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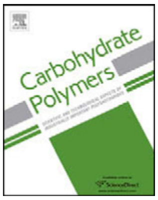

A "click-chemistry" approach to cellulose-based hydrogels

Andreas Koschella[a], Matthias Hartlieb[a], Thomas Heinze[a,b,*]

[a] Friedrich Schiller University of Jena, Institute for Organic Chemistry and Macromolecular Chemistry, Center of Excellence for Polysaccharide Research, Humboldtstraße 10, D-07743 Jena, Germany
[b] Åbo Akademi University, Fibre and Cellulose Technology, Porthansgatan 3, FI-20500 Åbo, Finland

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ABSTRACT

Cellulose derivatives bearing azide- and alkyne moieties were prepared by conversion of cellulose *p*-toluenesulphonic acid ester with sodium azide, on one hand, and propargylamine, on the other. The products obtained were carboxymethylated to yield water soluble multifunctional cellulose derivatives. Elemental analysis, FTIR- and NMR spectroscopy were applied to prove the structure of the polymers. SEC of the hydrogel components revealed values of the degree of polymerization (DP) between 43 and 200 that are acceptable values after this multi-step reaction starting from celluloses with DP 600. The copper(I)-catalyzed 1,3-dipolar cycloaddition reaction (Huisgen-reaction) was applied for the cross-linking. Gel formation occurred within 55 and 1600 s after mixing of the aqueous solutions of both components and copper(I) catalyst. The gelation time was found to depend on both the degree of functionalization and the amount of copper(I) catalyst. FTIR spectroscopy revealed incomplete conversion of the reactive sites. The gels contain up to 98.4% water. Freeze-drying led to spongy materials with a porous structure as visualised by SEM.

1. Introduction

Hydrogels are three-dimensional networks of hydrophilic polymers that are cross-linked either chemically via covalent bonds or physically by ionic-, van der Waals-, hydrophobic, or hydrogen bond interactions (Yu & Ding, 2008). Their most important property is the capability of remarkable solvent uptake. Such materials play an enormous role in our daily lives as, e.g., superabsorbers in diapers and other hygiene products (Buchholz, 1996). Due to their high water content and tissue like structure, hydrogels are valuable materials for advanced applications in fields where biocompatible devices in chemical and biochemical applications are needed (Bajpai, Shukla, Bhanu, & Kankane, 2008; Calvert, 2009; Lee & Mooney, 2001; Park & Park, 1996; Yu & Ding, 2008).

Due to its inherent hydrophilicity the renewable resource cellulose is a valuable starting material for hydrogels. It is already reported that physical gels from hydroxypropylmethyl cellulose, methyl cellulose, and hydroxypropyl cellulose possess thermoresponsive properties and are therefore only stable above the lower critical solution temperature (LCST). Moreover, chemical cross-linking can be achieved by either difunctional molecules (e.g. divinyl sulphone) or by radical cross-linking with high-energy irradiation. It is also possible to prepare hydrogels from solutions of unmodified cellulose (Chang & Zhang, 2011). Yang et al. dissolved cellulose in an ionic liquid followed by coagulation with water yielding a gel that is physically cross-linked by hydrogen bonds (Li, Lin, Yang, Wan, & Cui, 2009). Zhang et al. prepared gels by dissolving cellulose in an aqueous 6 wt% NaOH solution with 4 wt% urea and subsequent cross-linking with epichlorohydrin (Zhou, Chang, Zhang, & Zhang, 2007). Most of the cellulose-based hydrogels are hybrid systems. Biocompatibile cellulose and cellulose derivatives are combined with a variety of synthetic polymers, e.g., poly(acrylonitrile) (Pourjavadi, Zohuriaan-Mehr, Ghasempoori, & Hossienzadeh, 2007), poly(*N*-isopropylacrylamide) (pNIPAAm) (Ma, Zhang, Fan, Xu, & Liang, 2008), and poly(vinyl alcohol) (Alupei, Popa, Hamcerencu, & Abadie, 2002) in order to tune the properties. The disadvantage of most of these gels is that hazardous cross-linking agents like epichlorohydrin or glutaraldehyde have to be used (Shang, Shao, & Chen, 2008).

An alternative preparation method would be the cross-linking by 1,3-dipolar cycloaddition reaction of alkynes and azides. One example is a gel composed of 6-azido-6-deoxy cellulose and pNIPAAm-co-hydroxyethylmethacrylate (HEMA). The HEMA block is modified with an alkyne moiety and the gelation is accomplished by the 1,3-dipolar cycloaddition (Zhang, Xu, Wu, Zhang, & Zhuo,

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* Corresponding author at: Friedrich Schiller University of Jena, Institute for Organic Chemistry and Macromolecular Chemistry, Center of Excellence for Polysaccharide Research, Humboldtstraße 10, D-07743 Jena, Germany.
Tel.: +49 3641 948270; fax: +49 3641 948272.
E-mail address: Thomas.heinze@uni-jena.de (T. Heinze).

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2009). Because of the LCST of pNIPAAm, the gels produced are thermo-sensitive.

It was interesting to study hydrogels solely composed of cellulose derivatives that can be prepared in aqueous solution. This paper describes the synthesis of water-soluble cellulose derivatives capable of undergoing the copper-catalyzed 1,3-dipolar cycloaddition reaction. Hydrogels were prepared and characterized regarding their cross-linking- and swelling behaviour as well as their morphology in the dried state.

2. Experimental

2.1. Materials

Spruce sulphite pulp was purchased from Fluka. Other chemicals were supplied by Aldrich, Merck and Fluka. Cellulose was dried in vacuum at 100 °C for 2 d over KOH and LiCl was dried in vacuum for 1 d at 150 °C.

2.2. Measurements

FTIR spectra were recorded on a Nicolet Avatar 370 spectrometer using the KBr-technique. The ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker AVANCE 400 spectrometer in dimethyl sulphoxide (DMSO)-d_6 or D_2O at 70 °C. For ^{1}H-NMR spectra up to 200 scans and for ^{13}C-NMR spectra up to 26,000 scans were accumulated.

