, and Van Bekkum[14] first applied the TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical)-mediated selective oxidation to water-soluble polysaccharides. Since then, the TEMPO-NaBr–NaClO system is being applied to the oxidation of many polysaccharides (starch, pullulan, galactomannan, hyaluronan, maltodextrins, etc.).[15–17] From these data, it was shown unambiguously that this mild and selective method oxidizes only the primary hydroxyl group at C-6 with high selectivity while leaving secondary hydroxyl groups unaffected. This gives an interesting way to modify selectively the polysaccharides.

Until now, reports on TEMPO-mediated oxidation are mainly targeted on the modification of polysaccharides and the mechanism of the oxidation reaction. During our study on the chemical modification of polysaccharides and TEMPO-mediated selective oxidation, we found that this selective oxidation can be used to

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determine the degree of substitution at C-6 (DS_6) of the substituted polysaccharides.[14] In fact, the rationale is simple: when the primary hydroxyl group at C-6 of the polysaccharide is substituted by another group (such as carboxymethyl, hydroxypropyl, and methyl ether), it is no longer able to react in the TEMPO-mediated oxidation. The difference between the degree of oxidation of substituted and non-substituted polysaccharides gives information on the DS_6. The aim of this work is, by using TEMPO-mediated oxidation of polysaccharides, to establish an efficient method for the determination of DS_6 for substituted polysaccharides.

A series of hydroxypropyl guar gums with different molar degrees of substitution were studied. Figure 1 shows typical kinetic plots of TEMPO-mediated oxidation as monitored by the consumption of NaOH. The oxidation reaction is relatively quick at first, then the reaction rate decreases asymptotically corresponding to the decrease of primary hydroxyl groups remaining free at the C-6 position. The same kinetic behavior was observed in all of the oxidation experiments in this work. As the end point of the reaction is very important for the quantitative determination, we have set it artificially as follows: when the pH of the reaction mixture remains constant for about 15 min, the reaction was considered to have reached its end point and was stopped. Each oxidation experiment was performed two or three times and was reproducible, that is, the standard deviation for the final consumption of NaOH did not exceed 3%.

IR spectra of oxidized guar gum and hydroxypropyl guar gum were recorded to evaluate possible structural changes after oxidation. The IR spectra of samples in acidic form (Fig. 2a) revealed a carboxylic acid band at 1735 cm^{-1}, showing that there is evident transformation from hydroxyl to carboxylic acid group upon oxidation of guar gum and its derivatives. In the IR spectra of neutralized samples (Fig. 2b), the –COONa group gives rise to a band that is shifted to a longer wavelength (1550–1650 cm^{-1}) region. No absorption band was observed in the 1730 cm^{-1} region for the oxidized guar samples, showing that no carbonyl group is present; this is a good evidence that the secondary hydroxyl groups are not oxidized to carbonyl groups by the TEMPO-mediated oxidation.[15]

The ^{13}C NMR spectrum of the oxidized hydroxypropyl guar gum is shown in Figure 3. There is no resonance in the 195–205 ppm region in the spectra, indicating that no ketone group is present after the oxidation. Similar ^{13}C NMR signals of guar gum have been as-

