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Endoglucanase fragmentation of cellulose sulfates derived from different synthesis concepts

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Abstract

Nine cellulose sulfates, derived from different synthesis concepts, were characterised regarding the degree of substitution, substituent distribution in the anhydroglucose unit, molar mass distribution, intrinsic viscosity and solubility. The degree of substitution ranged from 0.37 to 1.46, and the degree of polymerisation amounted from 160 to 350. All samples were intensively incubated with an endoglucanase, and the degradation was monitored using multi-detected size exclusion chromatography. For samples with a degree of substitution up to 0.8 a severe fragmentation occurred, while for samples with higher substitution at least a significant modification of the derivatives could be achieved. The enzyme altered not only the molar mass distribution and viscosity but also its water solubility.

The investigations on cellulose sulfates with different substitution pattern indicated that a homogeneous substituent distribution resulted in the best solubility of the derivatives. Investigation of samples after enzymatic fragmentation suggested that the C_6 position inhibited the enzyme most effectively.

Keywords: Cellulose sulfate; Endoglucanase; Size exclusion chromatography

1. Introduction

Cellulose sulfate half-esters are among the first cellulose derivatives investigated in the early days of cellulose chemistry. The derivatisation procedures were based on heterogeneous reactions leading to a continuous dissolution of the reaction product. Water solubility of these derivatives required high degree of substitution (DS) above two. During the reactions an intensive hydrolysis of the polymeric chain could not be prevented. A literature review on cellulose sulfate synthesis was given by Philipp and Wagenknecht (1983) and by Krässig (1993).

The main applications for use of cellulose sulfates were focused on the viscosity effect for paints and printing colours. The corresponding derivatives had a high DS and were water-soluble as well as soluble in dimethylformamide (DMF) and dimethylsulfoxide (DMSO). Recently cellulose sulfates of low DS-values, derived from heterogeneous reactions, were also suggested to be used for the production of microcapsula (Lukanoff & Dautzenberg, 1994).

In the last decades intensive studies were performed on non-aqueous solvent systems for homogeneous cellulose derivatisation procedures. Solvent systems leading to derivatisation but offering suitable leaving groups for further reactions enabled the synthesis of cellulose sulfates with DS above 0.3 and satisfying water solubility. Derivatization concepts resulting in a preferred regioselective sulfation in C_6-, $C_{2/3}$- or $C_{2/6}$- position of the anhydroglucose units could be developed using cellulose nitrite (Wagenknecht, Nehls & Philipp, 1993), trimethylsilyl cellulose (Wagenknecht, Nehls, Stein, Klemm & Philipp, 1992) or cellulose acetates (Wagenknecht, 1996a,b; Philipp, Klemm, Wagenknecht, Wagenknecht, Nehls, & Stein et al., 1995) as intermediates and subsequent elimination of instable intermediate groups.

Cellulose sulfates with low degree of substitution and a preferred substitution in C_6-position could be obtained by a competitive esterification reaction of cellulose using chlorosulfuric acid and acetic anhydride in DMF. During the derivatisation the product was dissolved, and the acetyl groups could be removed after the reaction (Philipp et al., 1995).

Schweiger (1979) reported that cellulose sulfates with high degree of substitution could not be degraded by cellulases. Early investigations on the enzymatic degradation of cellulose derivatives revealed that cellulose sulfates with DS < 1 were degraded not as intensively as other cellulose

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derivatives of comparable DS (Husemann, 1954; Reese, 1957). Other authors compared the extent of the enzymatic degradation of different cellulose derivatives, including cellulose sulfates. They concluded that the degradation of water-soluble cellulose derivatives depended on the DS while the type of substituent was of minor importance, at least for substituents with a similar charge (Kasulke, Dautzenberg, Polter & Philipp, 1983). In addition the authors pointed out that enzymes could liberate more glucose from heterogeneously produced samples compared to those from homogeneous reaction systems. Accordingly the derivatisation procedure has to be considered for studies on enzymatic degradation.

