Query Details Back to Main Page

1. Please check and confirm organsiation division and organisation name are correctly identified for all the affiliations.

Changes added

2. Please check and confirm the inserted citation of Fig 3 is correct. If not, please suggest an alternative citation. Please note that figures and tables should be cited in sequential order in the text.

It is correct.

3. Please provide editor names for the reference Heinze et al. (2018).

Series Ed.: Kalia, S.

Synthesis and characterization of dicarboxymethyl cellulose

Ricardo Chagas, [1]✉

Email: r.chagas@fct.unl.pt

Martin Gericke, [2]

Ricardo B. Ferreira, [3]

Thomas Heinze, [2]

Luísa M. Ferreira, [4]✉

Email lpf@fct.unl.pt

[1] i3N/CENIMAT, Department of Materials Science, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

[2] Institute of Organic Chemistry and Macromolecular Chemistry, Center of Excellence of Polysaccharide Research, Friedrich-Schiller-University of Jena, Humboldtstr. 10, 07743 Jena, Germany

[3] LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisboa, Portugal

[4] LAQV-REQUIMTE, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

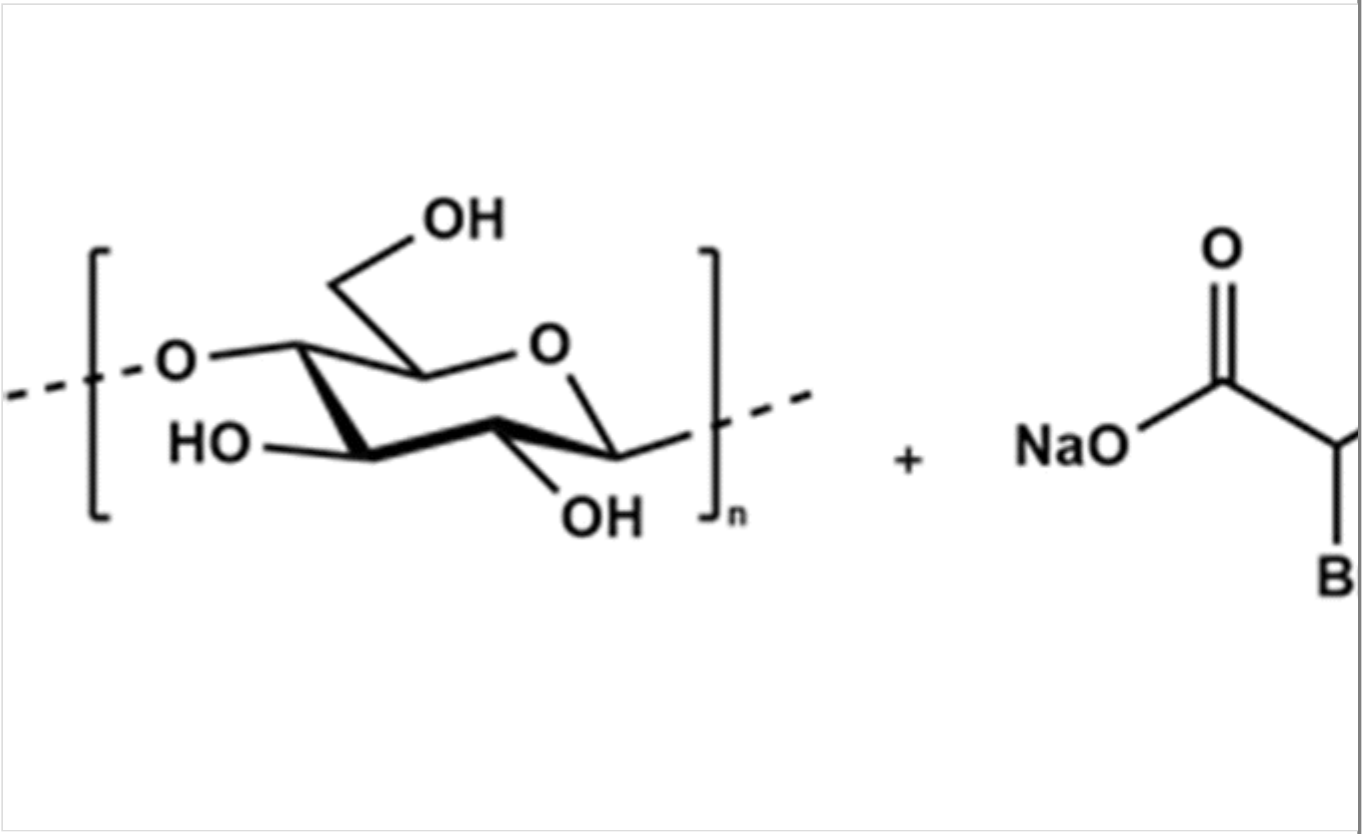
Received: 18 August 2019 / Accepted: 18 December 2019

Abstract

Synthesis of dicarboxymethyl cellulose (DCMC) under heterogeneous conditions was examined. Cellulose was etherified using sodium bromomalonate in isopropanol/water in the presence of NaOH. The reaction was performed with five different NaOH concentrations (5–30 w/v %) and the products were characterized by anion-exchange high-performance liquid chromatography (AE-HPLC), inductively coupled plasma atomic emission- (ICP-AES), Fourier transform infrared- (FTIR), and nuclear magnetic resonance (1D-/2D-NMR) spectroscopy. Adjusting the amount of NaOH resulted in increasing functionalization of the cellulose achieving an average degree of substitution (DS) between 0.05 and 0.51. Both ICP-AES and AE-HPLC gave comparable DS values. NMR spectroscopic analysis showed that etherification occurred preferably at *O*-6 and, to a certain extent, at the secondary positions depending on the reaction conditions.

AQ1

Graphic abstract



Keywords

Dicarboxymethyl cellulose
Cellulose ether
Heterogeneous derivatization
Cellulose functionalization

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s10570-019-02952-6) contains supplementary material, which is available to authorized users.

