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Synthesis and Bioactivity of Cellulose Derivatives

Kai Zhang,[1] *Dieter Peschel,*[2] *Erica Brendler,*[3] *Thomas Groth,*[2] *Steffen Fischer**[1]

Summary: Cellulose derivatives having carboxyl- or carboxymethyl- and sulphate groups were synthesized with control of reaction conditions to regulate the distribution of substituents and molecular weights of the products. Sodium cellulose sulphates (NaCS) were synthesized through acetosulphation of cellulose or direct sulphating of cellulose and cellulose-2.5-acetate (C2.5A). The properties of the products were controlled by choice of starting materials and reaction parameters like reaction temperature and duration. Cellulose sulphates containing carboxyl groups were prepared through oxidation with 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)/NaBr/NaClO in water or through carboxymethylation with chloroacetic acid after alkali treatment with sodium hydroxide. The biological activity of the products was analyzed with a binding assay to fibroblast growth factor (b-FGF). It was found that NaCS with maximum *O*-6-sulfation and intermediate to high *O*-2-sulfation was able to bind b-FGF comparable to natural heparin. Products being sulphated and afterwards carboxymethylated at all three positions were also able to bind substantial quantities of b-FGF.

Keywords: b-FGF; cellulose derivatives; growth factor; NMR; synthesis

Introduction

Cellulose is naturally in large amount produced. Many products have been synthesized from this polysaccharide like cellulose acetate and carboxymethyl cellulose (CMC).[1–3] Cellulose sulphate, as one representative of these products, has attracted much attention because of its interesting biological effects. In previous studies, NaCS has shown anticoagulant and antiviral activity.[4–6] Also it has expressed interesting and important characteristics like enzymatic degradability and solubility in water and in dimethyl sulphoxide.[7,8]

To this end many efforts have been made to synthesize NaCS with distinct substitution patterns. Philipp and Wagenknecht have developed a number of methods to prepare NaCS with specific substitution patterns and degree of substitution (DS) up to 3.0.[8–10] Later on Yamamoto et al. reported the synthesis and structure of sulphated cellulose with antiviral activity.[11] Recently, Hettrich and his colleagues have reported some new possibilities on the acetosulphation of cellulose. Acetosulphation in different solvents with different sulphating agents and acetylating agents has been shown.[12] However, little attention has been paid to the synthesis of polysaccharides containing both sulphate- and carboxyl groups. The presence of other charged groups may have an influence on the properties of NaCS.

COC and CMC are widely applied products. Cellulose can be oxidized by a TEMPO-mediated reaction.[13] TEMPO-oxidized cellulose can potentially be used as ion absorber[14] and can also be used to prepare cellulose nanofibres.[15] CMC is an

[1] Institute of Wood and Plant Chemistry, Dresden University of Technology, Pienner Straße 19, 01307 Tharandt, Germany
Fax: (+49) 35203 38 31201;
E-mail: sfischer@forst.tu-dresden.de
[2] Biomedical Materials Group, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Halle (Salle), Germany
[3] Institute of Analytical Chemistry, Freiberg University of Mining and Technology, Freiberg, Germany

important product from cellulose and possesses many useful properties like thickening, emulsification and good compatibility with skin. CMC is therefore widely used, e.g. as additive in food, medicine and cosmetic or as thicken former.[3,16]

Glycosaminoglycans like heparin or heparan sulphate were studied in respect to their ability to bind growth factors like b-FGF. It was found that the binding is based on the interaction between negatively charged sulphate and carboxyl groups in the polysaccharides and basic amino acids in the proteins.[17] Also other polysaccharides like alginates or hyaluronans, which do not possess sulphate groups, exert a certain binding of growth factors similar to heparin.[18] However, investigations of heparan sulphate oligomers have clearly shown a direct correlation between the sulphating degree especially at *O*-6-position and the b-FGF- binding ability.[19] Corresponding to that different sulphated polysaccharides like colominic acid, dextran or (1→6)-α-D-mannopyran have presented an increasing support of the mitogenic activity of FGFs, which depended on their sulfation degrees.[20,21] So far, only little attention[22] has been paid to the effect of sulphated as well as carboxylated cellulose concerning the binding of growth factors.