In all previous investigations degradation experiments were not performed with single enzymes but with mixtures and culture filtrates. The degradation of cellulose derivatives was monitored either by the increase of reducing sugars or by viscosity determination. This investigation deals with cellulose sulfates, which were prepared according to different synthesis concepts preferably in homogeneous reactions. The material was incubated with a pure endoglucanase I (EG), and the fragmentation is monitored by multi-detected size exclusion chromatography. The extent of the enzymatic degradation is compared to results previously obtained for carboxymethyl cellulose, methyl cellulose and cellulose acetate using the same enzyme and reaction conditions (Saake, Horner & Puls, 1998). In addition to this, our investigation should answer the question if cellulose sulfates are suitable substrates for enzyme-aided analysis of substituent distribution along the polymeric chain, analogous to a procedure recently developed for carboxymethyl cellulose (Horner, Puls, Saake, Klohr & Thielking, 1999).

2. Materials and methods

2.1. Synthesis

2.1.1. Homogeneous sulfation via commercial cellulose acetates (Ho 1)

Commercial cellulose acetate (CA) was dried at 105°C and dissolved in 150 ml freshly distilled dry DMF (water content 0.01–0.02%) under vigorous stirring. For samples 1 and 9 a 2.5-CA (partial substitution by NMR: C_2 0.85, C_3 0.80, C_6 0.75) was used while sample 3 was produced from a 2-CA preparation (partial substitution by NMR: C_2 0.65, C_3 0.55, C_6 0.65). A solution of 20% of a suitable sulfation reagent (see below) in dry DMF was added and the system was kept for 2 h at 20°C. The product was precipitated into the threefold volume of solution of sodium acetate in ethanol (4% w/w). The precipitate was filtered off and washed with ethanol containing 20–30% water and secondly with ethanol. For deacetylation the resulting Na-cellulose acetate sulfate was suspended in a solution of NaOH in ethanol (4% w/w) and kept for 15 h, employing a molar ratio of 3 mol NaOH/mol AGU (AGU = anhydroglucose unit). Finally, the suspension was neutralized to pH 8 with acetic acid. The resulting sodium salt of the cellulose sulfuric half-ester was washed with ethanol and dried at 50°C in vacuo.

Sample No. 1: 0.8 mol $ClSO_3H$/mol AGU.

Sample No. 9: 0.8 mol $CH_3C(O)SO_4H$/mol AGU (prepared from H_2SO_4 and $(CH_3CO)_2O$).

Sample No. 3: 1.2 mol $ClSO_3H$/mol AGU.

2.1.2. Preferred $C_{2/3}$-sulfation of cellulose via cellulose triacetate (Ho 2)

As a first step regioselectively substituted CA was produced by site-selective deacetylation: 200 g commercial CTA was dissolved in 3.6 l of DMSO at 80°C under vigorous stirring and application of vacuum. For partial deacetylation, a mixture of 191 g dimethylamine (6 mol/mol AGU), 280 ml H_2O (22 mol/mol AGU) and 200 g DMSO was added within 30 min and the solution was kept for 3.5 h at 80°C under stirring. After neutralisation with acetic acid, precipitation in an excess of ethanol, washing the precipitate with ethanol, and drying at 50°C in vacuo, a CA partially deacetylated in $C_{2,3}$-position of the AGU was obtained (partial substitution by NMR: C_2 0.65, C_3 0.70, C_6 1.00). The synthesis of cellulose sulfate from this CA was carried out in the same manner as described before (see Ho 1).

Sample No. 8: 1.0 mol H_2NSO_3H/mol AGU; 2 h at 80°C.

2.1.3. Preferred C_6-sulfation of cellulose by competitive esterification (HetHo)

5 g cellulose (air dry) were suspended in 100 ml dry DMF and stirred for ≥1 h at room temperature. Acetic acid anhydride and chlorosulfuric acid were added and the derivatisation was performed at 50°C as mentioned below. The cellulose dissolved during this procedure, and a clear solution was obtained. The precipitation, deacetylation and purification procedures were carried out in the same manner as described before (see Ho 1).

Sample No. 7: 0.8 mol $ClSO_3H$ and 0.8 mol $(CH_3CO)_2O$/mol AGU; 8 h.

Sample No. 5: 2 mol $ClSO_3H$ and 4 mol $(CH_3CO)_2O$/mol AGU; 3 h.

