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Efficient Method for the Preparation of Pure, Water-Soluble Cellodextrines

Tim Liebert, Marit Seifert, Thomas Heinze*

Summary: An efficient method towards water soluble cellodextrine mixtures is described and detailed information on structure analysis is given. For cellulose degradation in good yield, treatment of cellulose with 85% phosphoric acid for 30 minutes at room temperature and a hydrolysis of 20 hours at 55 °C are suitable. With a work up in tetrahydrofuran (THF), a yield of almost 70% raw product is achievable. After separation of a water insoluble fraction, a yield of 53% of cellodextrines with a degree of polymerization (mean weight average, $\overline{DP}_w$) of 7.5 and a polydispersity index of 1.7 is obtained. These cellodextrines are soluble in water, dimethyl sulfoxide (DMSO) and dimethyl acetamide (DMA)/LiCl. Detailed structure analysis by one and two-dimensional NMR, FTIR and mass spectroscopy revealed that the substance consists only of β-1 $\rightarrow$ 4 linked glucoses. A partial functionalization was excluded by ^{13}C NMR- and ^{31}P decoupled ^{1}H NMR spectroscopy.

Keywords: cellodextrine; cellulose degradation; gel permeation chromatography; NMR spectroscopy

Introduction

Numerous phenomena related to dissolution processes and functionalization steps of the polysaccharide cellulose or the interaction of cellulose with other biomolecules such as proteins can not be investigated directly with the high molecular weight cellulose itself. This is due to the extensive hydrogen bond system, which is responsible both for the inaccessibility of the molecule and for the insolubility in common solvents. Moreover, the high molecular mass of the polysaccharide interferes with a variety of analysis such as nuclear magnetic resonance (NMR) spectroscopy, which may yield spectra of limited resolution. Consequently, there is a need for suitable cellooligomers for such basic investigations. Cellobiose is not reasonable as mimic for cellulose because its reactivity is still drastically influenced by the reducing end group. Nevertheless, labeled cellobiose may be used to study the hydrogen bond network of cellodextrines and cellulose.[1,2] The water soluble cellooligomers, so called cellodextrines, can overcome this problem. Fractions of cellooligomers with adjusted molecular masses could be applied as model systems to extrapolate on the properties of the biopolymer. Thus, different paths for the cellulose degradation were investigated to prepare pure cellodextrines with defined size.

A variety of aerobic[3,4] and anaerobic[5,6] microorganisms can produce enzymes for the biodegradation of the polysaccharide.[7] Nevertheless, an enzymatic depolymerization of cellulose is slow and provides cellodextrines only to small amount at high costs in comparison to a chemically catalyzed hydrolysis.[8] The same is true for degradation reactions via functionalization.[9] An alkaline degradation leads to a diversity of structures dependent on the alkali used, the concentrations applied and the reaction temperature.[10]

Center of Excellence for Polysaccharide Research, Friedrich Schiller University of Jena, Humboldtstrasse 10, D-07743 Jena, Germany
**Member of the European Polysaccharide Network of Excellence (www.epnoe.eu)
E-mail: tim.liebert@uni-jena.de

The depolymerization of cellulose by thermo-mechanical treatment in ethylene glycol under high pressure yields celluloses with a degree of polymerization (DP) of about 50 at the best.[11] Consequently, acid hydrolysis of cellulose seems to be a good choice with regard to soluble products of small chain length. Carboxylic acids are not suitable for cellulose hydrolysis because their acidity is too low for effective splitting of the glycosidic bonds. The treatment of cellulose with sulphuric acid or nitric acid may lead to sulphation and nitration, respectively.[12] If hydrochloric acid is used, problems result from the heterogeneous reaction conditions.[13] Even in case of dissolution of the cellulose material at −30 °C, a yield of only 3% of cellooligosaccharides could be achieved.[14] In addition, precipitation of the depolymerized material with alcohols could result in rearrangement reactions. It can be avoided by a work up with aprotic solvents like ethers.[15] The application of phosphoric acid is advantageous because it is combined with decrystallization of cellulose to provide amorphous substrates for further hydrolysis.[16] Therefore, phosphoric acid is employed as swelling agent[17,18] and for effective hydrolysis of cellulose. Cellooligomers with DP values between 64 and 88[19] as well as defined fractions with DP of 7 and 15 respectively, were obtained. The depolymerization was carried out at room temperature leading to reaction times of several weeks.[20–22] The occurrence of rearrangement reactions was not investigated.