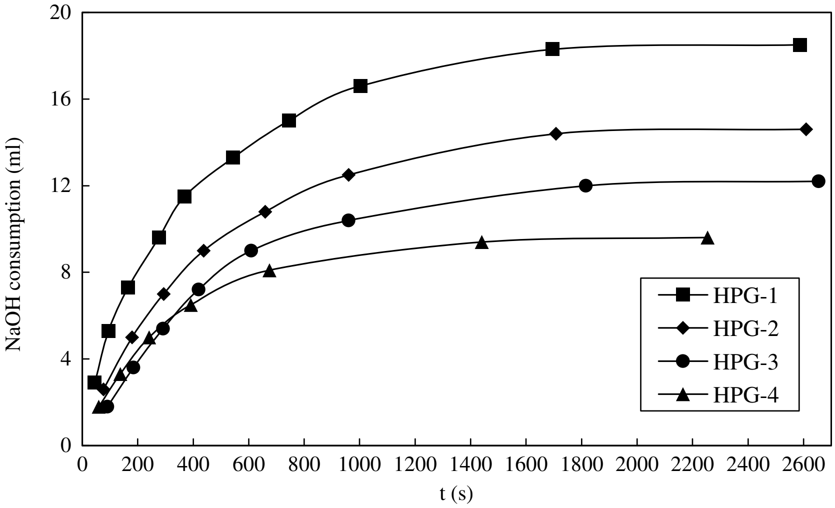

Figure 1. The kinetic course of TEMPO-mediated oxidation of hydroxypropyl guar gums with MS varying from 0.08 (■) to 1.08 (▲).

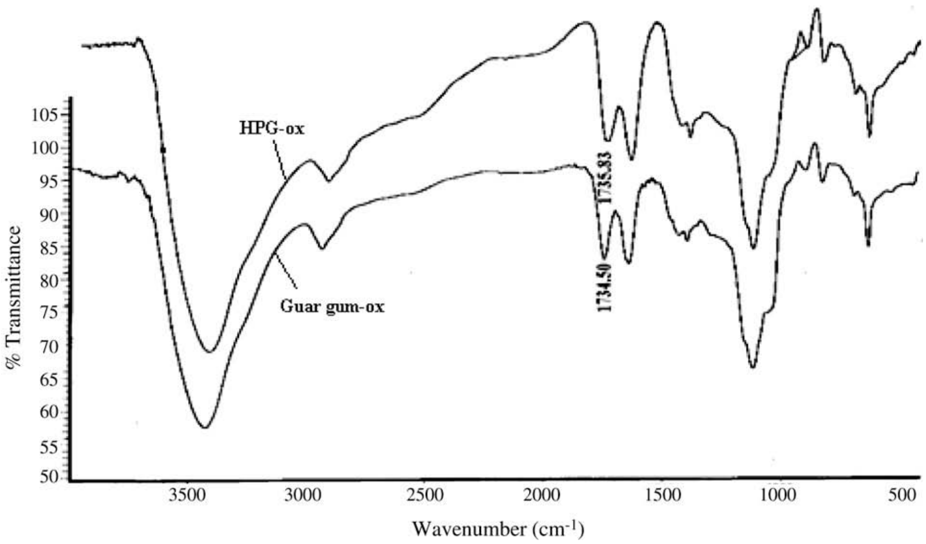

Figure 2a. IR spectra of oxidized guar gum and hydroxypropyl guar gum (HPG-3, MS = 0.62) in the acidic form.

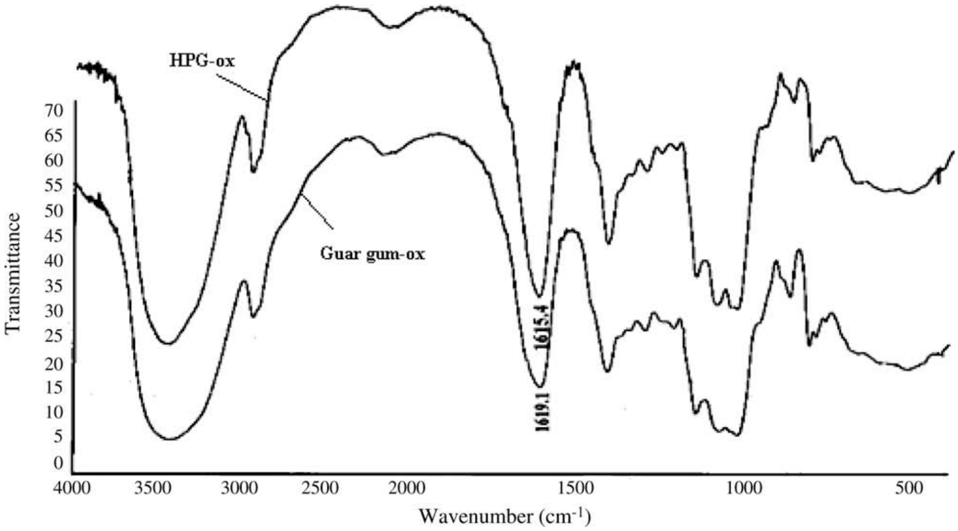

Figure 2b. IR spectra of oxidized guar gum and hydroxypropyl guar gum (HPG-3, MS = 0.62) as sodium salt.