2.1.4. Preferred C_2-sulfation by homogeneous reaction of cellulose nitrite (Ho 3)

10 g microcrystalline cellulose (MCC; dried at 105°C) was swollen for 24 h at room temperature in 300 ml of dry DMF. The cellulose was dissolved by adding 22.7 g N_2O_4 (4 mol N_2O_4/mol AGU) under vigorous stirring. After 3 h the cellulose trinitrite solution was cooled to the reaction temperature of −20°C. 10% SO_3 in dry DMF of the same temperature was slowly added and the solution was kept cool for 3 h. The reaction mixture was poured into acetone (water content 1%). The precipitate was washed with acetone containing 30% ethanol and then neutralized with 4% NaOH in ethanol in order to obtain a neutral

sodium cellulose sulfate. Finally the product was washed with ethanol and dried at 50°C in vacuo.

Sample No. 6: 2 mol SO_3/mol AGU

2.1.5. Preferred C_6-sulfation of cellulose by heterogeneous reaction (Het)

10 g cotton linters (air dry) was immersed in 300 ml of H_2SO_4/*n*-propanol (see below) at 0°C for 2 h. The product was filtered off, washed with *n*-propanol, neutralised with NaOH and washed with *n*-propanol containing 30–35% water. The sodium cellulose sulfate was dissolved in water and the insoluble portion was removed by filtration. After precipitation in *n*-propanol the product was washed with *n*-propanol and dried at 50°C in vacuo.

Sample No. 2: 1.2 mol H_2SO_4/mol *n*-PrOH.

Sample No. 4: 1.5 mol H_2SO_4/mol *n*-PrOH.

2.2. Characterisation of DS and substitution pattern

The total DS of sulfate ester groups was calculated from the sulfur content determined by elemental analysis (Carlo Erba). The ^{13}C-NMR spectra were recorded on Bruker MSL 400 and AM 300 spectrometers, at frequencies of 100.63 and 75.47 MHz, respectively. Between 100 and 500 scans were accumulated per spectrum. The chemical shifts were measured with respect to tetramethylsilane as a reference.

Partial degrees of substitution in the different position of the AGU were determined from the ^{13}C-NMR spectra of sodium cellulose sulfate samples dissolved in D_2O by integration of the signal areas, and comparing the signal integrals of the 'substituted' and 'non-substituted' C-atoms. It must be emphasized that the proton decoupling (nuclear Overhauser effect) influences the signal intensity of each of the various C-atoms of the AGU to a different degree; therefore, only a comparison of the signal integral of the same C-atom in the substituted and non-substituted state is valid. Signal integrals of different C-atoms should not be compared for quantitative evaluation of spectra recorded with normal decoupling routines (Nehls, Wagenknecht, Philipp & Stscherbina, 1994).

2.3. Enzyme-aided fragmentation

Endoglucanase I (EG) from *Humicola insolens* was a research preparation from Novo-Nordisk (Bagsværd, Denmark). 0.2% solutions of cellulose sulfates were incubated with 100 nkat of EG per mg of sample for 6 days at 45°C in 0.1 M aqueous sodium nitrate (pH 5.6), the eluent used for later SEC analysis. This procedure was chosen to avoid salts or buffers differing from the SEC eluent, which would strongly affect the refractive index signal and impede a proper evaluation of the chromatograms. The enzyme was inactivated by boiling, and the flocculated protein was removed by centrifugation.

2.4. Size exclusion chromatography

Analytical SEC was performed after sample filtration (0.45 μm regenerated cellulose). 100 μl of 0.2% cellulose sulfate samples were injected onto TSK-SEC columns (Toso Haas) coupled in line (TSK G5000PW$_{XL}$, G4000PW$_{XL}$, G3000PW$_{XL}$, each 300 × 7.8 mm and a G2500PW$_{XL}$ guard column 40 × 6 mm, 40°C). The eluent was 0.1 M $NaNO_3$ in water (0.4 ml/min). The multi-detector system consisted of a refractive index detector (Shodex RI-71), a two-angle light scattering detector (Precision Detector PD 2000), and a viscosity detector (Viscotek H502). The light scattering signal was only quantitatively used in order to ensure that no aggregates were present in the samples. The viscosity detector was used for molar mass determination, applying a universal calibration curve, which was based on dextran standards (Fluka). The software WINGPC 4.0 (Polymer Standard Service, PSS) was used for data capture and evaluation of the results. For the EG-fragmented samples broad distributions with oligomeric degradation products were often determined. In case that oligomeric product could not be reliably detected by the viscometer this particular part of the chromatogram was calculated by extrapolation. For this purpose the Mark–Houwing equation, obtained from the intrinsic viscosity and universal calibration in the high molar mass range of the chromatogram, was extrapolated into the low molar mass region.