Introduction

Cellulose ethers are produced commercially in large scales and are significant to several industries from agriculture, laundry products, mining to cosmetics. Many of these ethers are water-soluble and some specific derivative present ionic behavior. One example of these ionic cellulose derivatives is carboxymethyl cellulose (CMC) which is described, in aqueous solution, as a polyelectrolyte with a low degree of ionization at low pH value (Heinze et al. 2018; Metodiev 2013). The ionic property

can have significant importance in several areas where this characteristic is crucial. Some examples can include its ability for the complex formation or its cation ion exchange ability (Heinze et al. 2018; Hen 1991; Li et al. 2017). Carboxymethylcellulose, commercially produced since the 1920s in Germany, is one of the most important ionic cellulose products (Balser et al. 2004). Large scale production of CMC is carried out almost exclusively using slurry processes by conversion of alkali cellulose swollen in a hydro-alcoholic NaOH solution with monochloroacetic acid or its sodium salt as etherifying agent (Ramos et al. 2005). Commercially available CMC present a DS in the range of 0.5 to 1.5 and its synthesis, distribution of carboxymethyl functions within the modified anhydroglucose unit (AGU), and properties are well studied (Heinze et al. 2018; Heinze and Pfeiffer 1999). This derivative is also used as ion exchange resin for adsorption of heavy metal ions (e.g. Cu^{2+}, Ni^{2+}, Cr^{3+}, Fe^{3+}) or for the purification of enzymes and proteins (Nada and Hassan 2006; Vaz and Filho 2019). Immobilized CMC is frequently used as an ion exchange resin though, it is a weak cation exchange resin. This occurs since its pH working windows is between 5 and 9 due to its pKa value (i.e. 4.5; Kastner 1999). The replacement of the carboxymethyl group by an easier ionizable group could enhance the working window of this family of cellulose derivatives. This can be achieved by the introduction of a substituent with two carboxylic acid groups (e.g. introduction of a malonic acid moiety) producing dicarboxymethyl cellulose (DCMC) as the final product.

Information regarding synthesis and characterization of dicarboxymethyl cellulose is available but scarce. Its synthesis was described using halomalonic acids in isopropanol at 130 °C. The resulting polymers were described as having a degree of substitution (DS) between 0.3 and 1.5 and a pKa of about 2.1 (Diamantoglou et al. 1977; Kötz et al. 1991). This polymer has the potential to be a relevant ionic cellulose ether with ion exchange capacity working at low pH value (2.5–5) though, new viable routes for its synthesis were not explored yet.

Due to this, it is of great interest to study the production of DCMC by the conventional slurry process. DCMC will be prepared from microcrystalline cellulose in a hydro-alcoholic media, at mild temperature, avoiding the use of closed reactors. The influence of the base concentration on the functionalization pattern of DCMC will be addressed. The synthesized products will be characterized by several analytical techniques.

Materials and methods

Materials and equipment

Microcrystalline cellulose (MN 400) was purchased from Macherey-Nigel (Düren, Germany). Malonic acid (99% purity), sodium hydroxide (> 97% purity) and bromine (reagent grade, minimum 98% purity) were purchased from Sigma Aldrich. Methanol (ACS reagent), isopropanol (ACS reagent), toluene (ACS reagent) and acetic acid (glacial) were purchased from Supelco.

Infrared spectra were recorded on a Perkin Elmer Spectrum Two in KBr pellets (10 mg of sample/100 mg of KBr). ^{1}H and ^{13}C NMR spectra were recorded on a Brucker ARX400 at 400 and 100 MHz, respectively. Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) was performed using a Horiba Jobin-Yvon Ultima model equipped with a 40.68 MHz RF generator, a Czerny–Turner monochromator with 1.00 m (sequential), and an autosampler AS500. Intrinsic viscosity was measured in a Micro Ostwald viscometer (Type I). Size exclusion chromatography (SEC) was performed on a Jasco SEC system equipped with a SEC-pump PU-980 and a RI-2031 Plus refractive index detector.

*Synthesis of sodium bromomalonate (**2**)*

To produce sodium bromomalonate (**2**), the corresponding acid bromomalonic acid was first synthesized according to literature (Barbucci et al. 1990). Bromine (15.5 g, 5 mL, 0.097 mol) was added dropwise to a stirred and ice-cooled suspension of solid malonic acid (10 g, 0.097 mol) in diethyl ether (100 mL). The malonic acid is consumed and the product bromomalonic acid readily dissolves in the reaction mixture. The reaction mixture was left at room temperature for 1 h after which diethyl ether was removed under reduced pressure. The solid residue obtained was kept over potassium hydroxide in a vacuum desiccator. The resulting solid was washed with toluene to obtain a hygroscopic, pale orange powder (14 g, yield: 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.18 (–CBr*H*–) ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.39 (–*C*OOH), 44.91 (–*C*BrH–). IR (FTIR-ATR) 3470, 1694, 1414, 1335, 1193, 1175, 938, 793, 726 cm^{-1}.

Bromomalonic acid (10 g, 54.7 mmol) was dissolved in water (15 mL) and the pH value was adjusted to 7 by adding a solution of NaOH (4.37 g, 109.3 mol) in water (15 mL) to produce sodium bromomalonate (**2**). Water was removed under reduced pressure and the solid obtained was washed with acetone, dried under vacuum (45 °C), and stored at − 20 °C until needed (11.5 g, yield: 93%). IR (FTIR-ATR) 3438, 1586, 1415, 1336, 1192, 1174, 937, 793, 727, 628 cm^{-1}.

*Synthesis of dicarboxymethyl cellulose (**3**) in isopropanol/aqueous NaOH*

Derivatization of the cellulose was carried out by a slurry method as previously described for carboxymethylation of cellulose with some modifications (Heinze and Pfeiffer 1999). Air-dried microcrystalline cellulose (**1**, 400 mg, 2.5 mmol of anhydroglucose units/AGU) in 15 mL isopropanol was stirred vigorously, while the appropriate quantity of aqueous NaOH was added dropwise during 10 min at room temperature. Stirring with a magnetic stirrer was continued for 1 h and the desired quantity of sodium bromomalonate (**3**) was added. After complete dispersion, the mixture was heated to 60 °C for 5 h under vigorous stirring. If the heterogeneous mixture clumped during the reaction, this was detached from the glass with a metal spatula. When finished, the reaction mixture was filtrated, suspended in 100 mL of 70% (v/v) aqueous methanol and neutralized with glacial acetic acid. The product (**3**) was washed three times with 20 mL of 80% (v/v) aqueous methanol and subsequently with 20 mL methanol. The products were further purified by dialysis against deionized water for 48 h using 6–8 KDa regenerated cellulose membranes (Spectra/Por, Spectrumlabs). After dialysis, the samples were separated between water-soluble and insoluble fractions by centrifugation (4300 *g*, 15 min) and the soluble fraction was freeze dried. Remaining analysis were performed in the soluble fractions.

Sodium content determination by ICP-AES

A known amount (approx. 5 mg per sample) of DCMC was weighted in glass vials and suspended in 1 ml pure nitric acid. The samples were incubated at 60 °C for 1 h prior to analysis. Sodium was quantified by ICP-AES. The DS was calculated from the sample sodium percentage (%Na) based on Eq. 1 analog to the procedure described in the literature for carboxymethyl starch (Tuting et al. 2004).

$$DS_{ICP-AES} = \frac{162 \times (\%Na/2/23)}{100 - (147 \times (\%Na/2/23))} \quad 1$$

Therein, the term “162” represents the molecular mass of an anhydroglucose unit of cellulose and “147” accounts for the net increase in molecular mass of modified anhydroglucose unit with a sodium dicarboxymethyl group added. The sodium content is divided by 2 (since every substituent group presents two carboxylic acids) followed by the division by sodium molecular mass.