Elemental analysis was performed with a Vario EL III from Elementaranalysensysteme Hanau (Germany). The DS values were calculated from the elemental composition by an inhouse software. Both the calculated composition based on the DS values and found values are given in the experimental part.

Size exclusion chromatography in aq. 0.1 mol/L Na_2HPO_4 containing 0.1% NaN_3 was measured with a JASCO SEC system (degasser DG 980-50, pump PU 980, UV detector 975 working at $\lambda = 254$ nm, refractive index detector 930, columns Suprema 1000 and Suprema 30 from Kromatek, Great Dunmow, Essex, UK, with eluent flow rate of 1.000 mL/min). The eluent DMSO/LiBr was used in combination with the columns Novema 3000 and Novema 300 (Polymer Standards Service, Mainz, Germany). Pullulan- and dextran standards (for $M < 10^4$ g/mol) were used for calibration. Weight average ($\bar{M}_w$) and number average molecular mass ($\bar{M}_n$) as well as polydispersity index (PDI) were calculated.

Scanning electron microscopic images were obtained from a LEO-1450 VP with Cryo-Transfer (BAL-TEC-VCT 100), X-ray-microanalysis (Oxford-Link Isis) and a resolution of 4 nm. The samples were freeze dried gels with the volume of 1 mL.

Rheological measurements were performed on a HAAKE Mars rheometer with cone and plate geometry at room temperature. The measurement parameter were $\tau = 10000$ Pa and $f = 1\ s^{-1}$.

Ascorbic acid (0.2 mL, 0.1 M) was mixed with 0.2 mL aqueous $CuSO_4$ solution (0.02, 0.03, and 0.04 M). The polymer solution (0.2 mL, 5%, w/w) was mixed with 0.2 mL of the catalyst solution and 0.2 mL of the mixture were rapidly transferred to the rheometer. The time given in Fig. 2 and Table 2 includes the time starting from mixing the solutions, transfer to the rheometer and start of the measurement. The time dependence of the storage modulus (G') and the loss modulus (G'') was recorded.

For the determination of the degree of swelling, gels were prepared from 1 mL reaction mixture. Immediately after preparation, the wet gels were placed in tubes equipped with fritted glass plate. The hydrogels were allowed to swell in a water bath for 48 h at a temperature of 25 °C. Excess of water was wiped off and the tubes were centrifuged for 2 min at 2200 rpm and weighed to obtain m_s. The swollen hydrogels were dried for 24 h at 60 °C and weighed to obtain m_d. The maximum water content Q_{eq} was calculated according to Eq. (1).

$$Q_{eq} = \frac{m_s - m_d}{m_s} \times 100\% \quad (1)$$

Q_{eq}: maximum water content; m_s: mass of the swollen sample; m_d: mass of the dried sample.

The gel fraction F_p was calculated according to Eq. (2).

$$F_p = \frac{m_{th}}{m_d} \times 100\% \quad (2)$$

F_p: gel fraction; m_{th}: theoretical mass of the polymer in the gel (based on the stock solutions used); m_d: mass of the dried sample.

The degree of substitution (DS) of carboxymethyl groups was determined by HPLC after hydrolysis of the polymer with perchloric acid according to a previously published method (Heinze & Pfeiffer, 1999).

2.3. Methods

2.3.1. Synthesis of tosyl cellulose **2**

The synthesis was carried out according to (Rahn, Diamantoglou, Klemm, Berghmans, & Heinze, 1996).

Yield: 41.1 g (133.8 mmol, 91.6%).
Degree of substitution of tosyl groups, DS_{Tos}: 0.94 (based on elemental analysis).
Degree of substitution of 6-deoxy-6-chloro groups, DS_{Cl}: 0.06 (based on elemental analysis).
Elemental analysis: 48.21% C, 5.17% H, 9.76% S, 0.7% Cl.
FTIR (KBr): 3479 (ν OH), 2887 (ν CH_2), 1359 (ν_{as} SO_2), 1173 (ν_s SO_2) cm^{-1}.

2.3.2. 6-Azido-6-deoxy cellulose (**4**)

Tosyl cellulose **2** (12.01 g, 39 mmol, DS_{Tos} 0.94) was dissolved in 400 mL *N,N*-dimethyl formamide (DMF) under stirring. Sodium azide (12.03 g, 185 mmol, 4.8 mol/mol anhydroglucose unit, AGU) was added to the viscous solution. The reaction mixture was allowed to react for 24 h at 100 °C under stirring. After cooling down to room temperature, the solution was poured into 3 L of ice water. The precipitate was filtered off, washed with water (4 × 1 L) and ethanol (3 × 500 mL). The white powder was dried in vacuum at 40 °C.

Yield: 6.84 g (37.3 mmol, 95.6%).
Degree of substitution of 6-azido-6-deoxy groups, DS_{Azide}: 0.81 (based on elemental analysis).
DS_{Tos}: 0.01 (based on elemental analysis).
SEC: $\bar{M}_n$ 53,278 g/mol, $\bar{M}_w$ 216,140 g/mol, PDI 4.06.
Number average degree of polymerization, DP_n: 291.
Elemental analysis: 38.42% C, 4.81% H, 18.51% N, 0.12% S. [theoretical data: 39.65% C, 5.03% H, 18.52% N, 0.17% S].
^{1}H-NMR: (DMSO-d_6/LiCl) δ: 7.8–7.4 (H_{aryl}-Tosyl), 5.00–3.14 (H_{AGU}) ppm.
^{13}C-NMR (DMSO-d_6/LiCl) δ: 103.3 (C1), 79.6 (C4), 75.6–72.3 (C2, 3, 5), 60.1 (C6′), 51.5 (C6) ppm.
FTIR (KBr): 3441 (ν OH), 2920 (ν CH_2), 2108 (ν azide), 1373 (ν_{as} SO_2), 1161 (ν_s SO_2) cm^{-1}.