For absolute molar mass determination the knowledge of accurate sample concentration is essential. Unfortunately for cellulose sulfates no well-defined standard material is available for validation of the system. Therefore the samples with the best solubilities and detector responses after enzymatic degradation were assumed as 100% soluble (samples 1,2,3; DS: 0.37–0.75). Previous investigations on methylcelluose and cellulose acetate had shown that endoglucanase fragmentation strongly improved the solubility of the derivatives (Saake et al., 1998). The calibration with endoglucanase fragmented samples might include a small systematic error, but facilitates a comparison of samples within this set of data.

3. Results and discussion

3.1. Characterisation of cellulose sulfate

Nine cellulose sulfate sodium salts, derived from different synthesis procedures were included into the investigation. The degree of substitution was determined by elemental analysis for all samples. In addition the substitution pattern on the position C_2, C_3 and C_6 was determined by liquid ^{13}C-NMR spectroscopy. The DS values ranged from 0.37 to 1.46 in good agreement with the various methods applied (Table 1). The first six samples in Table 1, listed in order of their DS, were used to investigate the influence of the DS on endoglucanase fragmentation. Most of the samples were produced by homogeneous derivatisation procedures

Table 1
Degree of substitution and substitution pattern of cellulose sulfate sodium salts derived from different starting materials and synthesis concepts (CA: cellulose acetate; MCC: microcrystalline cellulose)

Sample no.	Starting material			Synthesis concept[a]	DS_{Sulfur}	DS_{NMR}	Pattern of substitution		
		DP_{Cu}	DS				C_2	C_3	C_6
1	2.5-CA	200	2.47	Ho1	0.37	0.33	0.09	0.04	0.20
2	Linters	1400	–	Het	0.50	0.50	0.05	0.0	0.45
3	2-CA	230	1.87	Ho1	0.75	0.70	0.20	0.15	0.35
4	Linters	1400	–	Het	0.80	0.80	0.08	0.03	0.69
5	MCC	150	–	HetHo	0.99	1.03	0.27	0.03	0.73
6	MCC nitrite	150	3	Ho3	1.46	1.35	0.74	0.12	0.49
7	Linters	375	–	HetHo	0.40	0.48	0.05	0	0.43
8	CTA (regio-CA)	305	2.39	Ho2	0.43	0.44	0.23	0.11	0.10
9	2.5-CA	200	2.47	Ho1	0.45	0.50	0.17	0.14	0.19

[a] See experimental section.

starting from partially substituted cellulose acetate dissolved in DMF (No. 1, 3, 8 and 9), solutions of cellulose in N_2O_4/DMF (No. 6) or by performing a simultaneous acetylation and sulfation (No. 5 and 7), generally followed by the removal of acetyl or nitrite groups. Two samples were produced in a heterogeneous reaction of cellulose in H_2SO_4/*n*-propanol (No. 2 and 4). While the heterogeneously prepared samples were preferably substituted in the C_6 position, the samples No. 1, 3 and 5 showed a substitution pattern with a preference of $C_6 > C_2 > C_3$. In addition to these samples three cellulose sulfates with very similar DS around 0.4–0.45, but different preferred positions of substitution were included (No. 7, 8, 9). Due to their low overall substitution these samples did not allow unambiguous conclusions on the effect of the substitution. Nevertheless they revealed first information on the influence of substitution pattern on degradation of cellulose sulfates. While sample 7 was dominantly substituted in C_6, sample 8 had a preferred substitution in C_2 compared to the substitution in C_3 and C_6. The last sample (No. 9) had a uniform substitution on all positions.

3.2. Effect of the degree of substitution on endoglucanase fragmentation of cellulose sulfates

Molar masses corresponding to DP values from 160 to 290 were determined in the SEC analysis of the original samples 1–6 (Table 2). The highest DP values were obtained for those cellulose sulfates, which were produced in a heterogeneous reaction of high molar mass cotton linters of DP 1400 (No. 2: DP 270; No. 4: DP 290) demonstrating the strong DP-decrease in this derivatisation procedure. DP values of the other samples ranged from 160 to 200, corresponding well with the viscosimetric DP determination of the starting materials (Table 1). This result demonstrated that only little or no decrease of DP occurred in homogenous reactions, starting with cellulose acetates or microcrystalline cellulose (MCC) with comparatively small chain lengths. High polydispersities (weight average molar mass/number average molar mass $= M_w/M_n$) of some samples can be attributed to oligomeric products in the SEC chromatogram. The yield of soluble derivatives detected after sample preparation and filtration in the SEC analysis varied from 79 to 96%. These values confirmed the good water solubility of the low DS cellulose sulfate samples.