This study describes the synthesis of NaCS and NaCS containing carboxyl groups with a profound characterization of the chemical structure. In addition, the biological activity of different cellulose derivatives was studied regarding their ability to bind b-FGF.

Experimental Part

Materials

Microcrystalline cellulose (MCC) with a DP of 275–277 was received from J. Rettenmaier & Söhne GmbH (Rosenberg, Germany). Native cellulose (AC, with 97.0% alpha cellulose) with an average DP of 1180 was purchased from Buckeye Technologies Inc. (Memphis, USA). Cellulose-2.5-acetate was received from M&G Group (Verbania-Pallanza, Italy). C2.5A and both celluloses were used without further treatment. *N,N*-Dimethyl formamide (DMF) was distilled and water was deionized before use. Unfractionated heparin from porcine intestinal mucosa was purchased from Calbiochem. Other chemicals were all of laboratory grades and used as received. Applied Dialysis membrane from Spectrum Laboratories Inc. (Rancho Dominquez, USA) has an approximate molecular weight cut off of up to 500 Daltons.

Sulphating of Cellulose and C2.5A

The sulphating could be carried out either as acetosulphation or direct sulphation.

For a typical acetosulphation, 5 g cellulose were suspended in 250 ml anhydrous DMF and the system was kept at RT for over 14 h. The reaction agent – chlorosulphuric acid or sulphuric acid and acetic anhydride in DMF – was prepared by slowly adding the sulphating agent into 50 ml DMF within 10 min under cooling, followed by the addition of acetic anhydride. After cooling to the RT, the reaction agent was dropped into the cellulose suspension under vigorous stirring within 15 minutes. The system was heated to 50 °C and kept for a period of 5 h. After a designated reaction time, the mixture was cooled down to RT and poured into a saturated ethanolic solution of anhydrous sodium acetate. Then the precipitate was washed with 4% sodium acetate solution in ethanol and deacetylated with 1 M ethanolic solution of sodium hydroxide for at least 15 h. The pH was adjusted to 8.0 with acetic acid/ethanol (50/50, m/m). After washing with ethanol the product was dissolved in water, filtered and lyophilized.

During the direct sulphation of cellulose and C2.5A acetic anhydride was not used. After the sulphation the mixture was cooled down to the RT, poured into the saturated ethanolic solution of anhydrous sodium acetate and washed with 4% sodium acetate solution in ethanol. After that the product was dissolved in water and

the pH was adjusted to 8.0 with acetic acid/ethanol (50/50, m/m). After washing with ethanol the product was dissolved in water, filtered and lyophilized. All products were dialysed in deionized water before they were used for elementary analysis and binding the b-FGF.

Oxidation of NaCS

2.5 g NaCS was dissolved at first in 150 ml water. Then the oxidation agent consisting of TEMPO, NaBr and NaOCl in water was prepared under continuous stirring until complete dissolution. The oxidation was started after adding the oxidation agent slowly to the solution of NaCS under stirring. The remaining NaOCl was dropped into the solution to maintain the pH at 10.5. After addition of the rest NaOCl the pH was maintained constant for up to 4 h using 0.5 M NaOH solution. Thereafter 5 ml of methanol were added to stop the oxidation and the pH was then adjusted to 7.5 with 0.5 M HCl solution.

After the oxidation the system was poured into 5 volumes of ethanol, stirred for 0.5 h and centrifuged. The precipitate was washed three times with ethanol/water (80/20, v/v), dissolved in water, filtered and lyophilized. The products were dialysed in deionized water before they were used for elementary analysis and binding the b-FGF.