2.4. Carboxymethyl-6- azido -6-deoxy cellulose (**6**)

6-Azido-6-deoxy cellulose **4** (6.01 g, 32.8 mmol, DS_{Azide} 0.81) was stirred with 100 mL DMSO for 2 h at room temperature. Isopropanol (150 mL) was added to the swollen polymer under stirring. A solution of 7.8 g (195.5 mmol, 6 mol/mol AGU) NaOH in 30 mL water was added to the suspension and the mixture was allowed to react for 2 h under stirring. Sodium monochloroacetate (22.6 g, 196 mmol) was added and the mixture was allowed to react for 4 h at 55 °C under stirring. Sodium monochloroacetate (8.5 g, 72 mmol) were added and stirring was continued for 1 h. After cooling to room temperature, the product was filtered off and washed with 500 mL ethanol. Purification was carried out by dissolving the crude product in water (120 mL) and reprecipitation in ethanol (700 ml). The product was washed with ethanol (2 × 500 mL), ethanol/water (5/1, v/v) (2 × 300 mL) and with ethanol (3 × 250 mL) and dried in vacuum at 40 °C.

Yield: 6.6 g (23.3 mmol, 71%).
DS_{Azide}: 0.81 (based on elemental analysis of compound **4**).
Degree of substitution of carboxymethyl groups, DS_{CM}: 1.57 (based on HPLC analysis of the hydrolyzed polymer).
SEC: $\bar{M}_n$ 45,934 g/mol, $\bar{M}_w$ 133,910 g/mol, PDI: 2.92.
DP_n: 162.
Diglycolic acid: 4.4% (based on HPLC analysis of hydrolyzed polymer).
Glycolic acid: 8.2% (based on HPLC analysis of hydrolyzed polymer).
^{13}C-NMR (DMSO-d_6/LiCl) δ: 175.8–175.3 (C8), 100.4 (C1), 79.5–71.4 (C2–5), 70–68.7 (C7), 68.1 (C6 (CM)), 55.8 (C6 (OH)), 48.8 (C6 (N_3)) ppm.
FTIR (KBr): 3439 (ν OH), 2926 (ν CH_2), 2115 (ν azide), 1607 (ν COONa) cm^{-1}.

2.4.1. *6-Deoxy-6-aminopropargyl cellulose (**8**)*

Tosyl cellulose **2** (12.0 g, 39 mmol, DS_{Tos} 0.94) was dissolved in 500 mL DMSO and propargylamine (20 g, 363 mmol, 9.3 mol/mol AGU) was added drop-wise to the viscous solution. The reaction mixture was allowed to react for 24 h at 80 °C under stirring. After cooling down to room temperature, the black solution was poured into 1.6 L acetone. The brown precipitate was filtered off and washed with acetone (4 × 400 mL). The raw product was reprecipitated from DMSO (100 mL) into acetone (1.2 L). After filtration and washing with acetone (2 × 400 mL), the product was dried in vacuum at 40 °C.

Yield: 5 g (22.4 mmol, 57.5%).
Degree of substitution of 6-deoxy-6-aminopropargyl groups, DS_{Amino}: 0.58 (based on elemental analysis).
DS_{Tos}: 0.25 (based on elemental analysis).
DS_{Cl}: 0.04 (based on elemental analysis).
Elemental analysis: 50.1% C, 5.96% H, 3.62% N, 3.62% S, 0.6% Cl. [theoretical data: 51.15% C, 5.92% H, 3.65% N, 3.6% S, 0.64% Cl].
^{1}H-NMR (DMSO-d_6/LiCl) δ: 7.78–7.42 (H_{aryl}-Tosyl), 5.0–2.86 (H_{AGU}), 2.3 (CH_3-Tosyl), 2.09 (H9) ppm.
^{13}C-NMR (DMSO-d_6/LiCl) δ: 129.9–126.1 (C_{aryl}-Tosyl), 103.3 (C1), 83.23 (C8), 80.42 (C9), 75.4-73.9 (C2–5), 61.1 (C6′), 45.9 (C6), 38.2 (C7), 21.5 (CH_3-Tosyl) ppm.
FTIR (KBr): 3442 (ν OH), 3297 (ν alkyne, shoulder), 2915 (ν CH_2), 1366 (ν_{as} SO_2), 1176 (ν_s SO_2) cm^{-1}.

2.4.2. *Carboxymethyl-6-deoxy-6-aminopropargyl cellulose (**10**)*

6-Deoxy-6-aminopropargyl cellulose **8** (4.5 g, 20 mmol, DS_{Amine} 0.58) was treated with 50 mL DMSO for 2 h at room temperature and subsequently isopropanol (140 mL) was added to the swollen polymer. A solution of sodium hydroxide (4.88 g, 122 mmol, 6 mol/mol AGU) in 20 mL water was added to the suspension and the mixture was stirred for 2 h at room temperature. Sodium monochloroacetate (8.86 g, 76 mmol) was added and the mixture was allowed to react for 4 h at 55 °C under stirring. After cooling to room temperature, the product was filtered off, washed with ethanol (300 mL), ethanol/water (4:1, v/v, 300 mL), and with ethanol (300 mL). The raw product was dissolved in 400 mL water, filtrated and precipitated in 1 L ethanol. The product was washed with ethanol (2 × 500 mL) and dried in vacuum at 40 °C.