Thus, in this paper we describe an efficient degradation method for cellulose with phosphoric acid, work up in an inert organic solvent, and structure analysis of the resulting cellodextrine mixture.

Experimental Part

Materials

Cellulose (Avicel, PH-101, DP = 153, ~50 μm, No. 11365) was purchased from FLUKA (Buchs, Switzerland), orthophosphoric acid (H_3PO_4, 85%, extra pure), tetrahydrofuran (THF), ethanol, isopropanol, acetone, dimethyl sulfoxide (DMSO), dimethyl acetamide (DMA), LiCl and NaN_3 was purchased from MERCK (Darmstadt, Germany). Deuterium oxide (D_2O) was obtained from ALDRICH (Taufkirchen, Germany). THF was purified by distillation; all other solvents were used as received.

Analytical Methods

^1H, ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectra of cellodextrines (30 mg in 1 ml D_2O) were measured with a Bruker AC-400 spectrometer at 40 °C. Fourier transformation infrared (FT-IR) spectra were recorded on a Nicolet AVATAR 370 DTGS spectrometer using the KBr-technique. Elemental analyses were performed by means of a CHNS 932 Analyzer (Leco). Mass spectroscopy was accomplished with a matrix assisted laser desorption/ionisation – time of flight (MALDI-TOF) system by Bruker Daltonics Bremen with the software flexControl and flexAnalysis. The samples were dissolved in a mixture of water, 2,5-dihydroxybenzoic acid, acetonitrile as well as trifluoracetic acid and ionized with the minimum intensity of a N_2 laser. Molecular weight and degree of polymerization of weight average ($\overline{DP}_w$) were estimated applying gel permeation chromatography (GPC) on a JASCO system and a refractive index detector (RI-930) calibrated with pullulan and dextran standards. The water-based system was equipped with three columns (HEMA 40) using an aqueous solution of 0.05% NaN_3 as eluent with a flow rate of 0.5 mL min^{-1} at 80 °C. The dimethyl sulfoxide (DMSO)-based system was equipped with two columns (NOVEMA 3000 and NOVEMA 300) using a DMSO solution with 0.25% LiBr as eluent with a flow rate of 0.5 mL min^{-1} at 70 °C.

Synthesis of Cellodextrine, Typical Procedure

Avicel (5 g) was added to phosphoric acid (60 mL 85%) and stirred for 0.5 h at room temperature before heating for 20 h at

55 °C. The clear solution of a light brown color was poured into THF (600 mL), the white precipitate was collected and washed with THF until it became neutral and powdery. This raw product (about 5.4 g moist mass corresponding to 3.39 g dried mass as determined in separate experiment) was given into 30 mL water, stirred at room temperature for 0.5 h and left 1 h for sedimentation. The water insoluble fraction was separated by filtration with a glass filter (G3). The filtrate was concentrated (about 3 mL) and precipitated into THF (30 mL). The water soluble fraction was collected and dried in vacuum at 60 °C.

Fraction B of Sample 3

A cellodextrine obtained in this way (sample **3B**) was soluble in water, DMSO and dimethyl acetamide (DMA)/LiCl. Yield: 2.65 g (53%). Elemental analysis: calculated for anhydroglucose unit (AGU): C, 44.45%; H, 6.22%; calculated for glucose: C, 40.00%; H, 6.71%; found: C, 41.56%; H, 6.51%.

FT-IR spectroscopy: 3491, 3446, 3380 (OH); 2895 (CH_2); 1640 (adsorbed water); 1420 (CH_2); 1373 (CH); 1316 (CH_2); 1229, 1199 (OH); 1161 (COC); 1028, 896 (CO) cm^{-1}.