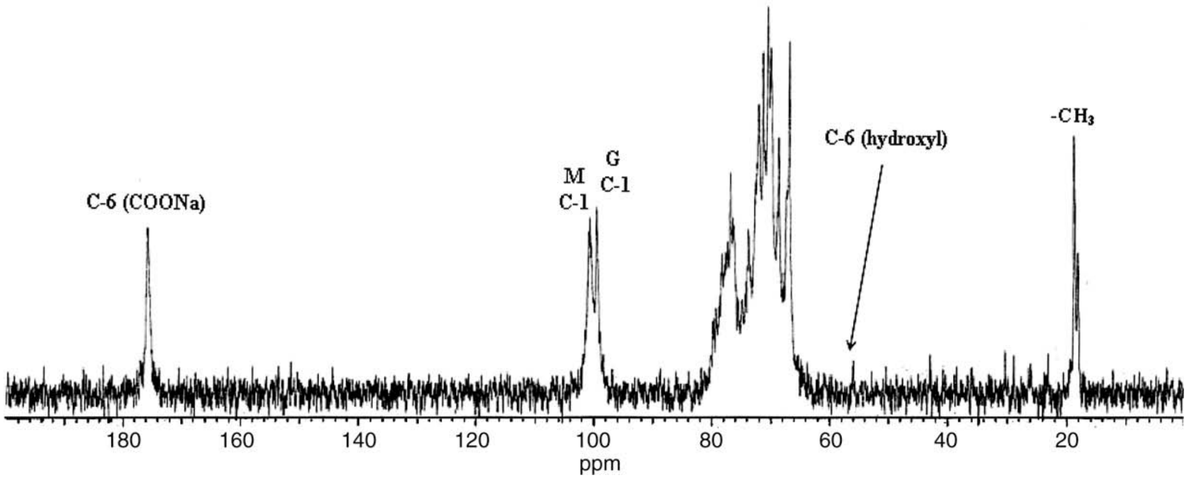

Figure 3. ^{13}C NMR spectrum of oxidized hydroxypropyl guar gum (HPG-3, MS = 0.62).

signed previously.[18] The signal due to the C-6 primary hydroxyl group at 64 ppm gradually decreased and that due to the C-6 carboxylate group at 175 ppm increased as the oxidation proceeded. This observation showed that the primary alcohol groups were transformed gradually into carboxylate, the same conclusion was obtained in the literature by Sierakowski et al.[19] and Kato et al.[17]

The guar gum sample was obtained from Jinkun Oil Field Company. The ratio of mannose to galactose monomeric units of the sample was determined by ^{1}H NMR spectroscopy (Fig. 4), which gave an average value of 1.8. For the purpose of using TEMPO-mediated oxidation for the quantitative determination of DS_6, the oxidation must proceed to 100% to ensure the accuracy of the measurement. Several oxidation experiments were conducted for the unsubstituted guar gum. The results are given in Table 1. The experimental value obtained for primary hydroxyl groups ($C\text{-}6_{OH\ (NS\text{-}EX)}$) of unsubstituted guar gum was in good agreement with the value calculated from the Man/Gal ratio of 1.8 ($C\text{-}6_{OH\ (NS\text{-}TH)}$), which shows that the oxidation reaction effectively proceeds to an almost quantitative yield.

Hydroxypropyl guar gums were prepared by the reaction of guar gum with propylene oxide under basic catalysis by a S_N2 mechanism, and the reaction occurs with a probability higher than 95% at C-1 of the propene oxide according to the literature.[20] In order to simplify the calculation, a probability of 100% was adopted as a first approximation. Thus, when the etherification occurs at the primary hydroxyl group of the polysaccharide, this functionality is replaced by the secondary hydroxyl group of the hydroxypropyl substituent which no longer suffers TEMPO-mediated oxidation. When the etherification reaction occurs on another position of the sugar unit, it will not change the molar content of the primary hydroxyl groups. The results of DS_6 determined by TEMPO-mediated oxidation for a series of hydroxypropyl guar gum samples with different MS are given in Table 2. The results show that more than 50% substitution occurs at C-6 of guar gum for a molar degree of substitution less than 1.0. There are no similar data of DS_6 in the literature for hydroxypropyl polysaccharides because the regioselectivity of hydroxypropylation strongly depends on reaction conditions, especially on the amount of base used. It is not possible to compare the accuracy of data obtained in this work.

