New Possibilities of the Acetosulfation of Cellulose

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Summary: Regioselectively substituted cellulose sulfates in C2/3-, C2/6-, or C6-position of the anhydroglucose unit are accessible by certain synthesis routes. Thereby, products with different properties and various application areas are resulted. Important characteristics of cellulose sulfates regarding their applications are solubility (e.g. in water), rheological behavior, different interaction with low or high molecular cations, thermo reversible gel formation, enzymatic degradability, anticoagulant and antiviral activity. In C6-position substituted cellulose sulfates can be synthesized in principle by acetosulfation. The acetosulfation is a quasi-homogeneous synthesis proceeding under gradually dissolution of the cellulose by using different reactivity of the primary and secondary OH-groups as soon as converting cellulose acetate sulfates. After precipitation of the polymer the acetyl groups are cleaved in alkaline solution. The focus of our study was firstly the investigation of the acetosulfation in different polar aprotic solvents by various sulfating and acetylating agents. In general it should be investigated if C6 substituted cellulose sulfates can be obtained by acetosulfation with different solvents and agents. The products were characterized by ^{13}C-NMR and Raman spectroscopy.

Keywords: cellulose; cellulose sulfate; esterification; raman spectroscopy

Introduction

Cellulose sulfates have so far received rather small attention within cellulose esterification reactions, as compared, for example with cellulose acetates or nitrates. Although sulfated polysaccharides like heparin or agar[-agar] with certain properties are encountered in nature. Possible application areas of cellulose sulfates in dependence on their properties can be: film-forming material, viscosity regulator, anionic polyelectrolyte component and substance with biological activity.[1–4] The properties of cellulose sulfates depend on the degree of polymerisation (DP), the degree of the substitution (DS), the distribution in the anhydroglucose unit (AGU) and the distribution along the chains.

The esterification of hydroxygroups of cellulose generally proceeds to the half-ester. These half-ester can be converted into the neutral sodium salts which are soluble in water in dependence on the synthesis condition from a DS value of 0.2–0.3. Generally, the term cellulose sulfate will be used to denote the acid half-ester or its sodium salt.

As fundamental routes of synthesis of cellulose sulfates can be considered:[1]

- sulfation of hydroxy groups of unmodified cellulose, usually starting under heterogeneous reaction conditions
- sulfation of hydroxy groups in partially functionalized cellulose esters or ethers with the primary substituent acting as a protecting group
- sulfation by displacement of an ester or ether group already present in the macromolecule

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Along the second and the third route regioselectively functionalized cellulose sulfates can be obtained. The sulfation of cellulose in a strictly heterogeneous system (first route) was discussed in a series of papers.[5–7]

The sulfation of hydroxy groups of partially modified cellulose esters and ethers can be realized in dipolar aprotic solvents (route two). Besides, the primary substituent presents itself as a protecting group and is not attacked by the sulfating agents. The acetyl group of partially esterified cellulose acetate proved to be a suitable stable protecting group in a subsequent sulfation.[8]

The influence of the sulfating agent, e. g. chlorosulfuric acid, amidosulfuric acid or sulfuryl chloride, on the distribution of the sulfuric half-ester within the AGU was investigated by Philipp et al.[9,10] Typically for the third route is the formation of labile ester or ether groups which are easily displaced by sulfating agents from its position in the AGU. Such labile groups can be used, e.g. the nitrite group as an ester or the trialkysilyl group as an ether.[11–14]

The acetosulfation is a quasi-homogenous synthesis proceeding under gradually dissolution of the cellulose by using different reactivity of the primary and secondary OH-groups as soon as converting cellulose acetate sulfates (Figure 1). The synthesis route consists of the competitive esterification of cellulose suspended in N, N-dimethylformamide (DMF) with a mixture of a sulfating and acetylating agent. After precipitation of the polymer the acetyl groups are cleaved in alkaline solution. The acetosulfation is a convenient method for preparation of regioselectively C6 substituted cellulose sulfates (Figure 1).[1,9]

The synthesis of sulfoacetate derivative of cellulose was described by Chauvelon et al.[15] The synthesis was arranged in glacial acetic acid as solvent and acetic anhydride and sulfuric acid as reagents. The water soluble products were distinguished by a $DS_{acetate}$ between 1.6 and 2.4 and $DS_{sulfate}$ of 0.3. The characterization of the products was carried out after cleavage of the acetate group and enzymatic hydrolysis.[16]

The focus of our study was the investigation of the acetosulfation in different polar aprotic solvents with various sulfating and acetylating agents. In general it should be investigated if C6 substituted cellulose sulfates can be obtained by acetosulfation with different solvents and agents. Hence cellulose sulfates with $DS < 1$ were of particular interest. The resulted cellulose sulfates should be dissolved completely in water.