Size exclusion chromatography

Size exclusion chromatography (SEC) was performed using a PSS SUPREMA precolumn/1000 A°/30 A° columns in sequence. The analysis was performed with a flow rate of 1 ml/min at 30 °C. A 0.1 M $NaNO_3$ aqueous solution with 0.05 wt% NaN_3 was used as mobile phase and pullulan standards were used for calibration.

Anion exchange chromatography

Anion exchange high-performance liquid chromatography (AE-HPLC) analysis of the DCMC samples was carried out on hydrolyzed samples as described in the literature (Heinze and Pfeiffer 1999; Qi et al. 2009). In brief, 100 mg of DCMC was dispersed in 2 mL $HClO_4$ (70%) and after 10 min at room temperature, 18 mL of water was added. This mixture was kept at 100 °C for 16 h. The solution obtained was neutralized with 2 M KOH and kept at 4 °C for 1 h to guarantee complete precipitation of the $KClO_4$. The precipitate was filtered off and washed three times with distilled water. The solution was reduced to approximately 3 mL and diluted with deionized water to give exactly 5 mL sample. Chromatographic experiments were carried out at 65 °C with 50 mM H_2SO_4 as eluent and a flow rate of 0.5 mL/min in a Bio-Rad Aminex HPX-87 H column. The $DS_{AE-HPLC}$ was calculated from the peak areas attributed to glucose (A_{gluc}; retention time: 20.1 min), mono-*O*-dicarboxymethylglucose (A_{mono}; retention time: 18.0 min), and di-*O*-dicarboxymethylglucose (A_{di}; retention time: 16.7 min) according to Eq. (2).

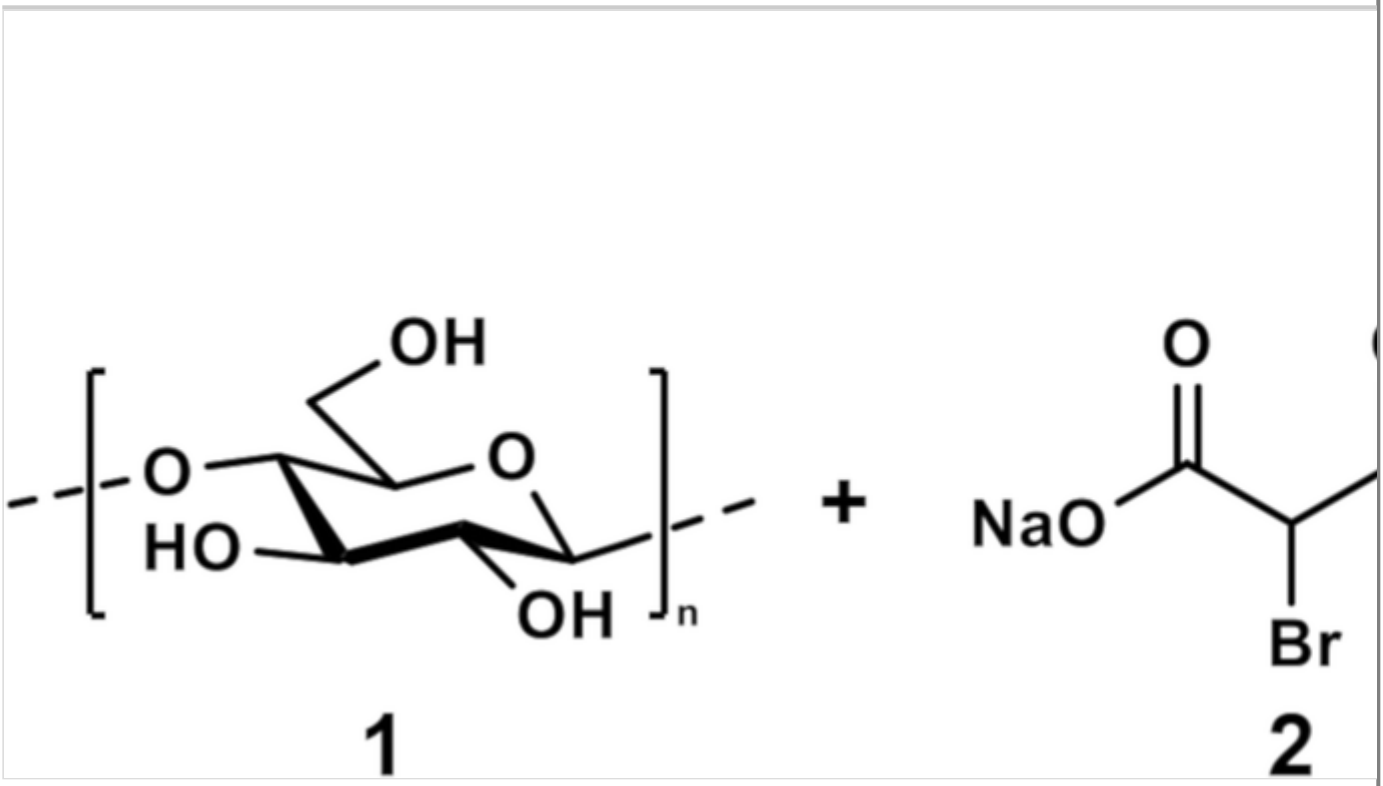
$$DS_{\mathrm{AE-HPLC}} = \frac{A_{mono} + 2 \times A_{di}}{A_{gluc} + A_{mono} + A_{di}} \quad (2)$$

Results and discussion

In this work, a slurry method previously described for the synthesis of carboxymethyl cellulose (CMC) was adapted for the efficient synthesis of dicarboxymethyl cellulose (DCMC). To have a reagent for the modification of cellulose, synthesis and purification of **2** was conducted. This compound will participate in a S_N2 reaction with cellulose under alkaline conditions to produce the cellulose derivative as represented in Fig. 1. This is the most common approach for the etherification of cellulose (e.g. to produce carboxymethyl cellulose) where there is an *O*-alkylation with alkyl halides (Klemm et al. 2005). In this case, sodium bromomalonate was used as the alkyl halide that will react with the free hydroxyls of the cellulose structure.

Fig. 1

Schematic representation of the reaction between the sodium bromomalonate (**2**) and the anhydroglucose unit of cellulose (**1**)



In the presented experiments, microcrystalline cellulose was slurried in isopropanol, activated with different quantities of NaOH and reacted with **2** at 60 °C (Table 1). During the reaction, gelation occurred after increasing the temperature to 60 °C. It was observed that increasing gelation occurs with an increasing quantity of NaOH. To aid the dispersion of the slurry, the gels were scraped from the glass with a spatula. The gels disintegrate forming homogeneous suspensions about 2 hours after the beginning of the reaction. After five hours, the products were filtered and washed. Afterward, the samples were purified by dialysis and separated between water-soluble and insoluble fractions by centrifugation. The water-soluble fractions were freeze dried, allowing the calculation of the water-soluble materials percentage, and were further analyzed for structural characterization of the product (Table 1).