Carboxymethylation of NaCS

In a typical case the NaCS was suspended in isopropyl alcohol. The reaction mixture was stirred for 0.5 h. Then NaOH solution (3.75 M) was dropped into the suspension and the mixture was stirred for 3 h. Following that the chloroacetic acid was added in solid state. Then the system has been kept at 55 °C for 5 h. After cooling down the solution to RT the reaction mixture was precipitated in five volumes of ethanol under stirring for 0.5 h. The precipitate was isolated by centrifugation and dissolved afterwards in water. The pH value was adjusted to 7.5 with acetic acid/water (50/50, v/v). The solution of the product was then precipitated again in 5 volumes of ethanol and a centrifugation was followed. After three times washing with ethanol/water (80/20, v/v) the product was dissolved in water, filtered and lyophilized. The products were dialysed in deionized water before they were used for NMR spectroscopy, elementary analysis and binding the b-FGF.

Characterization of Reaction Products

The ^{13}C-NMR spectra were recorded at RT using a Bruker DFX 400 spectrometer with samples dissolved in D_2O, with 30° pulse length, a frequency of 100.13 MHz, 0.3 acquisition time and relaxation delay of 3 s. Scans between 5000 and 20000 were accumulated.

^{1}H-NMR was analyzed according to ref.[23] and the spectra were obtained after the hydrolysis in 25% D_2SO_4/D_2O using a Bruker Ultrashield 500 Plus spectrometer with a frequency of 500.13 MHz and accumulation number between 8 and 16.

FT Raman spectra of the samples in small metallic discs were recorded on a Bruker MultiRam spectrometer with a liquid-nitrogen cooled Ge diode as detector. The spectra were recorded over a range of 3500–0 cm^{-1} using an operating spectral resolution of 3 cm^{-1}.

Elemental analysis was carried out using CHNS Elemental Analyser EuroEA 3000 from EuroVector (Italy). The contents of C, H, N and S were determined and the DS_S values were calculated.

Binding of b-FGF to the Cellulose Derivatives

The binding affinity of the synthesized cellulose derivatives to the growth factor b-FGF was performed with a competition assay using heparin agarose beads (Fluka, Biochemica). 25 ng of b-FGF obtained from InVitrogen were mixed with heparin agarose beads and PBS and agitated for 30 min at 200 rpm at RT to allow the binding of growth factors to the beads. The unbound growth factor was removed by two times washing with PBS. For the release of the growth factor from the beads cellulose derivatives or heparin (control) was added to the mixture and agitated for 30 min at

200 rpm at RT. After centrifugation the supernatants with the polysaccharides and the released b-FGF were applied to cellulose nitrate membrane in a slot-blot apparatus. A primary antibody against b-FGF (Sigma) and a horseradish peroxidase labelled secondary antibody (Dianova) were applied to the membrane to label bound growth factors. Detection was performed with ECL plus chemiluminescence kit and a CCD camera (Raytest, Diana 2). The quantification of the signals was done by ImageJ.

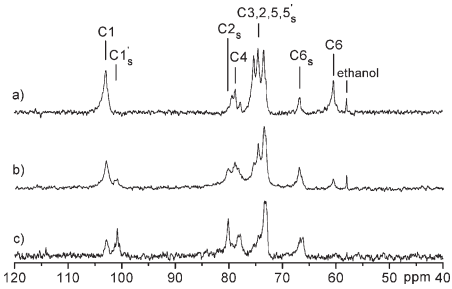

Figure 1.
^{13}C-NMR spectra of NaCS in D_2O at RT: a) CS5 ($DS_S = 0.37$), b) CS6 ($DS_S = 0.92$) and c) CS7 ($DS_S = 1.57$).