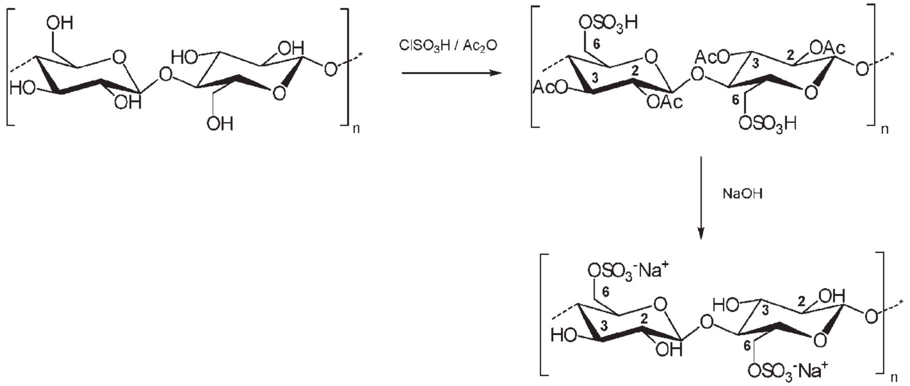

Figure 1.
Sulfation of cellulose by acetosulfation followed by deacetylation.

Experimental Part

Measurements

^{13}C-NMR Spectroscopy

The ^{13}C-NMR spectra were recorded by means of a Varian UNITY 400 NMR spectrometer at a frequency of 100.58 MHz. The samples were dissolved in D_2O and analyzed with high-resolution liquid ^{13}C-NMR. The measurements occurred using a quantitative method without Nuclear-Overhauser effect.[17]

FT Raman Spectroscopy

FT Raman spectra of all samples were acquired using a Bruker RFS 100 spectrometer with a liquid-nitrogen cooled Ge diode as detector. A cw-Nd:YAG-laser operating with an exciting line of $\lambda_{Nd:YAG} = 1064$ nm and maximum power of 1500 mW was applied as a light source for the excitation of Raman scattering. The spectra were recorded over the range of 3400–100 cm^{-1} using an operating spectral resolution of 4 cm^{-1}. The powders were analysed in small metallic discs which were placed across the normal sample holders. 180° backscattering geometry was applied. The spectra were averaged over 64 scans using 300-500 mW laser power output. The measurements were repeated three times for each sample under the same conditions. Then these three spectra were normalized and an average spectrum was calculated yielding the resulting spectrum for each of the samples.

Elemental Analysis

By means of elemental analysis (Company Carlo Erba) the contents of C, H, N und S were determined after quantitative combustion of the cellulose sulfate sample. The DS was calculated by means of sulfur content determined by elemental analysis (Equation 1):

$$DS_{Sulfat} = 162 \times \%S/(3200 - 102 \times \%S) \tag{1}$$

Solubility and Viscosity

The solubility of the cellulose sulfate was characterized by preparation of aqueous solutions (1%, w/w) and stirring for 18 h at room temperature. The solution turbidity was measured with a nephelometer 2100AN (Hach, Loveland, Colorado, USA). The calibration of the instrument was carried out with a formazin standard.

The solution viscosity of 1% (v/v) aqueous sample solution was determined by Ubbelohde viscometer.