Table 1

Conditions for and results of the synthesis of dicarboxymethyl cellulose (DCMC) by conversion of microcrystalline cellulose with sodium bromomalonate in isopropanol / water

mixtures (92:8) at 60 °C for 5 h

DCMC	Conditions		Results			
	Molar ratio[a]	ρ_{NaOH}[b], (%)	Water-soluble fraction (%)	$DS_{ICP-AES}$[c]	$DS_{AE-HPLC}$[c]	DP_W
3a	0.5:2:1	5	1.8	0.05	–[d]	–[d]
3b	1.0:2:1	10	37.3	0.32	0.40	269
3c	1.6:2:1	15	59.4	0.44	0.45	370
3d	2.2:2:1	20	67.9	0.46	0.51	326
3e	3.2:2:1	30	61.0	0.51	0.44	166

[a] Molar ratio (in molar equivalents) of NaOH: sodium bromomalonate: anhydroglucose units

[b] Mass concentration of NaOH

[c] Degrees of substitution (DS) determined for the soluble fraction by inductively coupled plasma-atomic emission spectrometer (ICP-AES), NMR spectroscopy, or high-performance liquid chromatography (AE-HPLC)

[d] Not determined due to small sample mass

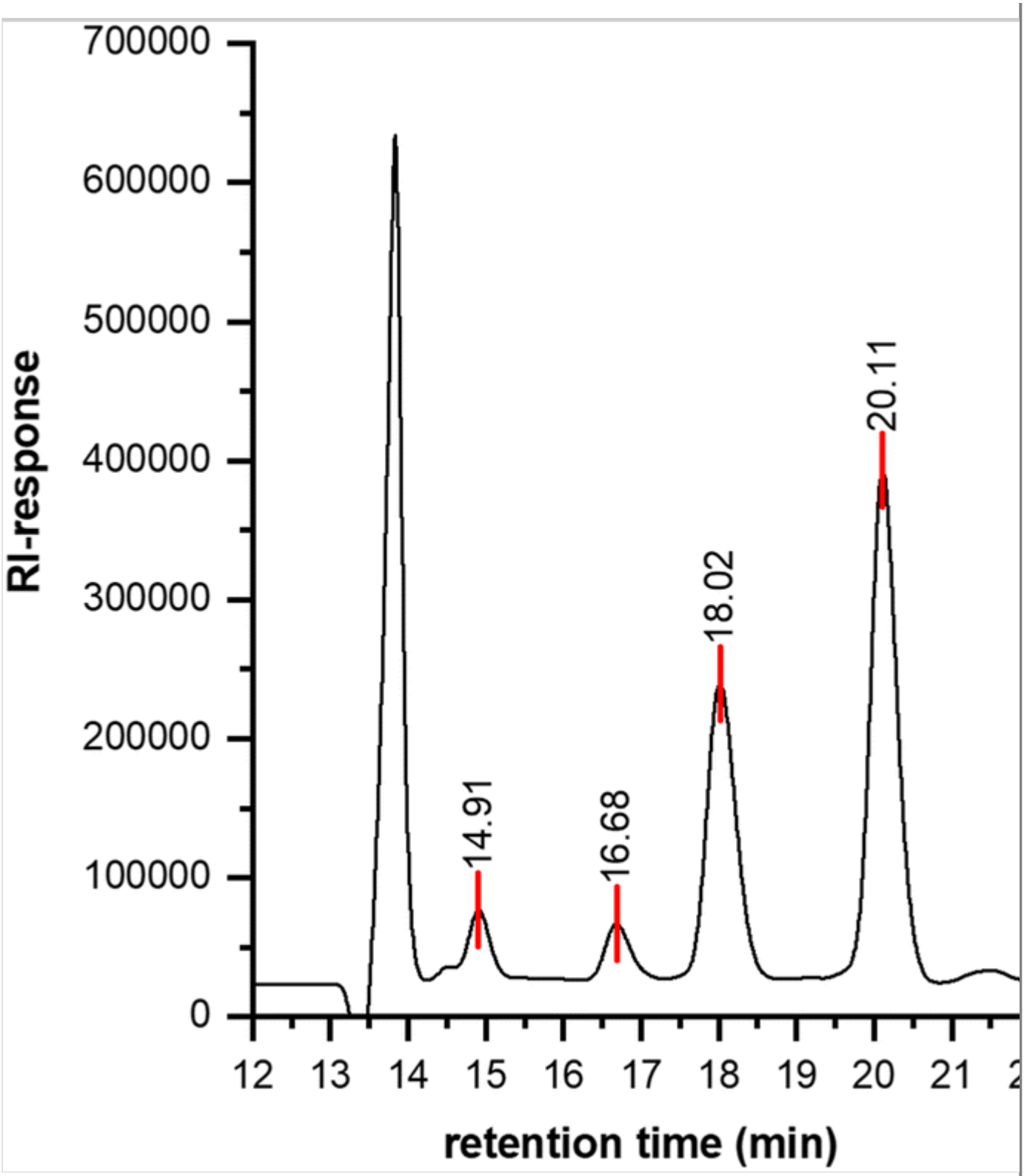
The product **3a**, obtained at the lowest NaOH concentration of 5% possessed only a minor water-soluble fraction (1.8%) and was not studied further. However, increasing the NaOH concentration to 10% (sample **3b**) resulted in a substantial increase in the water-solubility (37.3%). A further increase to 15–30% NaOH (sample **3c**–**3e**), increased the content of the water-soluble fraction to about 60%. However, no complete water solubility was achieved. The synthesis of DCMC is a heterogeneous process (cellulose and bromomalonate are both not soluble in the reaction mixture), which is prone to result in non-uniform distribution between individual polymer chains. Increasing the amount of NaOH improves the swelling of cellulose in the slurry, which improves even accessibility of sodium bromomalonate, and activates cellulose for etherification, resulting in higher DS and thus favors solubility in water.

The water-soluble fractions of each sample were characterized further. Elemental analysis demonstrated that no bromine was present in the products, confirming the absence of residual reagent. In addition, NMR spectroscopy confirmed the absence of sodium hydroxyl malonate that in principle can be formed as a by-product of hydrolysis of bromomalonate and can be identified by characteristic peaks (4.3 ppm

in ^{1}H-NMR, 75 ppm in ^{13}C-NMR; Kötz et al. 1991). The DS of DCMC samples was determined by different complementary techniques. From the sodium content determined by ICP-AES, the DS was calculated considering that each substituent contains two sodium counter ions. In addition, the DS was determined by a chromatographic method. The polymeric samples were hydrolyzed to monosaccharide compounds according to literature procedures described for CMC (Heinze and Pfeiffer 1999; Qi et al. 2009). The monosaccharides were separated and quantified by AE-HPLC. A representative chromatogram of DCMC is represented in Fig. 2. It features four major peaks that must be considered. The peak at 20.1 min was confirmed to be glucose by co-injection with an internal standard. Standards for the different alkylated ionic monosaccharides (i.e., 2,3,6-tri-*O*-dicarboxymethylglucose, 2,3-, 2,6 and 3,6-di-*O*-dicarboxymethylglucose and 2-, 3- and 6-mono-*O*-dicarboxymethylglucose) are not available. However, peaks could be assigned by comparison with previously published data for CMC (Qi et al. 2009).