Results and Discussion

Synthesis of NaCS

NaCS could be prepared either through acetosulphation or through direct sulphation with sulphating agent under different conditions as shown in Table 1. Figure 1 shows the ^{13}C-NMR spectra of three NaCS with different DS_S values. The chemical shifts of unsubstituted C_1–C_6 are located at 102.8, 74.5, 73.4, 79, 75 and 60.4 ppm. The signal of C_6 is shifted from 60.4 to 66.7 ppm after sulphation and that of C_2 from 74.5 to 80.2 ppm.[5,9,11,12] The substitution at *O*-3-position could be detected with ^{13}C-NMR spectroscopy (as a peak at around 82.1 ppm), but the calculation of the DS_S value at *O*-3-position is with this method almost not possible. The elemental analysis offers us a chance to determine the sulphur content in the products and therefore to calculate the total DS_S values (Table 1).

Figure 2 represents the Raman spectra of two NaCS with different DS_S values. A new peak at 1073 cm^{-1} appears in the spectra of NaCS while it is not a real peak in the spectrum of MCC. Also this peak is obviously sharper in the spectrum of NaCS with higher DS_S value than that in the spectrum of the NaCS with low DS_S value. In addition in the spectrum of NaCS with

Table 1.
Sulphation of cellulose and C2.5A and characterization of end products.

Samples	Starting Material	Molar ratio[a)]	T[b)] (°C)	DS_S (^{13}C-NMR)[c)]				EA[d)] DS_S
				DS_{S6}	DS_{S2}	DS_{S3}	Total	
CS1	MCC	0.85/8	50	0.46	0.05	0[e)]	0.51	0.49
CS2	MCC	0.85/8	70	0.38	0.03	0	0.41	0.48
CS3	MCC	0.70/8	70	0.30	0.04	0	0.34	0.41
CS4	AC	0.55/8	50	0.35	0.03	0	0.38	0.34
CS5	AC	0.85/8	60	0.33	0.04	0	0.37	0.44
CS6	AC	3/8	50	0.77	0.15	0	0.92	n. d.
CS7	MCC	6/4	40	1	0.57	0	1.57	n. d.
CS8	MCC	4.5/0	RT	1	0.80	n. d.[e)]	> 1.80	n. d.
CS9	MCC	13/0	RT	1	0.94	n. d.	> 1.94	2.28
CS10	MCC	3/4	50	1	0.70	n. d.	> 1.70	n. d.
CS11	MCC	3/8	50	1	0.69	0	1.69	n. d.
CS12	C2.5A	5/0	RT	0.24	0.07	0	0.31	n. d.

[a)] Molar ratio in mol sulphating agent/acetylating agent per mol AGU. Sulphating agent: chlorosulphonic acid for CS1-9 and CS12, sulphuric acid for CS10-11; acetylating agent: acetic anhydride;
[b)] T: Temperature in °C. The reaction duration was 5 h. By CS8 and CS9 they were 6 and 3 h respectively;
[c)] DS_{SX}: DS values of sulphate groups on carbon C_6, C_3 or C_2;
[d)] EA: elemental analysis; resulted DS_S values of the products as total DS_S values;
[e)] 0: no substitution at O-3-position and $DS_{S3} = 0$; n. d.: substitution not determined.

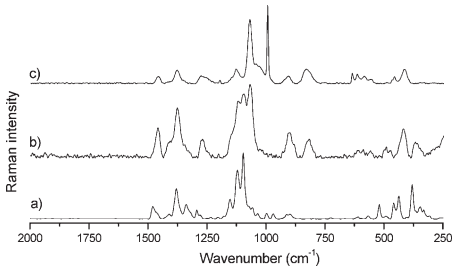

Figure 2.
Raman spectra of a) MCC, b) CS3 ($DS_S = 0.34$) and c) CS7 ($DS_S = 1.57$).