Sulfation of cellulose (example Sul-6)

The synthesis was accomplished according to Philipp et al.[9] 10 g (0.062 mol) linters cellulose (DP: 1090) was added in 350 ml N,N-dimethylacetamide (DMAC). The sample was heated at 80 °C for 6 h along with stirring. After cooling at room temperature the sample was stirred for further 18 h. The reagent mixture was prepared in a separate closed flask under cooling. First 64 ml of acetic anhydride was added to 150 mL DMAC. Subsequently 3 ml of sulfuric acid was added drop wise into the reagent flask along with cooling and stirring for further 15 min. After adding the reagents to the cellulose sample, the mixture was heated to 50 °C and stirred for 6.5 h. The polymer was precipitated in a mixture of 1400 ml ethanol, 53 g NaOH, 100 ml H_2O and 20 g sodium acetate. After filtration the polymer was washed three times with 300 mL of a 3% sodium acetate solution in ethanol. The cleavage of

Table 1.
Solvents, acetylating and sulfating agents for acetosulfation of cellulose.

solvents	N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMAC), dimethyl sulfoxide (DMSO), N-methyl-2-pyrrolidone (NMP)
sulfating agents	chlorosulfuric acid ($ClSO_3H$), amidosulfuric acid (NH_2SO_3H), sulfur trioxide DMF-complex (SO_3 x DMF), sulfuric acid (H_2SO_4), sulfuryl chloride (SO_2Cl_2)
acetylating agents	acetic acid anhydride (Ac_2O), acetyl chloride (CH_3COCl)

Table 2.
Reaction condition and some selected results of the sample with acetic anhydride as acetylating agents.

sample	solvent	molar ratio per AGU sulfating/acetylating agent	sulfating agent	temperature [°C]	reaction time [h]	water solubility
Sul-1	DMAC	1:1.2	H_2SO_4	50	4.0	−
Sul-2	DMAC	3:11*	H_2SO_4	50	6.0	+
Sul-3	DMAC	3:11	H_2SO_4	50	6.0	+
Sul-4	DMAC	3:11	H_2SO_4	70	6.5	+
Sul-5	DMAC	3:11	H_2SO_4	70	5.0	+
Sul-6	DMAC	1:11	H_2SO_4	50	6.5	+
Sul-7	DMAC	0.9:11	H_2SO_4	50	5.5	+
Sul-8	DMAC	3:11	NH_2SO_3H	50	5.0	+
Sul-9	NMP	0.9:11	NH_2SO_3H	50	6.0	+
Sul-10	NMP	2:11	NH_2SO_3H	50	6.0	+
Sul-11	NMP	0.5:11	NH_2SO_3H	70	6.5	+
Sul-12	DMSO	1:11	SO_3 x DMF	70	6.0	−
Sul-13	DMF	0.9:11	SO_2Cl_2	50	6.0	+
Sul-14	DMF	1:11	SO_2Cl_2	60	6.0	+
Sul-15	DMF	1:11	SO_2Cl_2	50	8.0	+

* At first a mixture of reagents with molar ratio with 3:3.6 was added to the reactor. After 30 minutes the residue 7.4 mol acetic anhydride per mol anhydro glucose unit (AGU) was added.

the acetyl groups was achieved with 330 ml of an alkaline solution (13 g sodium hydroxide, 30 g water filled up to 300 ml with ethanol) by stirring for 12 h. The pH was adjusted to 8.0 with acetic acid/ethanol (50/50, v/v). Finally the product was washed with ethanol and dried at 40 °C under vacuum.

Results and Discussion

The acetosulfation was carried out in four different polar aprotic solvents. Five different sulfating agents and two acetylating agents were used (Table 1).

The investigation are shown that good water solubility of the obtained cellulose sulfate is only resulted if the cellulose is dissolved under formation of the mixed ester during the course of the reaction. If a turbidity remains in the reactor, no clear water solubility of the product will receive. The results are discussed in dependence on the used acetylating agent.

Investigation with Acetic Anhydride as Acetylating Agent

Table 2 shows the reaction condition of synthesis with acetic anhydride as acetylating agent as well as the obtained DS and water solubility of the cellulose sulfates in the last two columns. The results in the Table show that a synthesis of cellulose sulfate with acetic anhydride is possible in the solvent DMAC, DMF and NMP.

However, the acetosulfation with DMSO as solvent did not lead to the desired product (Sul-12). DMSO reacts presumably with acetic anhydride according to the Pummerer reaction to the acetoxymethyl-methyl sulfide.[18,19] Hence acetic anhydride is not available as reaction partner any more. The formation of the cellulose mixed ester is prevented and no dissolution of the sample occurs. The synthesis remains heterogeneous. The

Table 3.
Properties of the water soluble cellulose sulfates.