Fig. 2

Representative anion-exchange chromatogram of dicarboxymethyl cellulose sample **3d** after acid-catalyzed depolymerization. Peak assignment: (20.11 min) glucose, (18.02 min) monosubstituted glucose with DCM moiety (16.68 min) disubstituted glucose with DCM moiety, (14.91 min) high molecular weight fragments



It must be considered that DCMC possesses twice as many anionic groups as CMC at same DS. Thus, it can be assumed that monosubstituted dicarboxymethylglucose features a similar retention time as disubstituted carboxymethylglucose. The peak with a retention time of 18.0 min is expected to correspond to monosubstituted dicarboxymethylglucose. For hydrolyzed CMC, a peak with a similar retention time

of around 18 min was observed for the disubstituted monosaccharide (Heinze and Pfeiffer 1999; Qi et al. 2009). The peak at 16.7 min. can be assigned to the disubstituted dicarboxymethylglucose with four negative charges. Trisubstituted carboxymethylglucose, which features 3 negative charges, shows a similar retention time of 17.0 min. It can be assumed that at high charge density, the difference in the number of negative charges (four vs three) has a less pronounced influence. Considering that (i) the highest DS values achieved herein are around 0.5 and (ii) the dicarboxymethyl substituent (DCM) is somewhat bulky and highly charged, it is unlikely that trisubstituted repeating units would occur in the DCMC samples prepared in this study. It is known for CMC that trisubstituted repeating units will only occur at DS > 1.0 (Bol et al. 2019; Heinze and Pfeiffer 1999). Thus, it can be concluded that the peak at 14.9 min is not related to the DCM substituted monosaccharide unit, i.e., these peaks were not considered for the DS calculation. It can be speculated that the peak corresponds to oligomers or high molecular weight fragments of the modified cellulose that was not completely hydrolyzed, similar to previously reported analysis of complex mixtures of carbohydrates (Bonn and Bobleter 1984). The DS was calculated from the areas under the peaks at 16.7, 18.0, and 20.1 (see Table 1). Both methods (ICP-AES and AE-HPLC) gave consistent results for all tested samples despite the apparent presence of larger molecular weight fragments. It can be speculated that the incomplete hydrolysis of the polymer chains did not affect the relative amounts of mono- and di-substituted sugar units in the hydrolysate.

Based on the calculated DS values, it can be concluded that the synthesis of DCMC can be enhanced by increasing the NaOH content in the reaction. This was even more accentuated at lower concentrations of NaOH (between 5 and 10% (w/v) of NaOH) where an increment of around 35% of the soluble fraction was observed for the sample 3b produced with a higher quantity of base. Moreover, an increase in the DS was observed as well. Regarding samples **3c**–**3e**, there was a much less pronounced increase both in the DS and in the water-soluble fraction of the products when increasing the NaOH content from 15 to 30% (w/v). Compared to the synthesis of CMC, a lower DS was achieved using comparable amounts of etherification reagent even with higher base concentration (Heinze and Pfeiffer 1999). For CMC produced in similar condition, it was shown that a maximum DS of 1.24 is achieved at 15% NaOH and the DS decreases again down to 0.95 upon increasing NaOH concentration until 30%. (Heinze and Pfeiffer 1999). For DCMC this decrease in the DS with increasing base content was not observed. The DS differences between samples **3c** and **3e** were marginal. The DCM substituent is bulkier than the CM group. It also introduces a higher charge density to the polymer

backbone because it carries two anionic groups in a rather rigid closely confined environment. Another possible reason for the overall lower DS of DCMC can be the insolubility of both starting materials in the reaction mixture. Oppositely to monochloroacetic acid sodium salt that is used in the production of CMC, sodium bromomalonate is insoluble in the isopropanol/water mixture which increases the heterogenicity of this reaction. Due to that, starting with freshly dried powdered sodium bromomalonate is crucial to avoid agglomeration and clumping in the reaction mixture.