$\overline{DP}_w$ derived from GPC in DMSO: 7.5

Polydispersity index: 1.7.

MALDI-TOFMS: peak with the highest intensity at 689 u (4 glucose units with sodium; $Glc_4 + Na$), and intensive peaks with a difference of m/z 162 with decreasing intensity up to 2796 ($Glc_{17} + Na$).

^{13}C NMR spectroscopy: 102.56 (1n), 102.35 (1i), 95.77 (1rβ), 91.83 (1rα), 78.71 – 69.49 (2 r,i,n – 5 r,i,n), 60.62 (6n), 60.08 (6r), 59.96 (6i).

^1H NMR spectroscopy: 5.22 (H-1rα), 4.66 (H-1rβ), 4.53 (H-1i), 4.50 (H-1n), 4.10 – 3.20 (H2 r,i,n – H6 r,i,n).

Results and Discussion

Degradation Studies

To find the optimal reaction conditions for the hydrolysis of cellulose with 85% phosphoric acid (H_3PO_4), the concentration of cellulose (Avicel) in the medium, the time of swelling and hydrolysis as well as the reaction temperature were varied. The results are presented in Table 1. For the desired dissolution of the cellulose in phosphoric acid, the concentration has to be less than 600 mmol AGU per L 85% H_3PO_4. Thus, a concentration of 514 mmol/L (calculated on the basis of an AGU with a molar mass of 162 g/mol), i.e. a molar ratio of AGU: H_2O: H_3PO_4 of 1: 16: 29 was applied, to avoid large excess of phosphoric acid and tetrahydrofuran (THF). Swelling of the cellulose at room temperature is necessary to provide a large surface easily accessible for degradation most notably in case of microcrystalline cellulose. A swelling time of 30 minutes is sufficient to initiate dissolution. It was observed that

Table 1.
Results for the degradation of cellulose by variation of the concentration of Avicel (anhydroglucose unit; AGU) in 85% phosphoric acid, time of swelling, time of hydrolysis and reaction temperature.

No	Concentration [mol/L] = n (AGU)/V(85%H_3PO_4)	Time of swelling [h]	Time of hydrolysis [h]	Reaction temp. [°C]	Dissolution/ color of the mixture	Precipitate	Yield [%]*
1	0.7710	0.5	19	60	No/black	black residue	–
2	0.6168	4	20	50	No/brown	white substance	10.0
3	0.5140	0.5	20	55	Yes/brown	white substance	53.5
4	0.5140	60	29	65	No/black	black residue	24.3
5	0.3084	0.5	4	50	No/white	white substance	5.2
6	0.3084	2	20	55	Yes/white	white substance	30.2
7	0.3084	2	19	60	Yes/black	white substance	19.0
8	0.1542	2	20	90	No/brown	black residue	–

* Of water soluble fraction (see experimental section).

hydrolysis at 60 °C and at higher temperature led to brown or black colored mixtures and consequently to black residues, on one hand. On the other, using a reaction temperature of 50 °C, the degradation of Avicel could not be accomplished satisfactorily. The yield of water soluble cellodextrines was at most 10% of the raw material. If we adjusted the temperature at 55 °C we obtained finally a white product in good yields.

The shortest period of hydrolysis is limited by the time needed for dissolution of the cellulose. An interruption of hydrolysis in the heterogeneous state afforded only a very low percentage of water soluble cellooligosaccharides as shown in Table 1, entry 5. However if the time of hydrolysis was set too long, after a homogeneous solution was achieved, the formation of glucose would be promoted unnecessarily.[19] A clear, highly viscous solution was available within one day. In our experiments the best results were obtained after 20 hours; entry 3 (Table 1) summarizes the optimal reaction conditions regarding the concentration of Avicel in phosphoric acid (514 mmol/L 85% H_3PO_4), the time of swelling (0.5 h), the time of hydrolysis (20 h) and the reaction temperature (55 °C).