Sample	Sulfur content [%]	DS	Viscosity [mm²/s]
Sul-2	16.0	1.63	1
Sul-3	18.4	2.21	1.4
Sul-4	17.4	1.89	1.4
Sul-5	17.3	1.93	1.5
Sul-6	9.5	0.68	3
Sul-7	6.9	0.45	5
Sul-9	7.74	0.52	38
Sul-10	12.4	1.04	29
Sul-11	5.1	0.31	12
Sul-13	6.5	0.41	20
Sul-14	7.3	0.48	33
Sul-15	7.6	0.51	40

Table 4.
Reaction condition and some selected results of the sample with acetyl chloride as acetylating agents.

Sample	solvent	molar ratio per AGU sulfating/acetylating agent	sulfating agent	temperature [°C]	reaction time [h]	DS	water solubility
Sul-16	DMF	0.9:11	$ClSO_3H$	50	12	0.15	−
Sul-17	DMF	1:11	SO_2Cl_2	50	12	0.25	−
Sul-18	DMF	1.5:11	$ClSO_3H$	70	8	0.17	−
Sul-19	DMF	1.5:6	$ClSO_3H$	70	10	0.71	−
Sul-20	DMF	1:6	SO_2Cl_2	70	6	0.44	−
Sul-21	DMF	0.9:6	$ClSO_3H$	60	10	0.43	−
Sul-22	DMAC	0.9:11	H_2SO_4	70	10	0.16	−
Sul-23	DMAC	1.5:6	H_2SO_4	50	8	0.82	+
Sul-24	NMP	1:6	NH_2SO_3H	70	7	0.49	+
Sul-25	NMP	1.5:6	SO_3 x DMF	60	9	0.78	+
Sul-26	NMP	0.9:6	NH_2SO_3H	50	8	0.54	−
Sul-27	DMSO	1.5:6	SO_3 x DMF	70	17	–	−
Sul-28	DMSO	1.5:6	SO_3 x DMF	70	10	–	−

obtained sulfide product was insoluble in water.

No dissolution of the cellulose was observed in synthesis Sul-1. Probably the used reagent amounts were not suffice for formation of the cellulose acetate sulfate. For every solvent the suitable molar ratio must be found out. The samples with sulfuric acid show (Sul-2 to Sul-5) that high DS could be attained. But a regioselectivity could not be detected on this high DS. Higher DS values were also obtained with lower reaction temperature at equal molar ratio of sulfating and acetylating agent (sample Sul-3 and Sul-4 as soon as Sul-14 and Sul–15).

The properties of water soluble cellulose sulfates are shown in Table 3. The viscosity of the obtained products varies from 1 to 40 mm^2/s. Noteworthy, the cellulose sulfates by using sulfuric acid are characterized by means of low viscosity. The degradation of the cellulose was higher as result of the formation of the acetylsulfuric acid in comparison with the other reaction systems.

Investigation with Acetyl Chloride as Acetylating Agent

The reaction condition of the samples with acetyl chloride as acetylating agent are shown in Table 4 as well as the obtained DS and water solubility of the cellulose sulfates. Less acetyl chloride as acetylating agents per mol AGU were required in comparison with acetic anhydride. Again no acetosulfation succeeded in the solvent DMSO due to concurrent side reaction with acetyl chloride to dimethyl sulfides.[19,20] No water soluble products could be achieved with the synthesis in DMF. During the synthesis no complete dissolution of the cellulose was observed. In any case, a turbidity could be ascertained in the reactor. However, the products of the study with acetyl chloride in DMF were exhibited DS between 0.15 and 0.71. Surprisingly these samples were soluble in DMSO. Water soluble cellulose sulfates with low viscosity were obtained in the solvents DMAC and NMP and various sulfating agents (Table 4 and 5).

Table 5.
Properties of the water soluble cellulose sulfates.

sample	Sulfur content [%]	DS	Viscosity [mm^2/s]
Sul-23	10.7	0.49	3
Sul-24	7.5	0.78	2
Sul-25	9.8	0.54	2

Table 6.
Comparison of the DS values resulted by elemental analysis and ^{13}C-NMR spectroscopy.