Yield: 2.21 g (6.4 mmol, 31.7%).
DS_{Amine}: 0.58 (based on elemental analysis of compound **8**).
DS_{CM}: 2.05 (based on HPLC analysis of the hydrolyzed polymer).
DS_{Cl}: 0.04 (based on elemental analysis).
SEC: $\bar{M}_n$ 14,815 g/mol, $\bar{M}_w$ 49,893 g/mol, PDI: 3.37.
DP_n: 43.
Diglycolic acid: 19.7% (based on HPLC analysis of hydrolyzed polymer).
Glycolic acid: 6.3% (based on HPLC analysis of hydrolyzed polymer).
^{13}C-NMR (DMSO-d_6/LiCl) δ: 176.2–175.4 (C11), 100.5 (C1), 79.5–71.8 (C2–5), 69–68.4 (C10), 67 (C6 (CM)), 55.9 (C6 (OH)), 46.7 (C6 (Amine)), 35.5 (C7) ppm.
FTIR (KBr): 3461 (ν OH), 3300 (ν alkyne, shoulder), 2924 (ν CH_2), 1601 (ν COONa) cm^{-1}.

2.4.3. *Preparation of hydrogels (sample **G9**)*

Carboxymethyl-6-deoxy-6-aminopropargyl cellulose (0.579 g, 1.66 mmol, sample **10**) and carboxymethyl-6-azido-6-deoxy cellulose 0.308 g, 1.09 mmol, sample **6**) were dissolved in 10 mL water under stirring. The copper(I)-catalyst was prepared by mixing aqueous solutions of $CuSO_4$ (5 mL, 0,04 mol/L) and ascorbic acid (5 mL, 1 mol/L). The solutions were mixed and homogenized rapidly. A clear gel of green colour was formed after 2.5 min at room temperature. The compositions of the gels prepared are summarized in Table 2.

3. Results and discussion

3.1. *Preparation of the cellulose derivatives*

The starting materials for the hydrogel preparation were synthesized from tosyl cellulose (Scheme 1), which can be easily prepared by conversion of cellulose dissolved in *N,N*-dimethyl acetamide (DMA)/LiCl with *p*-toluenesulphonic acid (tosyl) chloride in the presence of triethylamine according to (Rahn et al., 1996). Tosyl celluloses with different DS were used (sample **1**, DS_{Tos} 0.33; sample **2**, DS_{Tos} 0.94). A $DS_{Tos} < 1$ was chosen to ensure a preferred tosylation of position 6 with negligible functionalization of the secondary OH groups. The 6-azido-6-deoxy celluloses **3** and **4** were prepared by conversion of tosyl cellulose (samples **1** and **2**) with sodium azide in DMF solution for 24 h at 100 °C (Heinze, Koschella, Brackhagen, Engelhardt, & Nachtkamp, 2006). The products **3** (DS_{Azide} 0.15) and **4** (DS_{Azide} 0.81) were isolated by precipitation in water followed by washing with water and ethanol. Elemental analysis revealed an almost complete substitution of the tosylate moieties by azide groups (0.88% S in sample **3**, DS_{Tos} 0.05; 0.12% S in sample **4**, DS_{Tos} 0.01). This was confirmed by ^{13}C-NMR spectroscopy. The following peaks were assigned: 103.3 ppm (C1), 79.6 ppm (C4), 75.6–72.3 ppm (C2, 3, 5), 60.1 ppm (CH_2OH), 51.5 ppm (CH_2N_3). Signals of very low intensity belonging to the tosyl groups could only be detected in the ^{1}H-NMR spectrum. The presence of the azide group was clearly shown by FTIR spectroscopy due to the presence of an absorption band at 2108 cm^{-1}. Other bands appear at 3441 cm^{-1} (νOH) and 2910 cm^{-1} (νCH). The characteristic bands of the tosyl group at 1373 (ν_{as} SO_2), 1161 (ν_s SO_2) cm^{-1} could be seen as very weak signals only. The samples are soluble in DMA, DMF, and DMSO.

The 6-deoxy-6-aminopropargyl celluloses **7** and **8** were prepared by a comparable strategy. Tosyl celluloses **1** and **2** dissolved

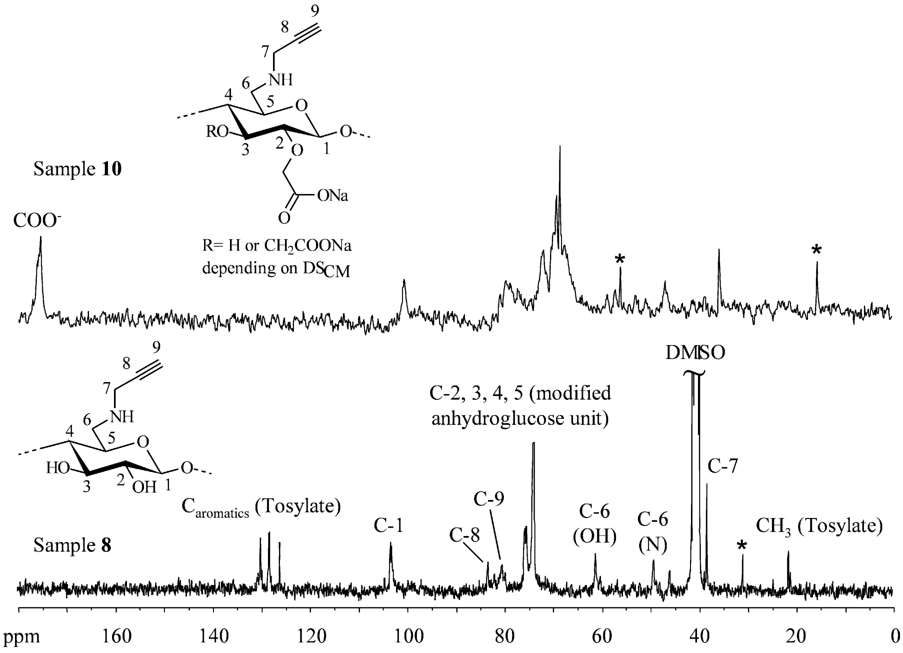


Fig. 1. ^{13}C-NMR spectra of 6-deoxy-6-aminopropargyl cellulose (**8**, degree of substitution of 6-desoxy-6-aminopropargyl groups, DS_{Amino} 0.58, recorded in dimethyl sulphoxide-d_6) and carboxymethyl-6-deoxy-6-aminopropargyl cellulose (**10**, DS_{Amino} 0.58, DS of carboxymethyl groups, DS_{CM}, 2.05, recorded in D_2O). Impurities (trapped solvents) are marked with an asterisk.

in DMSO were converted with propargyl amine for 24 h at 80 °C according to (Pohl & Heinze, 2008). The products were precipitated in acetone, washed, and dried. The elemental analysis revealed an incomplete nucleophilic displacement of the tosyl groups, i.e., there is a remaining DS_{Tos} of 0.05 (sample **7**) and 0.25 (sample **8**).