Different Work up

Although the composition of the hydrolyzate and the yield is basically determined by the reaction conditions, the work up of the degraded cellulose has a significant influence as well. As shown in Table 2, the precipitation of the raw product of sample **3** in different solvents is reflected by the yield considerably. In comparable experiments the best yield (67.7% of the raw product) could be observed for precipitation in THF. The products were dissolved in dimethyl sulfoxide (DMSO), and the filtrates were analyzed by GPC. The degrees of polymerization (weight average, $\overline{DP}_w$) indicated a separation during the work up in different solvents. Acetone gives a mixture with a $\overline{DP}_w$-value of 19.1. With ethanol predominantly smaller molecules were isolated ($\overline{DP}_w = 5.7$). The consistency of the precipitate was different. Thus, work up in isopropanol provided an ultra fine precipitate, which could not be collected in a conventional filtration. Long running centrifugation had to be applied. THF has proved to be the preferred separation medium regarding excellent yield, easy handling, and a raw product with a $\overline{DP}_w$ of 14.6.

Fractionation Studies

The desired cellodextrines should be completely water soluble.[22] Thus, fractionation of the decomposed cellulose was studied. In Table 3 the general procedure for the separation of the water soluble part out of the raw product obtained from THF and the assignment of the different fractions are summarized. Usually the fractionation was investigated by dispersing the wet raw product in water (about 180 g/L corrosponding to 0.7 mol/L dry mass), stirring the mixture at room temperature for 0.5 h and leaving it 1 h for sedimentation. The water insoluble part was separated by filtration, the filtrate was concentrated and precipitated into THF. Fraction B contained the water soluble cellodextrine mixture, which appeared to be additionally soluble in DMSO and in N,N-dimethyl acetamide (DMA)/LiCl. The upper limit for a clear solution was 30 g/L (0.19 mol/L). R, A and B were insoluble in common

Table 2.
The influence of different work up on the yield and on the degree of polymerization ($\overline{DP}_w$). The $\overline{DP}_w$ values (weight average: $\overline{DP}_w$) were derived from GPC in dimethyl sulfoxide (DMSO) of the raw product of sample **3**.

	Ethanol	Isopropanol	Acetone	Tetrahydrofuran
Yield of the raw product [%]*	46.6	55.5	53	67.7
$\overline{DP}_w$	5.7	14.6	19.1	14.6
Polydispersity index	1.3	1.9	4.0	2.1

* Over all yield.

Table 3.
General procedure of manufacturing and fractionation of the raw product, and the assignment of the different fractions.

Fraction	Manufacture and Isolation of the fraction	Component of the hydrolyzate
R	Hydrolysis of cellulose in H_3PO_4, precipitation in THF	Raw product
A	Raw product R in water, collecting of filter cake	Water insoluble part
B	Concentration of filtrate, precipitation in THF	Water soluble part (cellodextrine mixture)

solvents like methanol, ethanol, acetic acid, DMA, pyridine, acetone, THF and isopropanol.

The $\overline{DP}_w$ values of the fraction **B** (water soluble fraction) obtained for different hydrolysis (see Table 1) are shown in Table 4. Obviously, the reaction conditions for sample **4** promoted the hydrolysis of the cellulose much too strong.

Table 4.
The degree of polymerization values (of weight average: $\overline{DP}_w$) and polydispersity indices derived from GPC in DMSO of the water soluble fractions B separated from the various samples (of wet raw product).

Sample No.*	$\overline{DP}_w$	Polydispersity index
3	7.5	1.7
4	4.2	2.2
6	7.2	1.5
7	7.1	1.8

* The corresponding yields are given in Table 1.

The other attempts gave cellodextrine mixtures with $\overline{DP}_w$ of about 7 and a polydispersity index of about 1.5. However, the best yield (yields of the water soluble fractions **B** are listed in Table 1) of fraction **B** could be achieved for sample **3** with 53.5% (Table 1). A fractionation under the same conditions but starting with dried raw product of sample **3** gave identical yield and $\overline{DP}_w$, i.e. drying before fractionation is not necessary. A representative gel permeation chromatogram of such a sample is shown in Figure 1.