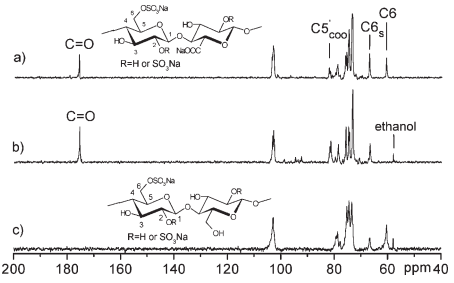

Figure 3.
^{13}C-NMR spectra of oxidized NaCS and NaCS in D_2O at RT: a) CS5-COC1, b) CS5-COC2 and c) CS5.

high DS_S value a new peak appears clearly at 994 cm^{-1}. Beside these findings a peak at 1461 cm^{-1} can be seen in both spectra of NaCS, which indicates the presence of cellulose II. In the spectrum of MCC there is no peak at 1461 cm^{-1} but two peaks at 1477 and 1455 cm^{-1} visible, which shows the presence of cellulose I.[24]

Based on the findings of the ^{13}C-NMR it can be found that the sulphation takes place primarily at *O*-6-position with the sulphating agents applied in this study. In NaCS with high DS_S values the *O*-2-position was also partly substituted (Figure 1). In CS9 the *O*-2-position could even be sulphated nearly completely with a DS_{S2} value of 0.94.[12] Also there was in CS9 a little sulphation on the *O*-3-position based on the results from elemental analysis.

From the results in Table 1 it can also be seen that the acetosulphation shows a preference to sulfate the *O*-6- and *O*-2-position in comparison to the direct sulphation. In almost all products obtained through acetosulphation there was no sulphation at *O*-3-position.

Oxidation of NaCS

NaCS could be oxidized homogenously in water and the products were soluble in water. The ^{13}C-NMR spectra of the products show a new signal at 175.5 ppm representing the shifted signal of C_6 after oxidation (Figure 3). Also the signals of the sulphated C_6 at 66.8 ppm are still visible. These results confirm that these substances contain both carboxyl and sulphate groups. Based on the above findings from ^{13}C-NMR, both functional groups are primarily selectively located at the *O*-6-position. As the substitution of sulphation in starting materials was homogeneous and both functional groups were located at the *O*-6-position, so the DS_{COO} values could be calculated through the change of amount of unsubstituted C6 using ^{13}C-NMR. Even more, the reduction of amount of the unsubstituted C_6 was just equal to the amount of C_6 that was substituted through carboxyl groups.

Moreover, a DS_{COO} value of 0.67 could even be reached under the applied reaction conditions (Table 2). According to the data in the Table 2 it is obvious that the increase of the oxidation agents can elevate the DS_{COO} values of the end products.

It is well known that the oxidation of native cellulose with TEMPO is very difficult.[13,25,26] In ref.[25,27] the native cellulose was not dissolved even after oxidation of 24 h at pH 10–11 and the end product was for the most part water-insoluble fraction. However, a regeneration or mercerization of cellulose could promote its solubility during the oxidation. Also with native cellulose as starting material, the carboxyl groups could not be introduced in great amount.[26]

But due to good solubility of NaCS in water the oxidation of NaCS was running homogeneously in water in this study and the end product was also in water soluble. Moreover, a DS_{COO} value of 0.67 could be simply reached. Because the crystallinity and the limited accessibility of the primary hydroxyl groups during the TEMPO-oxida-

Table 2.
Oxidation of NaCS with TEMPO/NaBr/NaClO at RT and pH 10.5.

Samples	Starting material	Mol ratio (mol per mol primary OH-groups)			Time (h)	DS (^{13}C-NMR)[a]	
		TEMPO	NaBr	NaClO		DS_{S6}	DS_{COO}
CS5-COC1	CS5	0.05	1.5	8	3	0.33	0.42
CS5-COC2	CS5	0.1	3	16	3	0.33	0.67

[a] DS values of sulphate groups and carboxyl groups, determined with ^{13}C-NMR.

tion are the main reasons for the low reactivity of native cellulose towards the oxidation. It is obvious that NaCS dissolved in water showed a better reactivity than native cellulose and due to the presence of sulphate groups the primary hydroxyl groups were more attendant to be oxidized.