The displacement of the tosyl groups became also obvious in the FTIR spectra: 3442 cm^{-1} (νOH), 3297 cm^{-1} (ν alkyne, shoulder), 2915 cm^{-1} (νCH_2), 1366 cm^{-1} ($\nu_{as}SO_2$), 1176 cm^{-1} ($\nu_s SO_2$) cm^{-1}. The structure was characterized by ^{13}C-NMR spectroscopy indicating following signals: 129.9–126.1 ppm (C_{aryl}-Tosyl), 103.3 ppm (C1), 83.23 ppm (C8), 80.42 ppm (C9), 75.4–73.9 ppm (C2–5), 61.1 ppm (CH_2OH), 45.9 ppm ($CH_2NHCH_2C{\equiv}CH$), 38.2 (C7), 21.5 (CH_3-Tosyl) (Fig. 1).

The 6-azido-6-deoxy cellulose derivatives **3** and **4** are soluble in DMA, DMF, and DMSO only and 6-deoxy-6-aminopropargyl cellulose derivatives **7** and **8** are insoluble due to their low DS. In order to get water-soluble cellulose derivatives, the compounds **3**, **4**, **7**, and **8** were carboxymethylated heterogeneously in an isopropanol slurry because the ionic moieties may induce water solubility (Pohl, Morris, Harding, & Heinze, 2009). In order to achieve a complete conversion of the starting materials (compounds **3**, **4**, **7**, and **8**) it was indispensable to dissolve or at least swell the polymers in DMSO followed by precipitation in isopropanol. Otherwise, the inner parts of the particles were inaccessible for the carboxymethylation reagents. After addition of aqueous NaOH, the activated polymer was allowed to react with sodium monochloroacetate. The products were washed with aqueous ethanol and reprecipitated from water into ethanol to remove side-products. HPLC analysis of the carboxymethylated products (samples **5**, **6**, **9**, **10**) after hydrolysis with $HClO_4$ revealed DS_{CM} values of 1.68 (sample **5**), 1.57 (sample **6**), 1.58 (sample **9**), and 2.05 (sample **10**). It must be noticed that by-products of the carboxymethylation (glycolic acid and diglycolic acid) could not be completely removed from the samples, even after multiple reprecipitation (Table 1).

The DS of azide- and alkyne moieties of the carboxymethylated compounds could not be obtained by elemental analysis due to the fact that these ionic compounds do not burn completely and, hence, incorrect results will be obtained. Therefore, the DS of CM-groups was determined by a HPLC method and under the assumption that the other substituents are stable during the carboxymethylation reaction

The molecular weight of the cellulose derivatives was characterized by means of SEC. However, due to the different solubility of the samples, only a rough comparison can be done. 6-Azido-6-deoxy cellulose (samples **3** and **4**) were measured in DMSO/LiBr and possess DP_n values of 104 (**3**) and 291 (**4**, Table 1). SEC of the carboxymethylated products in aqueous Na_2HPO_4 solution gave DP_n values of 196 (sample **5**) and 162 (sample **6**). DP_n values of 140 and 43 were calculated for the carboxymethyl-6-deoxy-6-aminopropargyl cellulose **9** and **10**. It can be concluded that the polymer was more severely degraded during tosylation and/or nucleophilic displacement reaction compared with the car-