To examine the possibility of extending the present method to other substituted polysaccharides, several samples of cationic guar gum, carboxymethyl cellulose, carboxymethyl pullulan, and methyl cellulose were subjected to the TEMPO-mediated oxidation. The results are given in Table 3. C140 and C170 are two kinds of cationic guar gum samples, *O*-(2-hydroxy-3-trimethylammonium)propyl ether-substituted from Jinkun Company, with a total

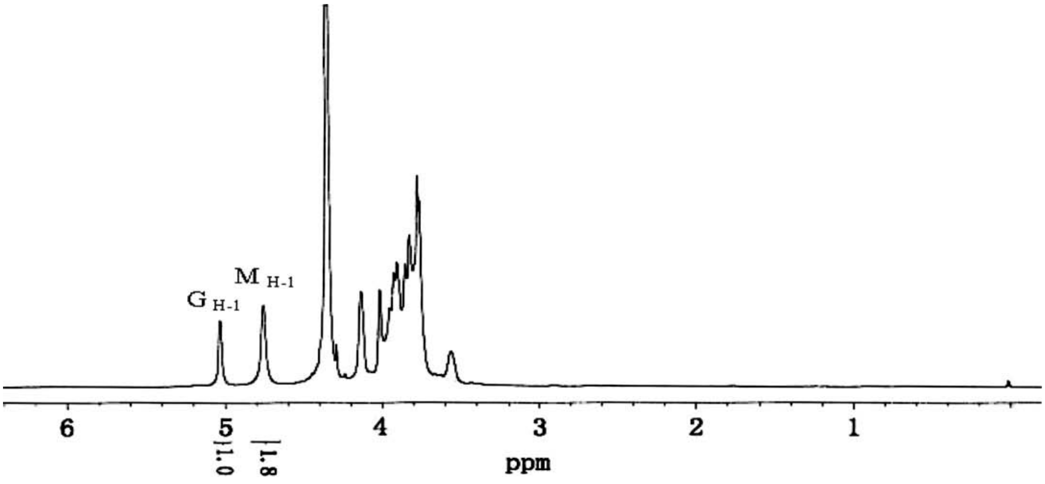

Figure 4. ^{1}H NMR spectrum of the native guar gum ($G_{H\text{-}1}$ is H-1 signal of galactose, $M_{H\text{-}1}$ is H-1 signal of mannose).

Table 1
Results of TEMPO-mediated oxidation for the unsubstituted guar gum

Guar gum	C-$6_{OH\ (NS\text{-}TH)}$ (mol %)	NaOH mol consumption/weight of sample (mol/g)	C-$6_{OH\ (NS\text{-}EX)}$ (mol %)
Sample 1	0.643	3.99×10^{-3}	0.646
Sample 2	0.643	3.83×10^{-3}	0.621
Sample 3	0.643	3.90×10^{-3}	0.632

Table 2
Results of TEMPO-mediated oxidation for hydroxypropyl guar gum

Sample	MS[a]	NaOH mol consumption/weight of sample (mol/g)	DS_6[b]
HPG-1	0.08	3.45×10^{-3}	0.06
HPG-2	0.34	2.21×10^{-3}	0.24
HPG-3	0.62	1.22×10^{-3}	0.40
HPG-4	1.08	0.9×10^{-3}	0.44

[a] Values obtained from NMR measurements.
[b] Values obtained from present method.