Carboxymethylation of NaCS

The synthesis of NaCS containing carboxymethyl groups with CMC as starting material was described by Vogt et al. and Schnabelrauch et al.[28,29] In order to get such products it is also possible to carboxymethylate NaCS (Table 3). The obtained products were soluble in water. In the Figure 4 new signals at 178.4, 178.5 and 179.3 ppm can be observed in the spectrum of CS2-CMC, which are the chemical shifts of carbonyl groups on different positions.[3,23] There are also other new peaks in the spectrum of CS2-CMC in comparison to that of CS2: peaks at 71.8, 70.9 and 70.2 ppm of the methylene groups on carbons C_2, C_3 and C_6; also peaks at 82.7 and 69.3 ppm as a result of presence of carboxymethyl groups in products. In addition to the peaks of carboxymethyl groups the signal of sulphated C_6 at 66.7 ppm is still visible in the spectrum of CS2-CMC.

Using ^{1}H-NMR the carboxymethylation of cellulose sulphate could be analyzed[30,31] and the DS_{CM} values of these products can be found in Table 3. A total DS_{CM} value of 1.47 could be reached and the *O*-6- and *O*-2-positions were preferred during the carboxymethylation in comparison to the *O*-3-position.

According to the results in Table 3, the heterogeneous carboxymethylation of NaCS took place preferentially at *O*-6-position and more carboxymethyl groups were introduced at *O*-2-position than at *O*-3-position. Also the presence of the sulphate groups may influence the carboxymethylation of NaCS. With more sulphate groups at *O*-6-position the carboxymethylation at this position may be hampered and that at the *O*-2-position may be preferred.

Binding Affinity of b-FGF to Cellulose Derivatives

As shown in Figure 5 NaCS (samples CS2–CS6) with DS_S values at *O*-6-position below 1 and at *O*-2-position below 0.57 were not able to bind substantial quantities of b-FGF. However, the products with maximum sulphation at *O*-6-position and intermediate to high sulphation at *O*-2-position could accomplish a binding of b-FGF (samples CS7-CS11), which was positively

Table 3.
Carboxymethylation of NaCS with $ClCH_2COOH$ and NaOH at 55 °C for 5 h.

Samples	Starting material	Mol ratio (mol per mol free OH-groups)		DS_{CM}[a]			DS_S[b]
		NaOH	$ClCH_2COOH$	DS_{CM2}	DS_{CM3}	DS_{CM6}	
CS2-CMC	CS2	4	2	0.3	0.25	0.46	0.41
CS12-CMC	CS12	4	2	0.54	0.26	0.67	0.31

[a] DS values of carboxymethyl groups, determined with ^{1}H-NMR;
[b] DS values of sulphate groups, determined with ^{13}C-NMR.

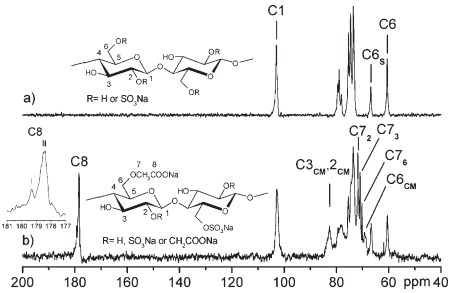

Figure 4.
^{13}C-NMR spectra of a) NaCS (CS2) and b) carboxymethylated NaCS (CS2-CMC) in D_2O at RT.