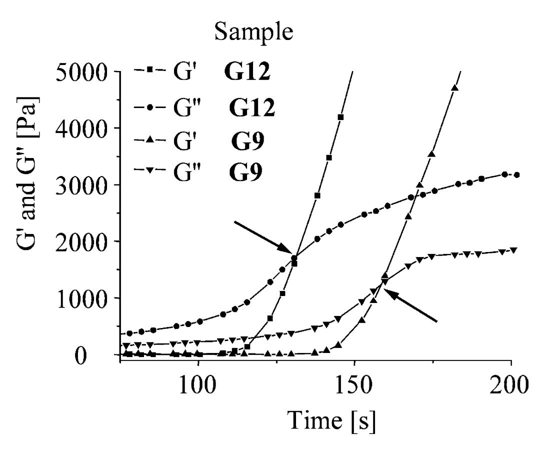
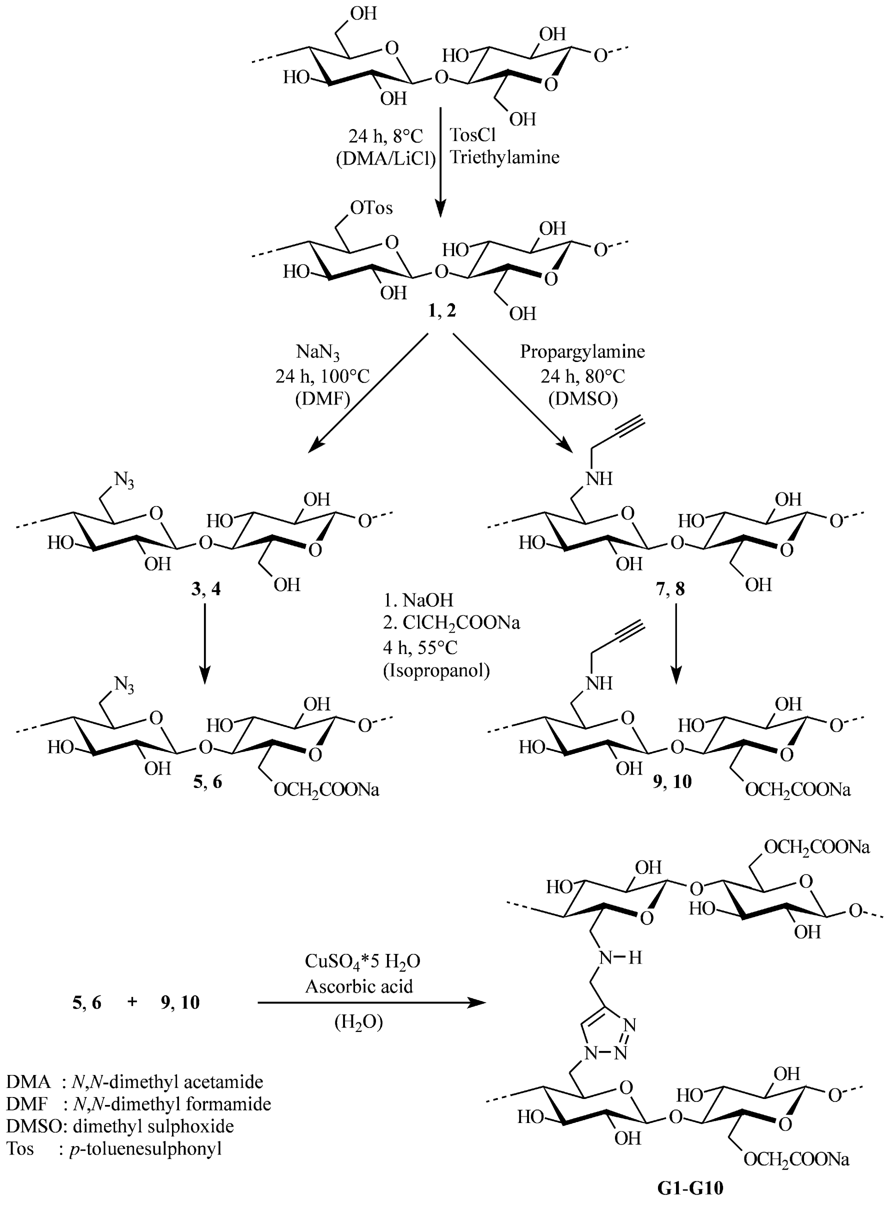


Fig. 2. Time dependency of storage (*G′*) and loss modulus (*G″*) during the copper-catalyzed 1,3-dipolar cycloaddition reaction of carboxymethyl-6-azido-6-deoxy cellulose and carboxymethyl-6-deoxy-6-aminopropargyl cellulose. For the starting materials of gels **G9** and **G12** see Table 2. No data acquisition from 0 to 30 s due to gel preparation.



Scheme 1. Preparation of hydrogels by copper-catalyzed 1,3-dipolar cycloaddition reaction of carboxymethyl-6-azido-6-deoxy cellulose and carboxymethyl-6-deoxy-6-aminopropargyl cellulose. (The real degree of substitution of the samples is not considered for clarity reasons.).

boxymethylation step. However, it must be taken into account that the molar masses measured may not represent the value of a single polymer chain because aggregation of polymer chains is likely to occur due to hydrogen bonds.

3.2. Preparation and characterization of the hydrogels

Gels were obtained within few minutes by mixing aqueous solutions of carboxymethyl-6-azido-6-deoxy cellulose (**5**, **6**) and carboxymethyl-6-deoxy-6-aminopropargyl cellulose (**9**, **10**) with a freshly prepared mixture of aqueous $CuSO_4$ solution and ascorbic acid (Table 2). The molar ratio between azide and alkyne was 1 for every sample. Oscillation rheology was applied to monitor the cross-linking reaction. Preliminary experiments showed that a frequency of 1 Hz and a stress of 10,000 Pa are appropriate measuring conditions. The solution prepared was rapidly transferred to the rheometer. The times given in Fig. 2 and Table 2 include the time required for gel preparation until the start of the measurement. Storage modulus G' and loss modulus G'' were recorded. At the beginning, i.e., before the gelation occurs, G' is lower than G''. The time where the cross-over is observed was set as gel point, i.e., the change from sol to gel state (Fig. 2). The cross-linking reaction is finished when both moduli do not increase further (Sahiner et al., 2006; Weng, Chen, & Chen, 2007). However, the end of the reaction could not be determined due to drying of the gel in the rheometer. Gelation times between 55 and 1594 s were measured (Table 2).

Increasing copper concentration caused a faster gelation of the mixture as exemplified by the comparison of **G1** (catalyst: 5 mmol/L, gelation time 1594 s) and **G3** (catalyst 10 mmol/L, gelation time 78 s). Increase of the catalyst concentration from 5 to 10 mmol/L while maintaining the gel composition constant led to an increase of the gelation rate by a factor of 20. Moreover, it was found that an increase of the DS of azide- and

Table 1
Cellulose derivatives used for the hydrogel preparation via copper-catalyzed 1,3-dipolar cycloaddition reaction.