Table 3
Results of TEMPO-mediated oxidation for other substituted polysaccharides

Sample	DS[a]	DS_6[b]	DS_6[a]
C140 (Cationic Guar)	0.14	0.07	–
C170 (Cationic Guar)	0.22	0.12	–
Carboxymethyl cellulose	0.80	0.42	–
Carboxymethyl pullulan	0.85	0.40	–
Methyl cellulose (A15C)	1.7	0.53	0.5

[a] Values obtained from NMR measurements.
[b] Values obtained from present method.

DS of 0.14 and 0.22, respectively. The DS_6 are 0.07 and 0.12, respectively, which means that about 50% of the cationic groups are at the C-6 position of the sugar unit. Some data for the substituent distribution on different hydroxyl groups of the sugar unity are shown in Table 3 for a few substituted polysaccharides. A DS_6 value of 0.42 for carboxymethyl cellulose with a total DS of 0.80 and a DS_6 value of 0.40 for carboxymethyl pullulan with a total DS of 0.85 were found, respectively. The results, which indicate that almost 50% of the carboxymethyl groups are at the C-6 position of the sugar unit, are similar to those of the cationic guar gum. In addition, we have tried to compare the results of a commercial methyl cellulose sample A15C from the DOW Chemical Co. (kindly provided by CERMAV, CNRS-Grenoble, France). This methyl cellulose sample has a total DS of 1.7, and the degrees of substitution—for positions C-2, C-3, and C-6—are 0.8, 0.4, and 0.5, respectively, as determined by NMR spectroscopy. This sample was analyzed by our method, and a value of 0.53 was found for DS_6 very close to the value of 0.50 determined by NMR. This can be considered as another evidence of the applicability of our approach for the determination of the degree of substitution at C-6 of polysaccharides.

In summary, the TEMPO-mediated oxidation of primary alcohol groups of polysaccharides is a highly selective reaction which proceeds with a quantitative yield. Studies on hydroxypropyl guar gum, cationic guar gum, carboxymethyl cellulose, carboxymethyl pullulan, and methyl cellulose (A15C) have demonstrated that this oxidation is a useful tool for the determination of the substitution pattern at C-6 of substituted polysaccharides.

1. Experimental

1.1. Materials and general methods

Hydroxypropyl guar gums with different degrees of molar substitution and cationic guar gums were obtained from Jinkun Oil Field Chemical Co.; samples of carboxymethyl cellulose and carboxymethyl pullulan were commercial products from Luzhou Chemical Co. (China); the sample of methyl cellulose (A15C) was kindly provided by CERMAV (CNRS-Grenoble, France). All polysaccharides were purified by dissolving in water and by precipitation with EtOH before use. TEMPO was purchased from Aldrich. All other chemicals were commercial products of analytical grade and were used without further purification.

The IR spectra were recorded on a Thermo Nicolet 670FT-IR instrument. To obtain the IR spectra of oxidized polysaccharides in their acidic forms and as sodium salt, 4 mL of polysaccharide solution (2 g/L) was treated with 2 g of Dowex 50W-X8 (H^+ form) ion exchange resin, which transformed the polysaccharide salts in their carboxylic acid forms. The filtered solutions were then used to prepare the test samples according to a published procedure.[15] ^{13}C- and ^{1}H NMR spectra were obtained with a VARIAN 400 MHz instrument.

1.2. Typical procedure for TEMPO-mediated selective oxidation

The oxidation experiment was carried out as follows: the polysaccharide derivative (1 g) was dissolved in distilled water (400 mL), and TEMPO (0.01 g) and sodium bromide (1.5 g) were