Sample	DS by elemental analysis	DS by ^{13}C-NMR			
		DS_{total}	DS_{C2}	DS_{C3}	DS_{C6}
Sul-11	0.31	0.32	–	–	0.32
Sul-13	0.41	0.48	0.05		0.43
Sul-15	0.51	0.55	–	–	0.55
Sul-10	1.04	1.13	0.38		0.75

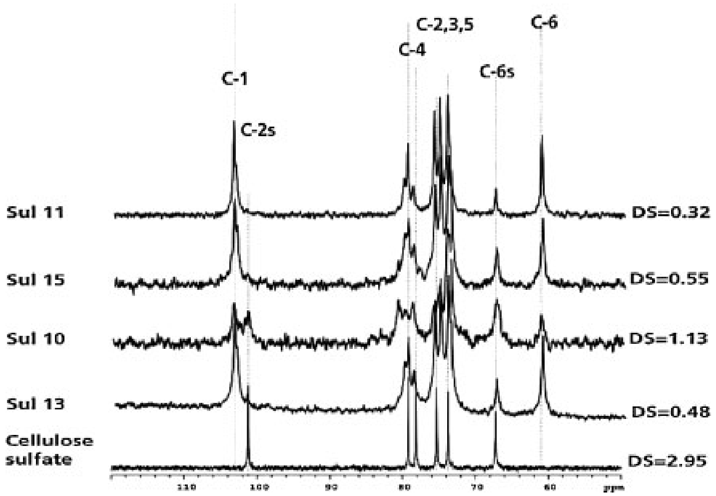

Figure 2.
^{13}C-NMR spectra of selected cellulose sulfate in comparison with nearly completely substituted cellulose sulfate (DS $>$ 2.9).

Regioselectivity of the Cellulose Sulfates

The regioselectivity of the cellulose sulfate was determined by means of ^{13}C-NMR-spectroscopy according to Nehls et al.[17] Figure 2 shows a selection of cellulose sulfates with DS up to 1 in comparison with a nearly completely substituted cellulose sulfate. The substitution of the sulfate half ester on position C6 appears by shifting the C6 signal from 61 to 67 ppm (Figure 2). The substitution on position C2 can be found by shift of the C1 signal from 103 to 101 ppm. The calculated DS values for the several C position are shown in Table 6. The regioselectivity of the acetosulfation can proved by the NMR data definitely.

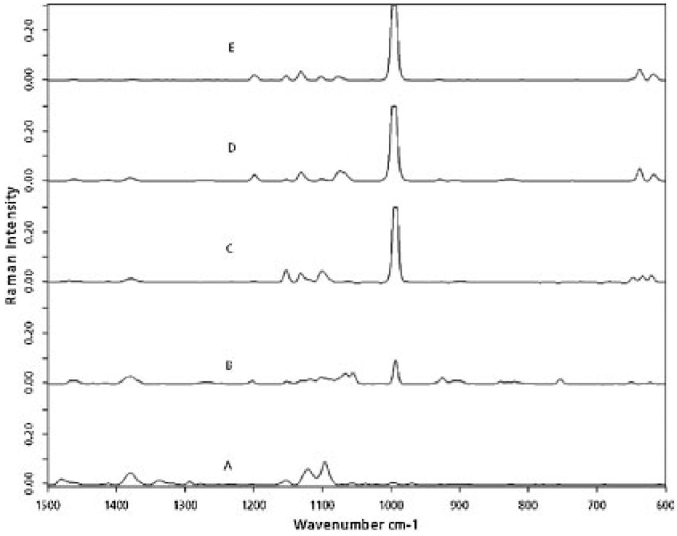

Figure 3.
FT-Raman spectra of selected cellulose sulfate in comparison with starting material (linters cellulose) in the range of 600–1500 cm^{-1}; A: linters cellulose, B: Sul - 7 (DS = 0.45), C: Sul - 10 (DS = 1.04), D: Sul-2 (DS = 1.63), E: Sul-3 (DS = 2.21).