Endoglucanase fragmentation did not only effect the molar mass of the samples. With the exception of sample 5 (DS 0.99) the water-solubility of the cellulose sulfates was increased by around 10% on average (Table 2). The ratio of molar mass before and after endoglucanase fragmentation, listed in Table 2 as 'fragmentability', underlines the strong

Table 2
Effect of endoglucanase fragmentation on solubility, intrinsic viscosity and molar mass of cellulose sulfates with different degree of substitution

Sample		Before EG-fragmentation				EG-fragmented material				Fragmentability
No.	DS_{Sulfur}	Yield[a] (%)	$[\eta]$ (ml/g)	M_w (g/mol)	M_w/M_n	Yield[a] (%)	$[\eta]$ (ml/g)	M_w (g/mol)	M_w/M_n	M_w/M_w before/after
1	0.37	96	53	41,000	9.9	100	–[b]	3200	6.4	12.8
2	0.50	80	104	58,000	5.1	100	–[b]	7500	10.0	7.7
3	0.75	91	83	44,000	4.1	100	13	18,000	5.2	2.4
4	0.80	82	101	71,000	1.8	97	54	26,000	10.8	2.7
5	0.99	81	105	42,000	3.2	81	62	38,000	2.1	1.1
6	1.46	79	103	52,000	2.6	91	114	60,000	6.1	0.9

[a] Recovery percentage of the sample after SEC analysis.
[b] Calculated by dextran calibration instead of universal calibration.

dependence of enzymatic degradation from the sample DS. An intense fragmentation of cellulose sulfates occurs up to DS 0.8. For samples No. 1 and 2 endoglucanase fragmentation was so severe that the viscosity of the samples dropped below the detection limits of the detector. Accordingly the molar mass determination was performed by a conventional dextran calibration curve. Since cellulose sulfates have a higher hydrodynamic volume compared to dextrans, the molar masses of 3200 and 7500 g/mol (DP_w of 16 and 35) were slightly overestimated.

Due to the intense fragmentation of these samples high polydispersities were obtained. The comparison of the elution curve of the intact sample No. 2 with the corresponding profile after enzymatic degradation revealed that oligomeric products dominated the elution profile after degradation. Nevertheless an intense tailing into the higher molar mass region could be detected (Fig. 1a). Up to a DS of 0.75–0.8 (Nos. 3, 4) the chromatograms showed strong signals of oligomeric products (Fig. 1b, No. 3), while for samples of higher DS no significant amounts of oligomers could be determined after fragmentation. Nevertheless the endoglucanase treatment had a strong effect on the viscosity signal. For sample No. 5 a 30% reduction in viscosity occurred while the molar mass, calculated from universal calibration, was reduced by only 10%. These findings indicated that enzymatic treatments modified the state of solution and accordingly the hydrodynamic volume of the molecule. For the DS 1.46 sample (No. 6) even an increase in viscosity and molar mass was determined after enzymatic treatment. This result could be explained by a 12% higher soluble portion of the enzymatically treated sample. SEC analysis showed a significantly increased high molar mass portion after endoglucanase treatment (Fig. 1c). This phenomenon indicated that the untreated sample contained aggregated or associated material, which was removed by filtration prior to SEC analysis. These structures were modified by the enzyme, and subsequently high molar mass material could be solubilised. Similar effects might be responsible for the more soluble fraction of the low DS samples. However, due to the intensive enzymatic hydrolysis at lower DS these fractions were hydrolysed to low molar mass products. Previously similar effects were reported for methyl cellulose and cellulose acetate after endoglucanase treatments (Saake et al., 1998). Besides of the increased portion of high molar mass products the elution curve of the endoglucanase fragmented sample No. 6 (DS 1.46) showed a shoulder at 25 ml, indicating the liberation of polymeric degradation products. The combination of both effects resulted in a polydispersity as high as 6.1.