Starting material	Product									
No.	Compound	No.	Degree of substitution of			DP_n[e]	PDI[f]	Yield [%]	Glycolic acid [%]	Diglycolic acid [%]
			Azide[b]	Amino[c]	CM[d]					
1[a]	6-Deoxy-6-azido cellulose	**3**	0.15	–	–	104	1.96	74.7	–	–
2[a]		**4**	0.81	–	–	291	4.06	95.6	–	–
3	Carboxymethyl-6-deoxy-6-azido cellulose	**5**	0.15	-	1.68	196	2.76	90.4	2.7	8.5
4		**6**	0.81	–	1.57	162	2.92	71.0	8.2	4.4
1	6-Deoxy-6-aminopropargyl cellulose	**7**	–	0.19	–	–	–	80.8	–	–
2		**8**	–	0.58	–	–	–	57.5	–	–
7	Carboxymethyl-6-deoxy-6-aminopropargyl cellulose	**9**	–	0.19	1.58	140	5.54	52.9	2.4	50.3
8		**10**	–	0.58	2.05	43	3.37	31.7	6.3	19.7

[a] Cellulose *p*-toluenesulphonic acid ester with degree of substitution of 0.33 (sample **1**) and 0.94 (sample **2**).
[b] 6-Deoxy-6-azido groups.
[c] 6-Deoxy-6-aminopropargyl groups.
[d] Carboxymethyl cellulose.
[e] Number average degree of polymerization calculated from the number average molar mass determined by SEC.
[f] Polydispersity index.

aminopropargyl groups accelerated the gelation time significantly. It was also observed that the gelation of a mixture of carboxymethyl-6-azido-6-deoxy cellulose with DS_N 0.81 (sample **6**) and carboxymethyl-6-deoxy-6-aminopropargyl cellulose with DS_{Amino} 0.19 (sample **9**) occurred very fast (sample **G4**, gel point 421 s). Both components are mixed in equimolar amounts with regard to azide- and alkyne groups.

The freshly prepared gels are transparent with a green-brownish colour (Fig. 3). They are capable of further swelling in water but lose their mechanical stability, i.e., the gels disintegrate. Therefore, it was impossible to monitor the swelling kinetics. However, maximum water content (Q_{eq}) in the range of 96.8–98.4% could be determined. Gravimetric investigations gave also information on the amount of gel fraction (F_p). Surprisingly low amounts in the range from 34 to 60% were measured indicating a comparably low yield of the cross-linking reaction. The highest gel fraction and, hence, a higher yield was found for samples with high DS of cross-linking groups. Noticeable, the maximum water content decreased with increasing gel fraction. An explanation is that a higher cross-linking density leads to a more rigid network with a lower absorption capacity than more flexible gels (Po, 1994). The gels were freeze-dried prior to further analysis obtaining a solid spongy mass. All structural features could be detected in the FTIR spectra (Fig. 4). The character-

Table 2
Composition and properties of hydrogels prepared from carboxymethylated 6-deoxy-6-azido cellulose and carboxymethylated 6-deoxy-6-aminopropargyl cellulose via copper-catalyzed 1,3-dipolar cycloaddition reaction.

Conditions							Result		
6-Deoxy-6-azido carboxymethyl cellulose[a]		6-Deoxy-6-aminopropargyl carboxymethyl cellulose[a]		Functional group [mmol/L]	Cu^{2+} [mmol/L]	Gel	Gel fraction [%]	Max. water content [%]	Gel point [s]
Sample	Conc. [wt%]	Sample	Conc. [wt%]						
5	6.15	**9**	5.0	30.5	5.0	**G1**	n.d.	n.d.	1594
5	6.15	**9**	5.0	30.5	7.5	**G2**	n.d.	n.d.	253
5	6.15	**9**	5.0	30.5	10.0	**G3**	34.3	98.4	78
6	1.07	**9**	5.0	30.5	5.0	**G4**	n.d.	n.d.	421
6	1.07	**9**	5.0	30.5	7.5	**G5**	n.d.	n.d.	96
6	1.07	**9**	5.0	30.5	10.0	**G6**	42.0	98.3	~55
6	2.89	**10**	5.0	82.5	5.0	**G7**	50.1	97.7	500
6	2.89	**10**	5.0	82.5	7.5	**G8**	59.9	96.8	292
6	2.89	**10**	5.0	82.5	10.0	**G9**	54.2	96.9	158
5	6.15	**10**	1.8	30.5	5.0	**G10**	n.d.	n.d.	341
5	6.15	**10**	1.8	30.5	7.5	**G11**	n.d.	n.d.	198
5	6.15	**10**	1.8	30.5	10.0	**G12**	45.0	98.45	131

Not determined (n.d.).
[a] The functional groups were mixed in equimolar ratio.

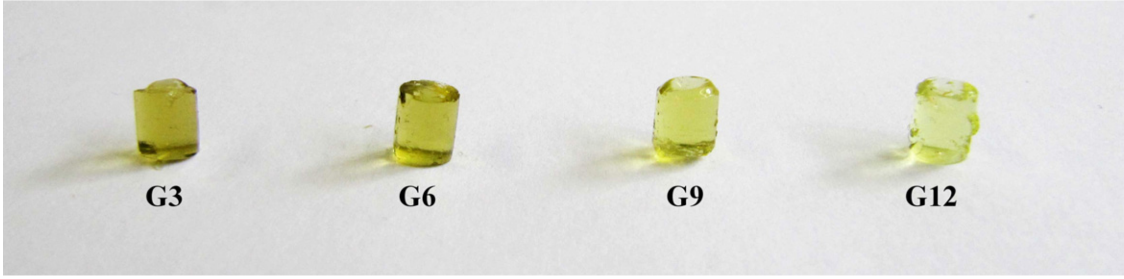


Fig. 3. Freshly prepared hydrogels obtained by copper-catalyzed 1,3-dipolar cycloaddition reaction of carboxymethyl-6-azido-6-deoxy cellulose and carboxymethyl-6-deoxy-6-aminopropargyl cellulose. For starting materials of the gels see Table 2.

istic band of the azide moiety at 2120 cm^{-1} was still present, which indicated that not all azide groups were consumed during the 1,3-dipolar cycloaddition reaction. However, remaining azide- or alkyne groups can be used to attach further functional moieties.

SEM images of **G3**, **G6, G9,** and **G12** were taken to visualise the porous structure of the dried hydrogels (Fig. 5). **G3** and **G6** exhibit an irregular but closed structure while **G9** and **G12** show porous topology as usual for freeze dried hydrogels. This might be caused by the high amount of diglycolic acid in compound **10**. This side product closes the pores of the gels. However, due to the good water solubility of glycolic acid and diglycolic acid they do not influence the swelling properties of the gels remarkably. The comparison of three gels with same composition but different amounts of catalyst is shown in Fig. 5. Obviously, the structure became more regular with an increasing copper concentration (**G7** 5 mmol/L, **G8** 7.5 mmol/L, and **G9** 10 mmol/L). The pore size of the samples was nearly the same.