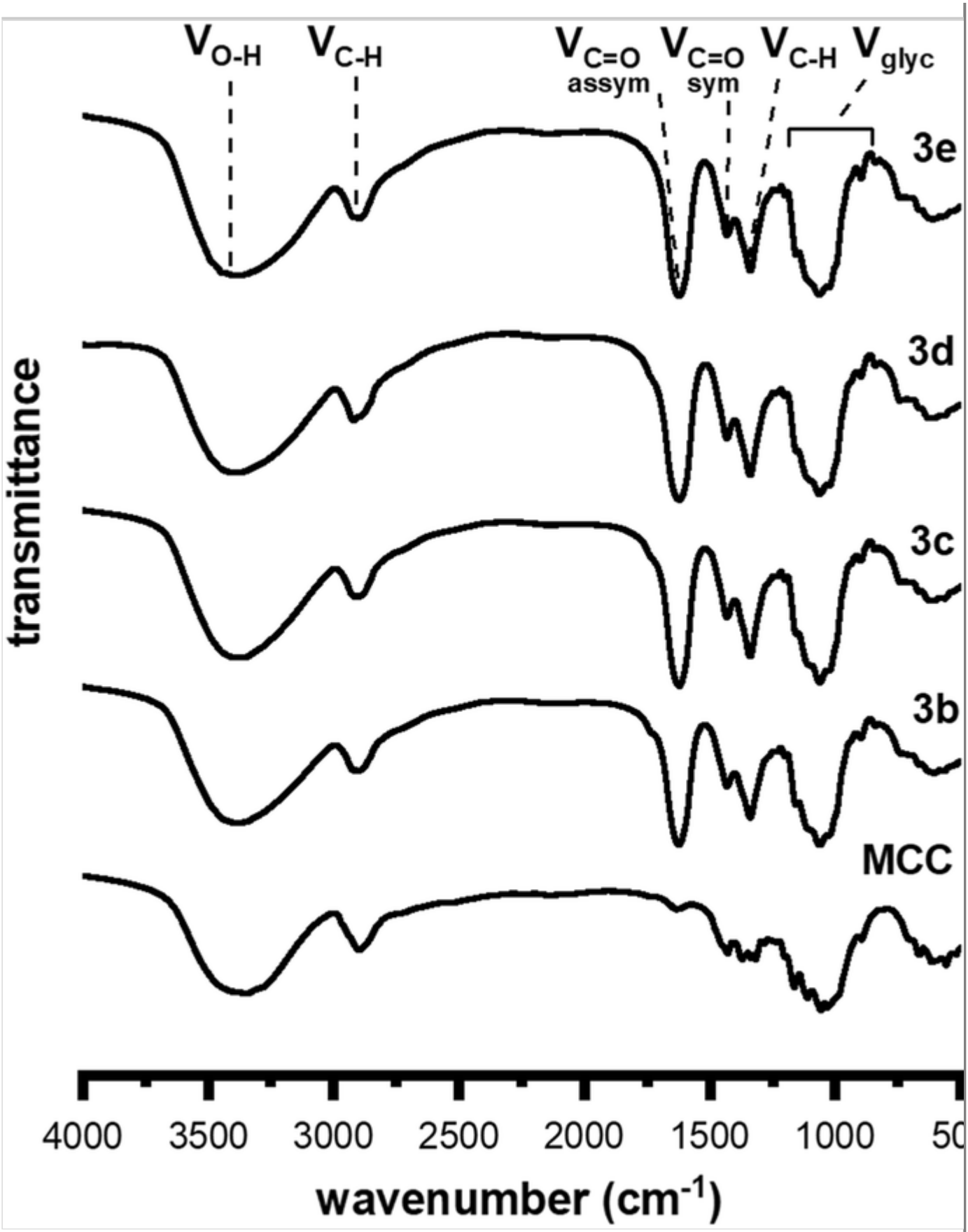
Structural characterization of DCMC

The infrared spectra of the DCMC products show the typical ~~peaks~~ bands related to the cellulose backbone, such as 1160, 1027 and 896 cm^{-1} from C–O and glycosidic bonds in cellulose (Xu et al. 2013). Upon increasing substitution, additional ~~peaks~~ bands emerged at 1625 cm^{-1} ($–COO^-$ asymmetric stretching), 1435 cm^{-1} ($–COO^-$ symmetric stretching) and 1340 cm^{-1} (C–H bending; Fig. 2). These bands correspond to the vibration of the carboxylate groups confirming the presence of the substituent. This is the first proof for successful modification of cellulose with DCM moieties (Fig. 3).

AQ2

Fig. 3

Infrared spectra (in offset) of dicarboxymethyl cellulose samples **3b**–**3e** and of microcrystalline cellulose (MCC)



To gain further information on the molecular structure, 1D- and 2D NMR spectroscopic experiments were performed. The ^{13}C NMR (Fig. 4.) and ^{1}H NMR

spectra (Fig. SI 2 in supplementary materials) of DCMC samples **3b**–3**e** feature the typical peaks of the cellulose repeating unit. By a combination of HSQC-DEPT (Fig. 5) and COSY experiments (Fig. SI 3 in supplementary materials), the peaks associated with C-1 to C-6 and H-1 to H-6 of the polysaccharide backbone could be assigned. Additional peaks occurred that could be correlated with derivatization with DCM groups. The peaks that occurred around 175 ppm in the ^{13}C NMR spectra are associated with the carbonyl moieties (C-8) in the newly introduced carboxyl groups. Due to the peak fine structure, it can be assumed that the substituent was introduced at different positions of the repeating unit. Around 67 ppm, a new peak of low intensity emerged. According to the HSQC-DEPT spectra (Fig. 5) it can be attributed to a secondary carbon atom, i.e., a substitution at the primary C-6 position occurred. For sample **3e**, no significant derivatization of C-6 was detected by NMR spectroscopy. It can be speculated that due to the much higher NaOH concentration employed, the reaction proceeded differently.

It has been proposed that the new peak detected around 82 ppm is attributed to the tertiary carbon atom of the DCM substituent (Kötz et al. 1991). This hypothesis could be confirmed in this work by 2D NMR techniques. The carbon peak shows a direct ^{1}J coupling with a proton peak at 4.1 ppm. This proton peak in return does not couple with any other protons (COSY spectrum) but shows ^{2}J coupling through space with the carbonyl carbon atom (HMBC spectrum). This finding clearly demonstrates that it is associated with the proton H-7 of the DCM group (Fig. SI 3 in supplementary materials). An increase in the signal intensity at 82 ppm is noticeable for samples **3b**–**3d**, which correlates with the increasing DS.

The ^{13}C NMR spectra feature an additional prominent peak at around 84 ppm that was previously assigned to an etherified C-3 based on the assumption that etherification at C-2 would result in a significant shift of the peak related to an adjacent C-1 (Kötz et al. 1991). However, it has been demonstrated in the literature that carboxymethylation of cellulose in position C-2 does not result in a significant splitting of the C-1 related peaks and the same could be expected for the derivatization with DCM groups (Baar et al. 1994; Kono et al. 2016a). Moreover, the 2D-NMR studies suggest a more complex interpretation of the spectroscopic data and the molecular structure of the DCMC samples obtained. As can be seen in the HSQC-DEPT spectrum, the carbon atoms associated with this peak at 84 ppm show a strong ^{2}J coupling not with one but with two different protons, i.e., the peak is associated with two carbon atoms with similar chemical shifts that fall together. One of the coupling protons is H-7, i.e., it can be concluded that DCMC is substituted not only in C-6 position but also at C-2 and/or C-3 and that the

corresponding peaks of the different C-7 species are separated by about 3 ppm. Similar results have been reported in the literature for CMC (Kono et al. 2016a, b). Two distinguished peaks have been reported therein that were associated with the methylene carbon atom of CM substituents at either C-6 (≈ 73 ppm) or C-2 (≈ 74 ppm). Based on the 1D- and 2D-NMR experiments performed it was not possible to assign the position of the two different C-7 species within the repeating unit of DCMC samples. For sample **3e**, almost no C-6 derivatization was observed suggesting that the peak around 82 ppm, which is rather prominent also for this sample, is attributed to a DCM substituent at a secondary C-2 and/or C-3 position.

The C-7 related peak at 84 ppm overlaps with another peak that can be assigned to an etherified secondary hydroxyl group. Previously, substitution at C-3 has been postulated (Kötz et al. 1991). This hypothesis is neither supported nor fully disproved by the NMR experiments performed in this study. However, it can be stated that the peak couples to a peak in the proton spectrum at around 4.4 ppm. This proton peak in return does not show any ^{3}J coupling in the COSY experiments with a H-2 or H-4 related peaks. Both peaks should occur in case of C-3 etherification. Thus, it is hypothesized that the peak at 84 ppm is attributed to a substituted C-2 position. Despite the efforts to gain high resolution spectra, the COSY experiments did not show a clear ^{3}J coupling between H-2 (substituted) and H-1. However, both peaks are overlapping, which makes it difficult to separate them.

To conclude, the successful derivatization of cellulose with DCM substituents was proven by NMR spectroscopy. It was demonstrated that the molecular structure of the DCMC samples prepared is rather complex. Depending on the reaction conditions, etherification clearly occurred at the primary hydroxyl group. To a certain extent, the secondary positions were modified as well even at DS ≤ 0.5. A peak assignment was postulated on the basis of 1D- and 2D NMR experiments.