It demonstrates the composition of the cellodextrines of molecules with $\overline{DP}_w$-values mainly between 3 and 10 impressively.

To gain additional fractions with varying $\overline{DP}_w$ values, alternative fractionation attempt were carried out. The raw product of sample **3** was dried and given into water with concentrations of 0.5 mol/L and of

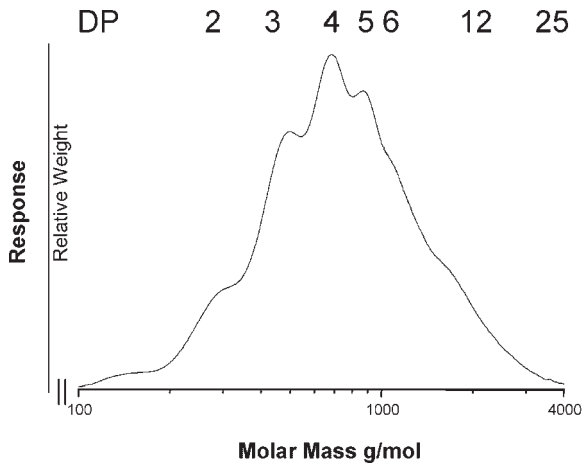

Figure 1.
Section of gel permeation chromatogram analyzed in water of the cellodextrine sample **3B** showing a mixture of cellooligomers. The corres-ponding $\overline{DP}_w$ (calculated from the molar mass determined versus the molar mass of an AGU) assigned on the top of the peaks.

Table 5.
Results for the fractionation experiment carried out with dried raw product of sample **3**. The yields of the fractionation step (regarding the starting raw product), the overall yields (regarding the starting cellulose material), and the degree of polymerization values (of weight average: $\overline{DP}_w$) as well as the polydispersity indices derived from GPC in DMSO.

Fraction	Yield [%]	Overall yield [%]	$\overline{DP}_w$	Polydispersity index
$\mathbf{B_{0.5}}$	71.7	44.5	12.2	3.0
$\mathbf{B_{0.1}}$	65.7	40.8	9.0	2.4

0.1 mol/L. The mixtures were agitated for 20 h at room temperature and left 1 h for sedimentation. After filtration, evaporation of the excess of water and precipitation into THF samples $\mathbf{B_{0.5}}$ and $\mathbf{B_{0.1}}$ were isolated (Table 5).

It can be shown that a longer reaction time and a lower concentration during the fractionation gives fractions with higher molecular weights but with a broader distribution compared to fraction **B** obtained from the short time fractionation of sample **3** ($\overline{DP}_w$ of 7.5, Table 4). Additionally, the lower concentration leads to a lower yield, which could be caused by the less efficient work up with the large excess of water. Repetition of the experiment does not show further fractionation. Therefore, the alternative fractionation can be utilized to vary the molecular weight of the products but the simple work up of the wet product (fraction **B**) is sufficient to gain the desired cellodextrines with a chain length between 2 to 12 and a small polydispersity index (1.7) in very good yields (53.5%) in a short time.

Structure Analysis

FTIR spectra of cellooligomers of all fractions were acquired and compared with those of cellulose spectra. No differences can be determined, i.e. no functionalization during the degradation is observed. Elemental analysis of the cellodextrines (**3B**) gave data appositely to the expected short chained cellulose structure. No