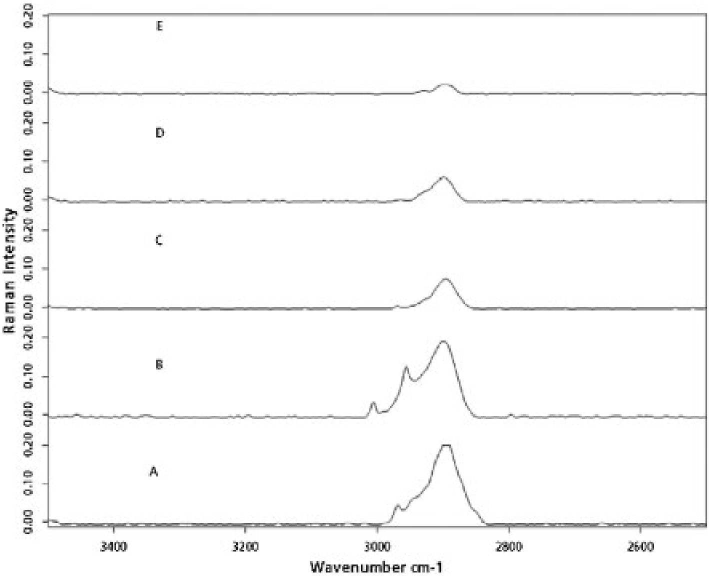

Figure 4.
FT-Raman spectra of selected cellulose sulfate in comparison with starting material (linters cellulose) in the range of 2500–3500 cm-1; A: linters cellulose, B: Sul - 7 (DS = 0.45), C: Sul - 10 (DS = 1.04), D: Sul-2 (DS = 1.63), E: Sul-3 (DS = 2.21).

Raman Spectroscopic Investigation of Cellulose Sulfates

Raman spectroscopy is an efficient method for structural characterization of native polymers. Thus the investigation of the different cellulose polymorphs were arranged by Raman spectroscopy.[21–25] However, the characterization of cellulose derivatives by Raman spectroscopy didn't arise very often. Figure 3 shows FT-Raman spectra of series of cellulose sulfates in the range of 600 and 1500 cm^{-1} with different DS. Noticeable, the increase of the Raman intensity at 993 cm^{-1} was observed with increase of the $DS_{sulfate}$. New peaks were appeared between 600 and 650 cm^{-1} as well. Although three signals were occurred at a DS up to 1.1 and only two signal were appeared at a $DS > 1.5$. The scissoring vibration at 1476 and 1455 cm^{-1} could assigned as typical Raman peak for modification cellulose I in the spectra of the linters cellulose (bottom spectrum). In contrast the scissoring vibration of cellulose II was assigned at 1461 cm^{-1}. This signal was decreased with increase of the DS_{sulfat} (upper spectra Figure 3). The modes between 1200 and 1400 cm^{-1} were involved methine, methylene and hydroxyl groups bending motions.[20] The signal at this range was decreased respectively disappeared with increase of $DS_{sulfate}$ (1378, 1334 and 1279 cm^{-1}). Further shifts in the range of the stretching vibration were declared between 1000 and 1200 cm^{-1}.

The symmetric and asymmetric stretching vibration of the CH and CH_2 could assigned in the Raman spectrum of cellulose between 2800 and 3000 cm^{-1}. Firstly a shift and then a considerably decrease of the Raman intensity were observed in this range with increase of the $DS_{sulfate}$ (Figure 4).

Conclusion

The present investigation show that the acetosulfation of cellulose can be carried out in different polar solvents using diverse sulfating and acetylating agents. The obtained cellulose sulfates were preferentially substituted at C6 position independent of the used solvents and agents. The products with DS > 0.25 were soluble in water. Dependent on the reaction conditions, the viscosities of the aqueous solution differ rigorously. The solvent dimethyl sulfoxide is unsuitable for the acetosulfation of cellulose due to concurrent side reaction.

The cellulose sulfates were characterized by ^{13}C-NMR and Raman spectroscopy, whereas the DS were determined by elemental analysis and ^{13}C-NMR spectroscopy. The investigation have demonstrated that FT Raman spectroscopy is a potentially useful method for the characterization of cellulose derivatives.

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