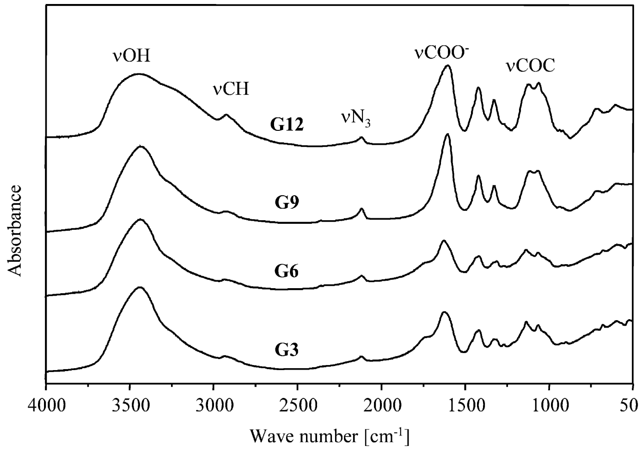


Fig. 4. FTIR spectra of selected freeze dried gels obtained by copper-catalyzed 1,3-dipolar cycloaddition reaction of carboxymethyl-6-azido-6-deoxy cellulose and carboxymethyl-6-deoxy-6-aminopropargyl cellulose. For starting materials of the gels see Table 2.

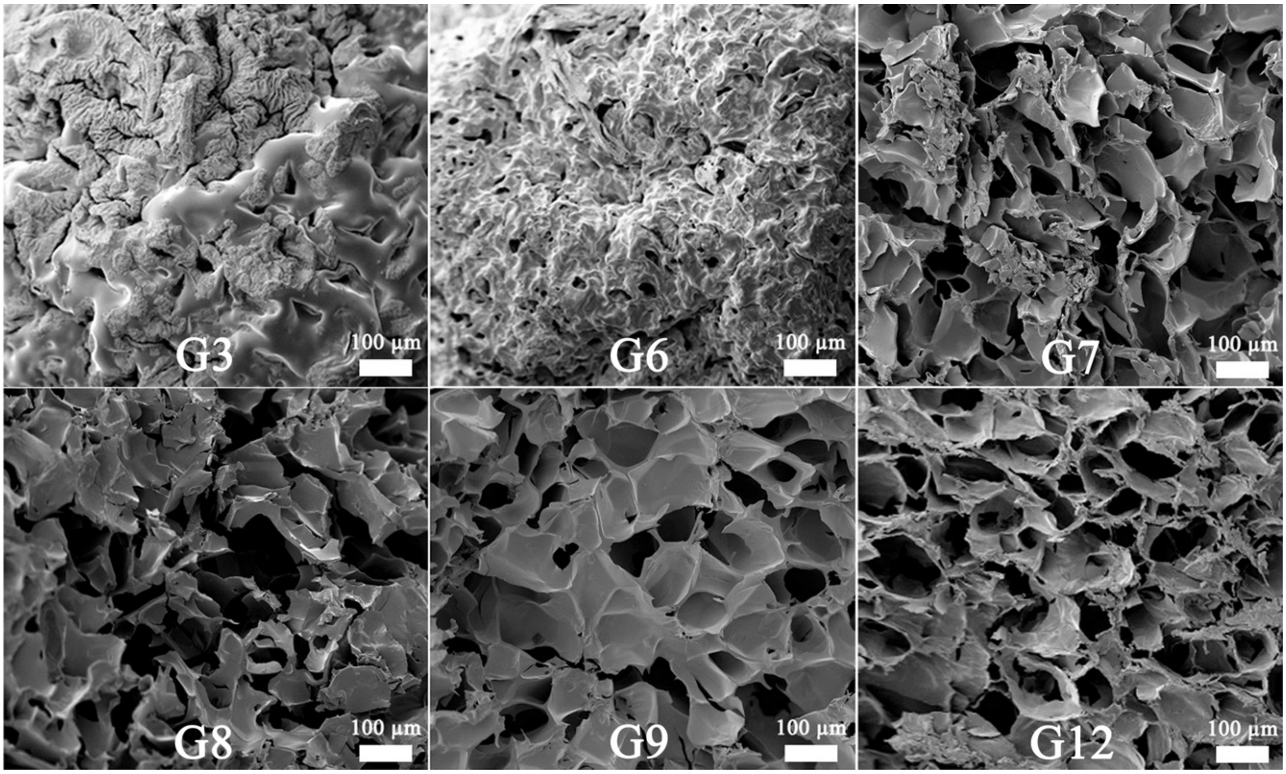


Fig. 5. SEM images of selected freeze dried gels obtained by copper-catalyzed 1,3-dipolar cycloaddition reaction of carboxymethyl-6-azido-6-deoxy cellulose and carboxymethyl-6-deoxy-6-aminopropargyl cellulose. The surface of the samples is shown. For starting materials of the gels see Table 2.

4. Conclusions

It was shown that copper mediated 1,3-dipolar cycloaddition of water soluble cellulose derivatives decorated with azide- and alkyne functions yields novel cellulose hydrogels. The gels were investigated via FTIR spectroscopy, oscillation rheology, SEM; also, the degree of swelling were measured. The gelation time can be controlled by the DS of azide- and alkyne groups as well as by the catalyst concentration. FTIR spectroscopy revealed remaining alkyne- and azide groups in the gels that might be used to bind bioactive molecules or other substances in the gels. The limiting factor for applications is the low mechanical stability, which is probably related to the low yield of the cross-linking reaction. Further investigations will be carried out to improve the mechanical stability of the gels. Moreover, it would be interesting to study the response of the gels to different stimuli, e.g., temperature and pH of the aqueous medium.

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