Comparing these data to earlier publications (Saake et al., 1998) it can be stated that endoglucanase fragmentation of cellulose sulfates was less intense compared to cellulose acetate and methyl cellulose. Fragmentation intensities of sulfate samples of low DS were comparable to results obtained earlier for carboxymethyl cellulose. Cellulose

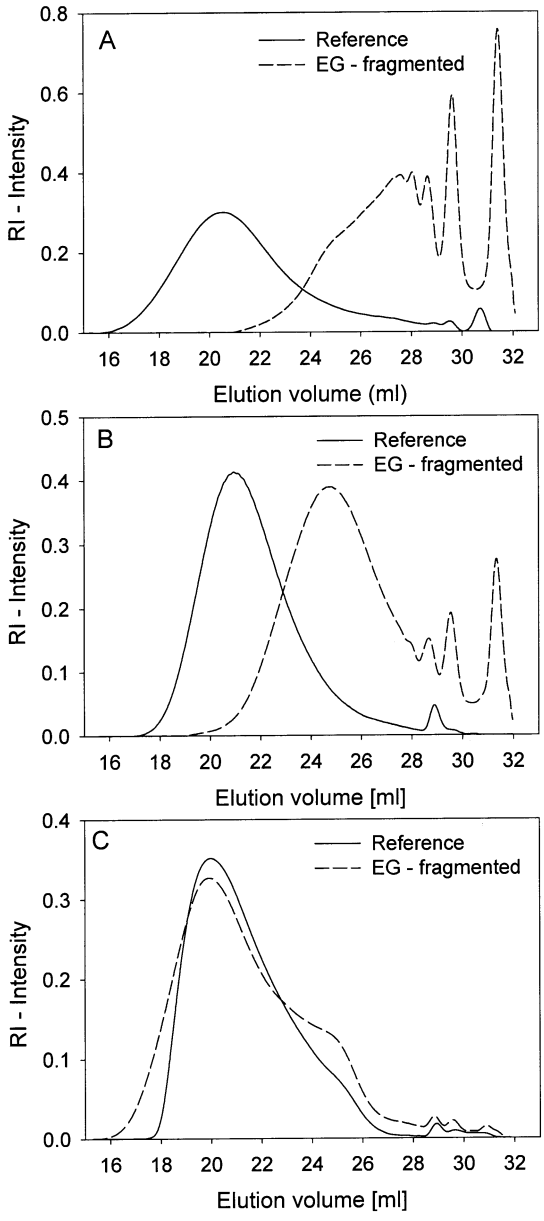

Fig. 1. Influence of endoglucanase fragmentation on SEC elution curves of cellulose sulfates with different degree of substitution. (A) No. 2, DS 0.5; (B) No. 3, DS 0.75; (C) No. 6, DS 1.46.

sulfates of DS 0.99 and DS 1.46 showed a limited release of oligomeric fragments compared to carboxymethyl cellulose. However, it has to be considered that the cellulose sulfates included in this study had lower starting DPs.

In general this comparison reveals a slight inhibition in the degradation of cellulose sulfates of DS $>$ 1, compared with cellulose acetate, methyl cellulose or carboxymethyl cellulose. Nevertheless at least up to DS 1.5 a significant modification of molar mass distribution and solubility could be achieved. Therefore it can be expected that enzymatic approaches can contribute to the modification and characterisation of cellulose sulfates.

Table 3
Effect of endoglucanase fragmentation on solubility, intrinsic viscosity and molar mass of cellulose sulfates with different substitution pattern

Sample		Before EG-fragmentation				EG-fragmented material[b]			Fragmentability
No.	Substitution pattern	Yield[a] (%)	$[\eta]$ (ml/g)	M_w (g/mol)	M_w/M_n	Yield[a] (%)	M_w (g/mol)	M_w/M_n	M_w/M_w before/after
7	C_6	67	129	71,000	1.7	66	13,600	10.0	5.2
8	$C_2 \gg C_6$	79	203	71,000	2.0	77	6400	3.2	11.1
9	$C_2 \approx C_3 \approx C_6$	92	31	38,000	4.0	100	6300	8.1	6.0

[a] Recovery percentage of the sample after SEC analysis.
[b] Calculated by dextran calibration instead of universal calibration.