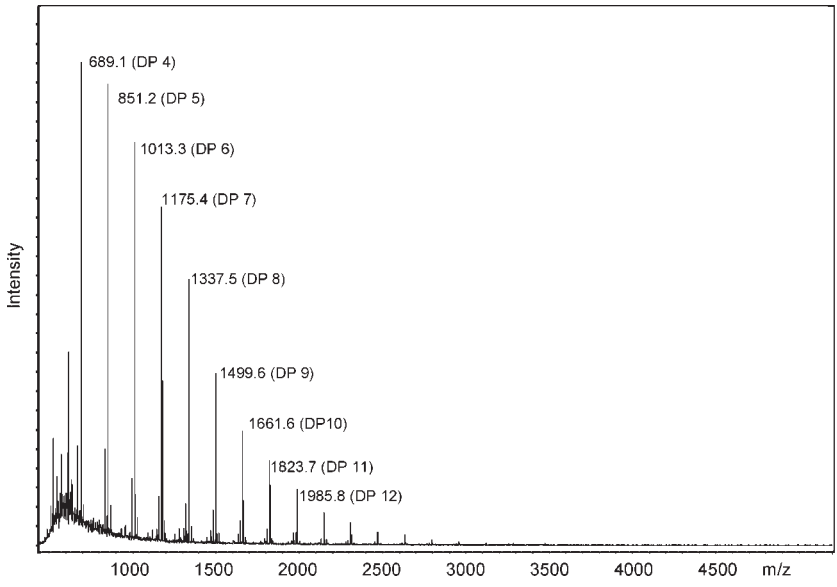

Figure 2.
MALDI TOFMS (ionization N_2 laser) of sample **3**. The corresponding $\overline{DP}_w$ (calculated from m/z versus molar mass AGU) are assigned on the peaks.

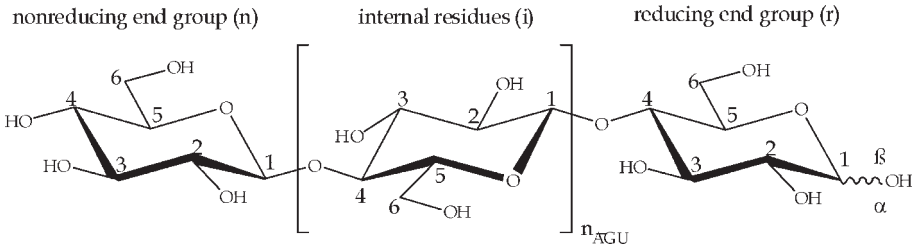

Figure 3.
Schematic structure of cellodextrines and assignment of the C atoms in the different glucose groups. n_{AGU} notes the number of the internal residues of anhydroglucose units (AGU).

phosphorous was determined. MALDI-TOF mass spectrometry was applied for a further proof of identity and documented the primary structure of the oligomers (Figure 2). It can be seen that the main peaks have a difference of m/z 162, which is an evidence for the pure cellodextrine structure consisting only of glucoses. The mass spectra support the results of the GPC (see Figure 1), i.e. most of the oligomers have 4–10 glucose units. It has to be mentioned that the absolute values of the molecular weight found for the oligomers with this MS method are m/z 41 higher than expected because adducts with sodium are formed. Therefore, the highest peak in the MS for the cellotetrose is m/z 689 instead of m/z 648.

NMR spectroscopy was used to confirm the structure of the cellodextrins shown schematically in Figure 3 and to exclude side reactions during the degradation. The ^{13}C NMR spectrum of the water soluble cellodextrines mixture in D_2O represented in Figure 4 reveals signals for all of the carbons in position 1 and 4 at the end groups and at the glycosidic bonds. It is a spectrum with a good spectroscopic resolution suitable for the NMR investigation of the interaction with cellulose solvents.

The assignment of the signals is in accordance with ref. [15]. The carbons at the reducing end groups exhibit a splitting into signals for the α-anomer at 91.8 ppm and for the β-anomer at 95.8 ppm. The appearance of comparably large signals for C-1rα and C-1rβ indicates a relative large portion of end groups as can be expected with a polymer with a $\overline{DP}_w$ of 7.5.