Fig. 4

^{13}C NMR spectra of dicarboxymethyl cellulose samples **3b**–**3e** with a postulated peak assignment, recorded in D_2O

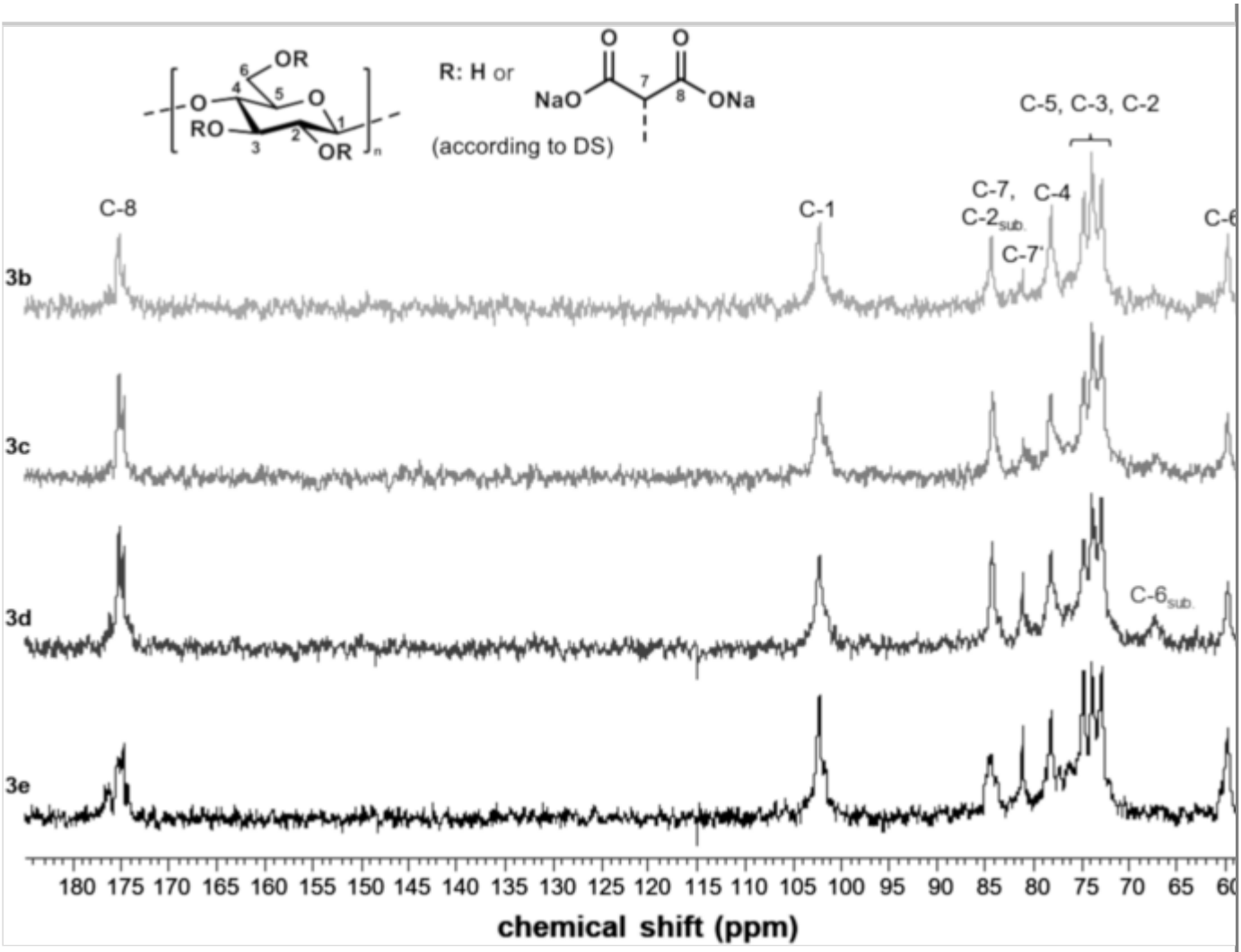
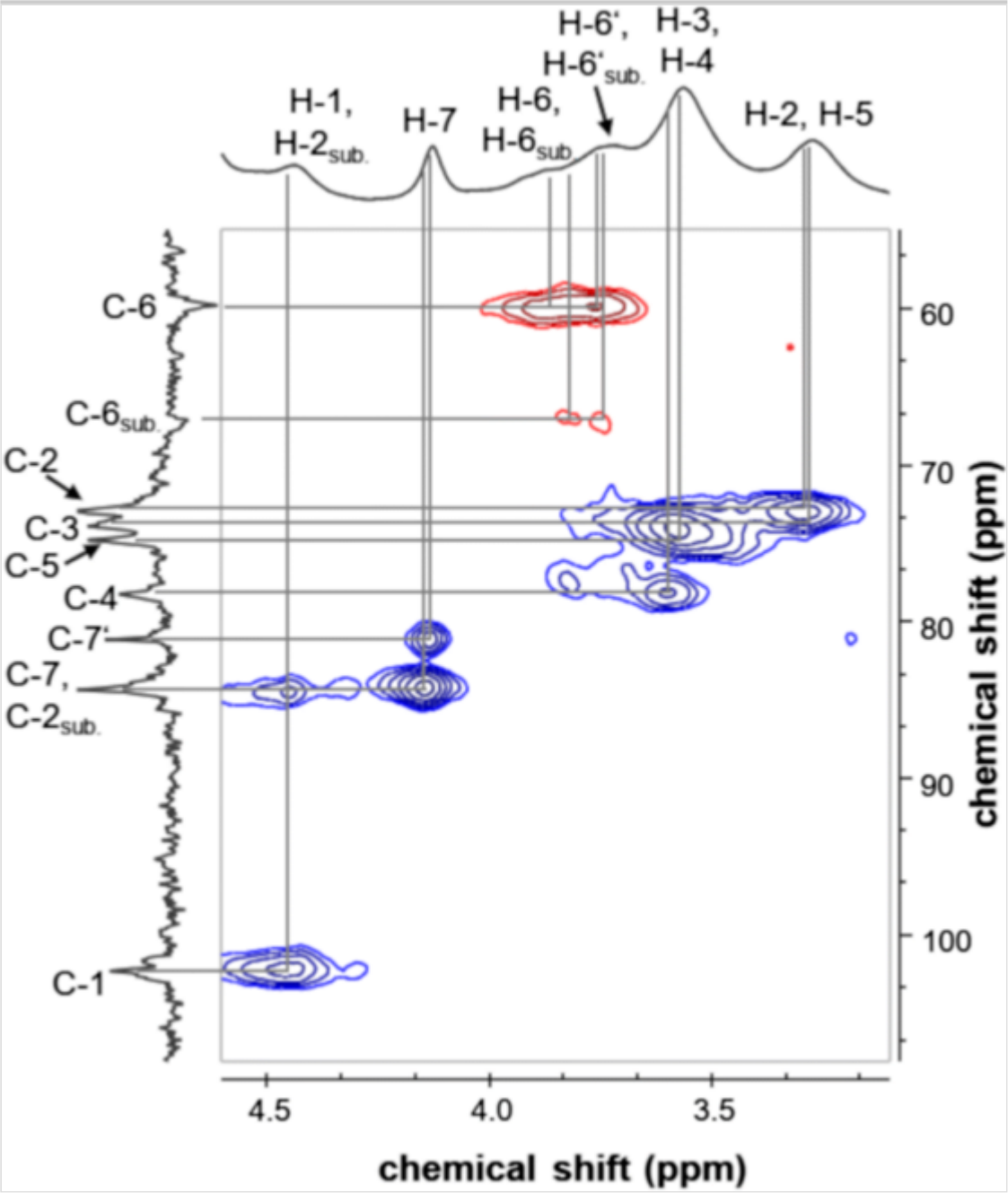