3.3. Effect of the substitution position on endoglucanase fragmentation of cellulose sulfates

Samples 7, 8 and 9 were included in this part of the study to obtain informations on the effect of different substitution patterns on the enzymatic degradation. All samples ranged in the DS between 0.4 and 0.45. Sample No. 7 had a preferred substitution in C_6, and No. 8 in C_2, while sample No. 9 excelled by a uniform distribution. SEC of the starting material resulted in molar masses of 71.000 g/mol for sample Nos. 7 and 8 representing DP values of 350 and 345. For sample No. 7 this value correlated well with the viscosimetric DP determination of the starting linters (DP 375). The DP of sample No. 8 had to be considered somehow critical, since it was 12% higher than the DP determined for the starting cellulose acetate. Both, samples 7 and 8 had narrow molar mass distributions without oligomeric degradation products and accordingly low polydispersities (Table 3, Fig. 2a). Sample 9 with the homogeneous substituent distribution was only half in chain length (DP 180) compared to both samples mentioned earlier. This can be explained by the low DP of the starting material (DP 200). SEC of sample No. 9 revealed some oligomeric products resulting in a higher polydispersity of 4. Regarding the solubility of the samples major differences could be observed. The sample with homogeneous substituent distribution (No. 9) had the highest water solubility (92%, Table 3) followed by No. 8 (79% water solubility). Lowest water solubility (67%) was obtained for sample No. 7, which was preferably sulfated in C_6 position. It was remarkable that the ratio between intrinsic viscosity and molar mass varied strongly between these samples, indicating that the synthesis concept and substitution pattern have a strong impact on the macromolecular properties.

Due to the low DS of samples endoglucanase treatments led to intense fragmentation of all cellulose sulfates. Accordingly no viscosimetric detection could be performed, and molar masses had to be calculated using dextran calibration. Solubility of samples substituted preferably in C_6 and C_2 was not influenced by enzymatic fragmentation. For the homogeneously substituted sample 9, which excelled by the best solubility prior to enzyme treatment, a further increase of 8% occurred (Table 3). This effect confirmed that differences in solubility observed for the starting materials did not result from molar mass, but from the substitution pattern.

After fragmentation the C_6 substituted cellulose sulfate (No. 7) had a molar mass twice as high compared to all other samples. This was remarkable since this material excelled by the lowest DS value within this set of data. A tailing to high molar mass fragments was evident from the SEC elution curve and resulted in a high polydispersity index. The other two samples did not differ in average molar mass, but showed different degradation patterns. The homogeneous substituted sample had a significant higher polydispersity, which could be attributed to a pronounced fraction of residual higher molar mass material and high amounts of monomeric degradation products

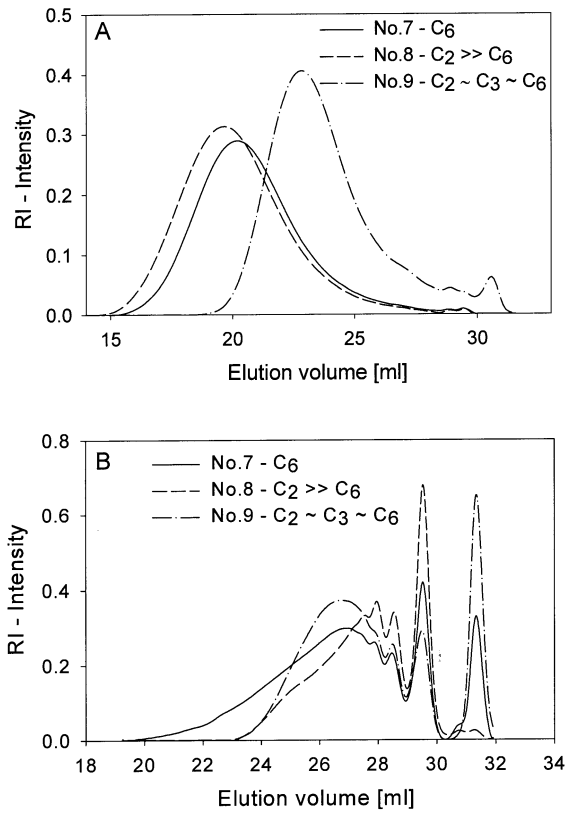

Fig. 2. Influence of endoglucanase fragmentation on SEC elution curves of cellulose sulfates with different substitution patterns. (A) Reference; (B) Endoglucanase fragmented samples.