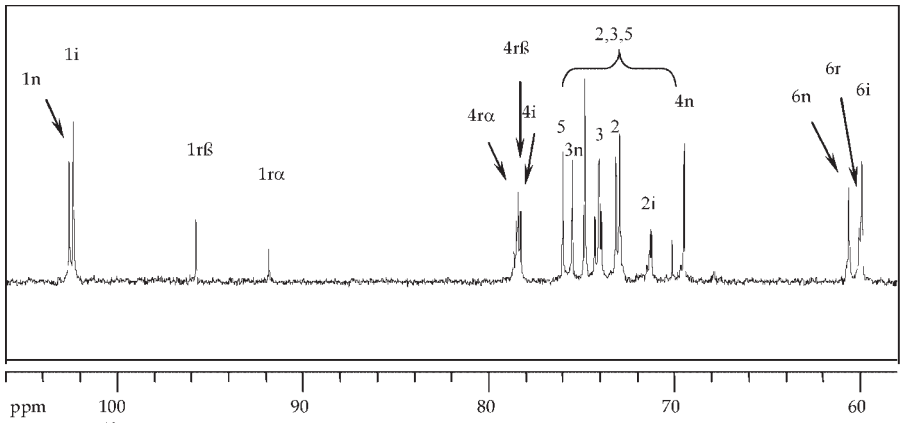

Figure 4.
^{13}C NMR spectrum of cellodextrines in D_2O, and the assignment of the signals to the C atoms (see Figure 3) according to[15].

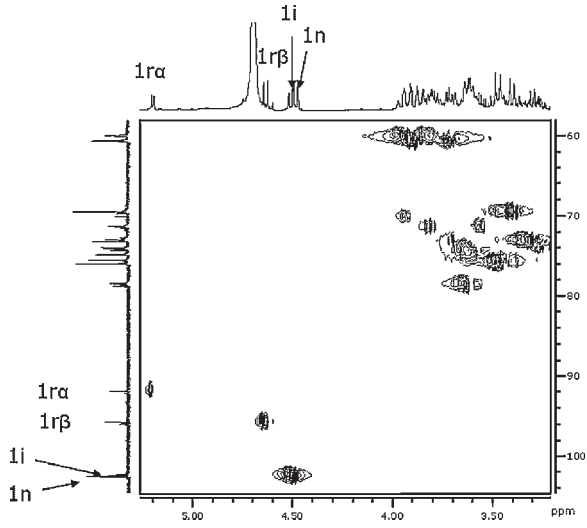

Figure 5.
$^{1}H,^{13}C$ HSQC NMR spectrum of the cellodextrines in D_2O.

Even the carbons in position 6 show a shift according to their position in the chain. There is only one signal at 102.1 ppm for bonds between two glucoses, namely the internal β-1,4 bond. The small shoulders at this signal are caused by the different chain lengths, i.e. a different number of neighboring groups. No hints for side structures, e.g. α-linkages or functionalization reactions are obtained. The ^{13}C NMR spectrum shows no signal at 63 ppm, which would be the chemical shifts for phosphate esters at position 6.[24]

In carbon-hydrogen correlation spectra ($^{1}H,^{13}C$ HSQC NMR spectroscopy) three distinguished cross-peaks for the three possible structural units in position 1, i.e. non-reducing end, reducing end and

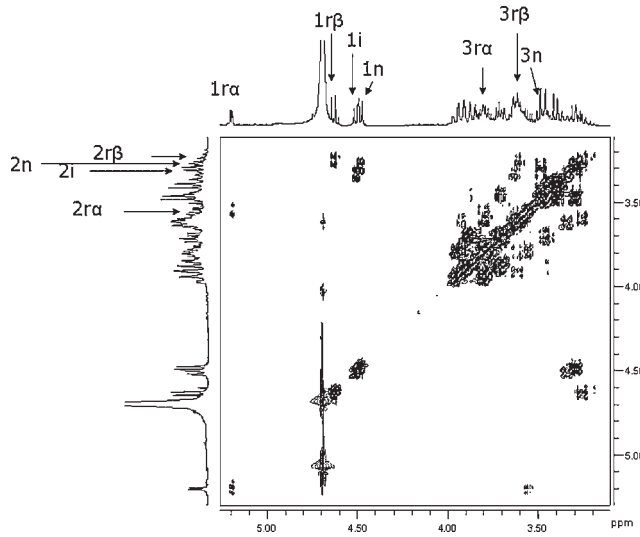

Figure 6.
$^{1}H,^{1}H$ COSY NMR spectrum of cellodextrines in D_2O.

internal glucose are visible (Figure 5) revealing the proposed structural uniformity (Figure 3), i.e. exclusive linkage of the glucoses via β-1 $\rightarrow$ 4 bonds.