Fig. 5

HSQC-DEPT spectrum of dicarboxymethyl cellulose sample **3d** with a postulated peak assignment, recorded in D_2O



Conclusion

Heterogeneous synthesis of dicarboxymethylcellulose (DCMC) by conversion of cellulose with sodium bromomalonate in isopropanol/water in the presence of NaOH resulted in products with a DS of up to 0.51. Adjusting the amount of NaOH resulted in increased functionalization and partly in different functionalization patterns within the AGU. Only minor DS differences in samples using 15 to 30% (w/v) of NaOH appeared. The molecular structure was characterized comprehensively by complementary chromatographic (AE-HPLC and ICP-AES) and spectroscopic techniques (FTIR, 1D-/2D-NMR).

The present work lays the groundwork for the synthesis of novel DCMC derivatives. These ionic cellulose derivatives possess a pronounced negative charge density that is of great interest for applications, e.g., as adsorbent material for water remediation, protein adsorption, or drug delivery. Future experiments will include the comprehensive characterization of polyelectrolyte properties and acid-base behavior. Moreover, the preparation of ion exchange materials by cross-esterification of DCMC for the adsorption of charged molecules in aqueous solutions is currently under investigation.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Acknowledgments

This work was supported by the Associate Laboratory for Green Chemistry-LAQV which is financed by national funds from FCT/MCTES (UID/QUI/50006/2019) and by Fundação para a Ciência e a Tecnologia (FCT) under the Ph.D. Grant SFRH/BD/84749/2012. The authors thank Dr. W. Günther (University of Jena) for the fruitful discussion on the NMR data and Dr. Andreas Koschella (University of Jena) for his help during this work. The authors also acknowledge the Portuguese Nuclear Magnetic Resonance Network (RNRMN), supported by FCT, Portugal (ROTEIRO/0031/2013–PINFRA/22161/2016, co-financed by FEDER through COMPETE 2020, POCI, and PORL and FCT through PIDDAC).

Electronic supplementary material

Below is the link to the electronic supplementary material.

Supplementary material 1 (PDF 378 kb)

References

Baar A, Kulicke W-M, Szablikowski K, Kiesewetter R (1994) Nuclear magnetic resonance spectroscopic characterization of carboxymethylcellulose. Macromol Chem Phys 195:1483–1492

Balser K, Hoppe L, Eicher T, Wandel M, Astheimer H-J, Steinmeier H, Allen JM (2004) Cellulose esters. In: Ullmann's Encyclopedia of Industrial Chemistry

Barbucci R, Casolaro M, Fini A (1990) Thermodynamics of protonation and copper (II) complex formation of a-aminomalonic acid. Dalton Trans 1:207–211

Bol M, Dobos MA, Lebioda S, Saake B, Mischnick P (2019) Methanolysis of carboxymethyl cellulose: a comprehensive study. Cellulose 26:383–397

Bonn G, Bobleter O (1984) HPLC-analyses of plant biomass hydrolysis and fermentation solutions. Chromatographia 18:445–448

Diamantoglou M, Mägerlein H, Zielke R, Maegerlein H, Zielke R (1977) Polycarboxylate aus Polysacchariden, Holz und holzähnlich zusammengesetzten Stoffen als neuartige Sequestriermittel. Tenside Detergents 14:250–256

Heinze T, El Seoud OA, Koschella A (2018) Etherification of cellulose. In: Cellulose derivatives. Springer series on polymer and composite materials. Ed.: Kalia, S., Springer International Publishing, Cham, pp 429–477

AQ3

Heinze T, Pfeiffer K (1999) Studies on the synthesis and characterization of carboxymethylcellulose. Angew Makromol Chem 266:37–45

Hen J (1991) Sulfonate-containing polymer/polyanionic cellulose combination for high temperature/high pressure filtration control in water base drilling fluids. United States Patent 5008025

Kastner M (1999) Protein liquid chromatography vol 61. Elsevier, Amsterdam

Klemm D, Heublein B, Fink HP, Bohn A (2005) Cellulose: fascinating biopolymer and sustainable raw material. Angew Chem Int Ed 44:3358–3393

Kono H, Oshima K, Hashimoto H, Shimizu Y, Tajima K (2016a) NMR characterization of sodium carboxymethyl cellulose 2: chemical shift assignment and conformation analysis of substituent groups. Carbohydr Polym 150:241–249

Kono H, Oshima K, Hashimoto H, Shimizu Y, Tajima K (2016b) NMR characterization of sodium carboxymethyl cellulose: Substituent distribution and

mole fraction of monomers in the polymer chains. Carbohydr Polym 146:1–9

Kötz J, Nehls I, Philipp B, Diamantoglou M (1991) Zum Polyelektrolytverhalten von Dicarboxymethylcellulose. Das Papier 45:226–231

Li ZS, Wang YT, Pei YQ, Xiong WF, Xu W, Li B, Li J (2017) Effect of substitution degree on carboxymethylcellulose interaction with lysozyme. Food Hydrocolloids 62:222–229

Metodiev A (2013) Electric properties of carboxymethyl cellulose. In: Van De Ven T, Godbout L (eds) Cellulose-fundamental aspects. IntechOpen, London

Nada AAMA, Hassan ML (2006) Ion exchange properties of carboxylated bagasse. J Appl Polym Sci 102:1399–1404

Qi HS, Liebert T, Meister F, Heinze T (2009) Homogenous carboxymethylation of cellulose in the NaOH/urea aqueous solution. React Funct Polym 69:779–784

Ramos LA, Frollini E, Heinze T (2005) Carboxymethylation of cellulose in the new solvent dimethyl sulfoxide/tetrabutylammonium fluoride. Carbohydr Polym 60:259–267

Tuting W, Albrecht G, Volkert B, Mischnick P (2004) Structure analysis of carboxymethyl starch by capillary electrophoresis and enzymic degradation. Starch-Starke 56:315–321

Vaz RP, Filho EXF (2019) Ion exchange chromatography for enzyme immobilization. In: Inamuddin (ed) Applications of ion exchange materials in biomedical industries. Springer International Publishing, Cham, pp 13–27

Xu F, Yu J, Tesso T, Dowell F, Wang D (2013) Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: A mini-review. Appl Energy 104:801–809