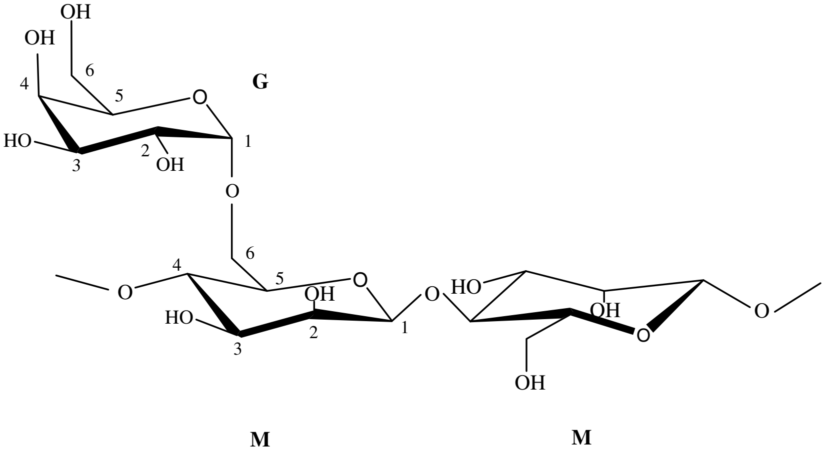

Figure 5. Schematic representation of the guar gum molecule. G is the galactose unit, M is mannose unit.

added. The mixture was stirred and cooled to 0 °C, and N_2 was passed through the reactor during the whole process. The pH of the solution was brought to 9 with NaOH. At time zero, 20 mL of a 13% sodium hypochlorite solution, whose pH had previously been adjusted to 10, was added to the mixture. The reaction rate was then monitored by the consumption of a solution of NaOH. The pH of the mixture, controlled with a pH-meter, was maintained at 10.2. At the end of the oxidation, the pH of the solution was adjusted to 8, and the oxidized polysaccharide was precipitated with EtOH, washed several times, and dried under diminished pressure at room temperature.

1.3. Determination of DS_6 for hydroxypropyl guar gums

For a proper calculation of DS_6, the following definitions are adopted for a substituted polysaccharide:

DS_6: degree of substitution at C-6 per sugar unit,
MS: molar degree of substitution per sugar unit,
$(MW)_0$: average molecular weight of the sugar unit for the unsubstituted polysaccharide,
$(MW)_S$: average molecular weight of the sugar unit for the substituted polysaccharide, thus:
$(MW)_S = (MW)_0 + MS \times$ (molecular weight of the substituent $- 1$).
$\text{C-6}_{OH(NS\text{-}EX)}$: molar content of primary hydroxyl groups at C-6 per sugar unit for the unsubstituted polysaccharide determined by TEMPO-mediated oxidation.
$\text{C-6}_{OH(NS\text{-}TH)}$: molar content of primary hydroxyl groups at C-6 per sugar unit for the unsubstituted polysaccharide calculated according to the structure of the polysaccharide.

For example: $\text{C-6}_{OH(NS\text{-}TH)} = 1$ for cellulose. In the case of guar gum, however, the theoretical value of $\text{C-6}_{OH(NS\text{-}TH)}$ is less than 1, because there are no primary hydroxyl groups at C-6 of the mannose units which are linked to galactose units (Fig. 5). In the present work, the mannose to galactose ratio R = M/G is determined by NMR spectroscopy as 1.8. An average theoretical value of $\text{C-6}_{OH(NS\text{-}TH)}$ can be calculated for guar gum:

$$\text{C-6}_{OH(NS\text{-}TH)} = \text{C-6}_{OH(NS\text{-}EX)} = \frac{M}{M+G} = \frac{R}{R+1} = 0.643\ (\text{mol}\ \%)$$

$\text{C-6}_{OH(S\text{-}EX)}$: molar content of primary hydroxyl group at C-6 per sugar unit for substituted polysaccharide obtained by TEMPO-mediated oxidation.

Then the DS_6 can be determined as follows:

$$DS_6 = [\text{C-6}_{OH(NS\text{-}EX)} - \text{C-6}_{OH(S\text{-}EX)}] \times \frac{\text{C-6}_{OH(NS\text{-}TH)}}{\text{C-6}_{OH(NS\text{-}EX)}}$$

$$\text{with, C-6}_{OH(NS\text{-}EX)} = \frac{\text{NaOH mol consumption} \times (MW)_0}{\text{weight of sample}}\ (\text{mol}\ \%)$$

$$\text{C-6}_{OH(S\text{-}EX)} = \frac{\text{NaOH mol consumption} \times (MW)_S}{\text{weight of sample}}\ (\text{mol}\ \%)$$

In case of hydroxypropyl guar gum: $(MW)_S = 162 + MS \times (58 - 1)$.

Acknowledgments

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