In addition to the information about the purity of cellodextrines synthesized, the HSQC spectrum can be used for the assignment of the ^{1}H signals of the different protons in position 1 as shown in Figure 5, which agreed with results described in ref. [23]. Four doublets with centers at 4.50 (H-1n), 4.53 (H-1i), 4.66 (H-1rβ) and 5.22 (H-1α) ppm are observed corresponding to the four possible structures at position 1. The broadening of the signals is again due to different chain lengths of the oligosaccharides, i.e. a different number of neighboring groups as observed in ^{13}C NMR spectra. Further evidence for the structural uniformity of the cellodextrins was gained from ^{1}H,^{1}H COSY NMR spectroscopy (Figure 6). Complete assignment of all the signals between 3.2 and 4.0 ppm for the protons bound directly to the ring carbons is not possible because of the complexity and overlapping splitting patterns.

Nevertheless, it is obvious that the proton signals of position 1 only yield cross peaks with signals in the region 3.3 ppm to 3.6 ppm. In Figure 6 the regions for signals of the coupling protons in position 2 are assigned.

Especially the H-1i signal has only one distinguished cross peak to the H-2i signal excluding the possibility for a transglycosidation as observed for degradation reactions with HCl and precipitation in protic media. In addition the assignment for protons in position 3, with separate cross peaks is given.

To provide an evidence for the absence of phosphate functions, ^{31}P NMR spectra were recorded that showed no significant ^{31}P signals. Moreover, the comparison of a ^{1}H NMR spectrum and a ^{31}P decoupled ^{1}H NMR spectrum displayed no difference indicating that no phosphorus is bound to the cellodextrines (Figure 7).

Conclusion

The method for the preparation of water soluble cellodextrines described herein is shown to be very efficient regarding the high yields, short reaction time, and low costs. While easy to obtain, the cellodextrines provide an excellent solubility both in protic and aprotic solvents compared to cellulose as well as in ionic liquids having

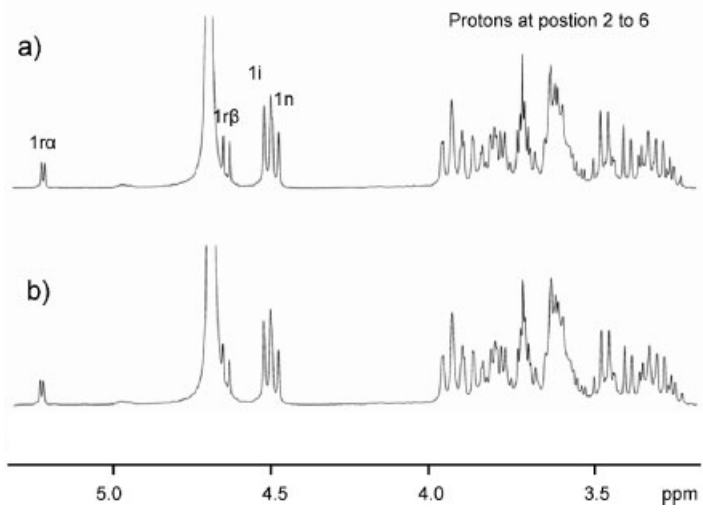

Figure 7.
Comparison of ^{1}H NMR spectrum (a) and ^{31}P decoupled ^{1}H NMR spectrum (b) of cellodextrines in D_2O.

typical reaction centers like cellulose. The molecular mass of the cellooligomer generates spectra of high resolutions and its primary structure comparable to that of cellulose allows an extrapolation on the polymer characteristics. First NMR experiments of the cellodextrines dissolved in ionic liquids such as 1-ethyl-3-methylimidazolium acetate are described in ref. [25]. The results indicating a reaction of the solvent molecule with the reducing end group of the cellooligomer. This effect is not known up to now and is still not completely understood. To investigate such effects in detail it is necessary to completely assign the NMR spectra of the cellodextrines. For this purpose two dimensional NMR studies are now under progress.

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