correlated to the overall degree of sulphation. The sample CS9 with complete sulphation at *O*-6-position, a high sulphation at *O*-2-position (0.94) and additional sulphation at *O*-3-postition has shown a binding effect to b-FGF of more than 60% respective to heparin. Because the cooperative activity between heparinoids like heparan sulfate and FGF is due to the presence of negatively charged substituents of the polysaccharides and the basic amino acids of FGF, a higher degree of sulphation or carboxylation can lead to a better binding to the growth factors. Hence, the finding of this study is in good agreement with previous work showing that the increase of a-FGF dependent proliferation of 3T3-L1 fibroblasts was related to the sulphation degree of (1→6)-α-D-mannopyran sulphate[21] or sulphated colominic acid.[20] On the other hand the cellulose derivative CS12-CMC with a low sulphation on C_6 (0.24) but a DS_{CM} value of total 1.47 with carboxymethyl groups at three positions provoked a prominent binding to b-FGF. The results of Hatanaka[22] have shown that CMC with a DS_{CM} value of 1.5–2.4 or polyacrylic acid could not provoke a significant increase in the a-FGF dependent proliferation. This leads to the conclusion that carboxyl or carboxymethyl groups alone do not have a sufficient a-FGF binding ability. There must also be sulphate groups in the compound.

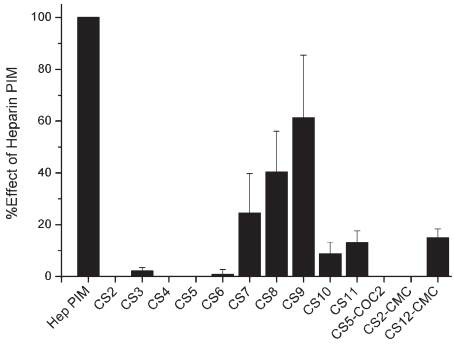

Figure 5.
Effects of cellulose derivatives and natural heparin on the competitive release of b-FGF. Hep PIM: heparin from porcin intestinal mucosa. CS2-11: NaCS with different substitution patterns and DS_S values. CS5-COC2: oxidized NaCS with CS5 as starting material. CS2-CMC and CS12-CMC: carboxymethylated NaCS with CS2 and CS12 as starting material respectively.

Conclusion

The present work shows that it was possible to prepare NaCS with different DS_S values through quasi-homogeneous acetosulphation or direct sulphation. The reaction conditions had influences on the DS_S values of end products. With a higher amount of chlorosulphonic acid and less acetic anhydride a higher DS_S value could be attained. Almost all NaCS obtained through acetosulphation showed a preferred sulphation at *O*-6- and *O*-2-position and no sulphation at *O*-3-position in comparison to the direct sulphation. The primary hydroxyl groups in the synthesized NaCS could be converted into carboxyl groups selectively at *O*-6-position through a TEMPO mediated oxidation. Because of the water solubility of NaCS a homogeneous oxidation could be carried out and the end products were in water soluble. Besides that a relatively higher content of carboxyl groups has been achieved compared to that of oxidized MCC. Also the hydroxyl groups in NaCS could be carboxymethylated with chloroacetic acid after alkali treatment with sodium hydroxide solution, but this procedure was not selective and the carboxymethyl groups were found at all three positions on the basis of the findings of ^{13}C-NMR, although the *O*-6-position was a little more preferred.

The rarely sulphated NaCS with preferred sulphate groups at *O*-6-position have not shown significant release of b-FGF. NaCS with maximal sulphation at *O*-6-postion and middle to high sulphation at *O*-2-position was able to bind b-FGF in a way almost comparable to natural heparin. Carboxymethylated NaCS having a low DS_S value at *O*-6-position and a high DS_{CM} value of 1.47 with a distribution of carboxymethyl groups over all three positions have shown also a prominent binding to b-FGF.