(31–31.5 ml, Fig. 2b). Considering that the sample preferably substituted in C_2 had a starting DP twice as high as the homogeneous substituted sample, the fragmentability of 11.1 calculated for this sample is much higher than for No. 7 and 9 (Table 3). Accordingly it can be concluded that the C_2 substituted sample was more accessible to endoglucanase action. Especially a substitution in the C_6 position seems to reduce the enzymatic degradation. This is in contrast to what is known from the enzymatic degradation of methylcellulose. Regioselectively substituted 6-*O*-methylcellulose was degraded by the cellulase of *Trichoderma viride*, while 2,3-di-*O*-methylcellulose was not (Kondo & Nojiri, 1994, Nojiri & Kondo, 1996). This contrast can be explained either by the different enzyme source or by the different charge of the substrates used in both studies. Certainly the latter point is most important.

3.4. Evaluation of the influence from the derivatisation procedure

Commenting on the enzymatic degradation of samples according to their DS or substitution pattern the question arises whether the different derivatisation procedures are influencing the observations. This evaluation of these effects can be facilitated by comparisons of the fragmentability index and the polydispersity index as presented in Tables 2 and 3. For samples 1, 9 and 3, produced by homogeneous sulfatation from CA (Ho 1), the fragmentability decreases with increasing DS (0.37, 0.45, 0.75) in the order $12.8 > 6.0 > 2.4$. Samples 7 and 5 (DS 0.4, 0.99) derived from the concept of preferred C_6-sulfation by competitive esterification (HetHo) show a decrease in fragmentabilty from 5.2 to 1.1 with the DS. The preferred C_6-sulfation of cellulose by heterogeneous reaction (Het) applied to samples 2 and 4 led to DS of 0.50 and 0.80 and a fragmentability of $7.7 > 2.7$. As could be expected the dependence of enzymatic fragmentation from the DS is valid for all synthesis concepts. However, samples from the homogeneous reaction (Ho1) seem to have the higher fragmentability based on a comparative DS.

Another interesting feature is the evaluation of polydispersity index determined after endoglucanase fragmentation of samples with a DS up to 0.8, which are highly accessible for the endoglucanase. High polydispersities of 10.0–10.8 were observed for samples 2, 7 and 4 which are derived from heterogeneous reaction systems. The homogeneous produced samples 1, 3, 8 and 9 have comparably lower polydispersities of 6.4, 5.2, 3.2 and 8.1. This might be an indication for a more homogeneous substitutent distribution along the polymeric chain.

For the production of samples with different partial substitution the application of different derivatisation procedures is a prerequisite. Therefore the effect of different reaction conditions can overlay the effect of partial substitution regarding the enzymatic fragmentation as deducted from samples 7, 8 and 9. However, since all three samples are derived from homogenous reaction systems the conclusion regarding the stronger inhibition of enzymes by the C_6 substitution seem to be valid.

4. Conclusion

The investigation demonstrated that cellulose sulfates could be intensively fragmented by endoglucanase at least up to a DS of 0.8. A significant modification of the derivatives could be achieved up to DS 1.46. The enzyme did not only modify the molar masses and viscosity of samples, but as well the solubility in the aqueous system.

While only the liberation of glucose was detected in previous studies, the SEC investigation enabled us to gain more detailed information. For samples with DS < 1 the intensity of fragmentation was comparative to carboxymethyl cellulose. For higher substituted samples the fragmentation was inferior to carboxymethyl cellulose, cellulose acetate or methyl cellulose. Nevertheless, it could be demonstrated that even at higher substitution levels up to DS 1.46 the enzymatic modification or enzyme-aided analysis of cellulose sulfates can be pursuit.

The investigations on samples with different substitution pattern indicated that a homogeneous substituent distribution in all three positions of the anhydroglucose unit resulted in improved solubility of the derivatives.

Results obtained after enzymatic fragmentation strongly suggested that the C_6 position inhibited the enzyme most effectively. This is note worthy since for methyl cellulose endoglucanase tolerates substituents in the C_6 position.

For low DS values ($<$ DS 0.8) samples resulting from homogenous reaction systems are more intensively fragmented and the resulting products have lower polydispersities, which might be an indication of a more homogeneous substituent distribution along the polymeric chain. It can be assumed that homogeneously produced samples of higher DS contain only few non-substituted regions, assessable to enzymatic action. Accordingly samples of higher DS are probably more intensively degraded, when they have been prepared under heterogeneous reaction conditions.

Further studies on cellulose sulfates from homogeneous and heterogeneous reactions should be extended to samples with higher substitution degree to complete the understanding of the different influencing factors in this system.

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