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[1] D. Klemm, T. Heinze, B. Philipp, W. Wagenknecht, *Acta Polymerica* **1997**, *48*, 277.
[2] E. Samios, R. K. Dart, J. V. Dawkins, *Polymer* **1997**, *38*(12), 3045.
[3] T. Heinze, A. Koschella, *Macromol. Symp.* **2005**, *223*, 13.
[4] T. Groth, W. Wagenknecht, *Biomaterials* **2001**, *22*, 2719.
[5] Z. M. Wang, L. Lin, B. S. Zheng, N. Normakhamatov, S. Y. Guo, *Int. J. Biol. Macromol.* **2007**, *41*, 376.
[6] N. D. Christensen, C. A. Reed, T. D. Culp, P. L. Hermonat, M. K. Howett, R. A. Anderson, L. J. D. Zaneveld, *Antimicrobial Agents and Chemotherapy* **2001**, *45*(12), 3427.
[7] B. Saake, J. Puls, W. Wagenknecht, *Carbohydr. Polym.* **2002**, *48*, 7.
[8] B. Phillipp, W. Wagenknecht, *Cellul. Chem. Technol.* **1983**, *17*, 443.
[9] I. Nehls, W. Wagenknecht, B. Philipp, A. Zschunke, *Acta Polymerica* **1985**, *36*(12), 657.
[10] B. Phillipp, I. Nehls, W. Wagenknecht, *Carbohydr. Res.* **1987**, *164*, 107.
[11] I. Yamamoto, K. Takayama, K. Honma, T. Gonda, K. Matsuzaki, K. Hatanaka, T. Uryu, O. Yoshida, H. Nakashima, N. Yamamoto, Y. Kaneko, T. Mimura, *Carbohydr. Polym.* **1991**, *14*, 53.
[12] K. Hettrich, W. Wagenknecht, B. Volkert, S. Fischer, *Macromol. Symp.* **2008**, *262*, 162.
[13] P. S. Chang, J. F. Robyt, *J. Carbohydr. Chem.* **1996**, *15*(7), 819.
[14] T. Saito, A. Isogai, *Carbohydr. Polym.* **2005**, *61*, 183.
[15] T. Saito, S. Kimura, Y. Nishiyama, A. Isogai, *Biomacromolecules* **2007**, *8*, 2485.
[16] H. Zhao, F. Cheng, G. Li, J. Zhang, *Int. J. Polym. Mat.* **2003**, *52*, 749.
[17] J. Schlessingr, A. N. Plotnikov, M. Mohammadi, *Molecular Cell* **2000**, *6*, 743.
[18] I. Freemann, A. Kedom, S. Cohen, *Biomaterials* **2008**, *29*, 3260.
[19] D. A. Pye, R. R. Vives, J. E. Turnball, P. Hyde, J. T. Callagher, *J. Biol. Chem.* **1998**, *273*, 22936.
[20] M. Kunou, M. K. Koizumi, K. Shimizu, M. Kawase, K. Hatanaka, *Biomacromolecules* **2000**, *1*, 451.
[21] M. Kunou, K. Hatanaka, *Carbohydr. Polym.* **1995**, *28*, 107.
[22] K. Hatanaka, T. Ohtsuki, M. Kunou, *Chem. Lett.* **1994**, *23*, 1407.
[23] A. Baar, W.-M. Kulicke, K. Szablikowski, R. Kiesewetter, *Macromol. Chem. Phys.* **1994**, *195*, 1483.
[24] K. Schenzel, S. Fischer, *Cellulose* **2001**, *8*, 49.
[25] A. Isogai, Y. Kato, *Cellulose* **1998**, *5*, 153.
[26] T. Saito, A. Isogai, *Biomacromolecules* **2004**, *5*, 1983.
[27] C. Tahiri, M. R. Vignon, *Cellulose* **2000**, *7*, 177.
[28] S. Vogt, T. Heinze, K. Röttig, D. Klemm, *Carbohydr. Res.* **1995**, *266*, 315.
[29] M. Schnabelrauch, T. Heinze, D. Klemm, I. Nehls, J. Kötz, *Polym. Bull.* **1991**, *27*, 147.
[30] F. F.-L. Ho, D. W. Klosiewicz, *Anal. Chem.* **1980**, *52*, 913.
[31] T. Heinze, K. Pfeiffer, *Angew. Makromol. Chem.* **1999**, *266*, 37.

