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Thermoresponsive xylan hydrogels via copper-catalyzed azide-alkyne cycloaddition

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ABSTRACT

In the present work, hydrogels of birch wood xylan and thermoresponsive poly(ethylene glycol)-b-poly(propylene glycol)-b-poly(ethylene glycol) (PEG-PPG-PEG) were prepared using copper catalyzed alkyne-azide cycloaddition (CuAAC) in aqueous reaction conditions. First, reactive azide groups were introduced on the backbone of xylan by etherification of 1-azido-2,3-epoxypropane in alkaline water/isopropanol-mixture at ambient temperature, providing degree of substitution (DS) values up to 0.28. On the second step, the azide groups were reacted with propargyl bifunctional PEG-PPG-PEG utilizing CuAAC, leading to formation of crosslinked hydrogels. The novel xylan derivatives were characterized with liquid and solid state nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FT-IR) and elemental analysis (EA). The temperature controlled swelling behavior of the developed hydrogels was evaluated in the range of 7–70 °C by water absorption and compressive stress–strain measurements, which showed a reduction in water content and change in stiffness with increasing temperature. The morphology of the hydrogels at different temperatures was studied by scanning electron microscopy (SEM), which showed a reduction in pore size with increasing temperature.

1. Introduction

Hemicellulose is the most abundant polysaccharide family next to cellulose, present as a component in most plant cell walls. In hardwood species, the main hemicellulose type is xylan, which predominantly consists of D-xylopyranoside units connected by β-(1→4)-linkages along with acetyl-, glucuronic acid and species dependent side groups (Deutschmann & Dekker, 2012). Among other methods, xylan can be conveniently extracted from biomass by alkali extraction (Ebringerová & Heinze, 2000; Glasser, Kaar, Jain, & Sealey, 2000) where the acetyl groups are also hydrolyzed rendering the backbone hydroxyl groups available for derivatization reactions.

Several chemical modifications targeting the hydroxyl groups have been reported for xylan, including etherification using epoxides and alkyl halides in alkaline media (Bigard et al., 2011; Ebringerová, Hromádková, Kacuráková, & Antal, 1994; Fang, Fowler, Tomkinson, & Hill, 2002; Jain, Sjöstedt, & Glasser, 2001; Kataja-aho, Haavisto, Asikainen, Hyvärinen, & Vuori, 2008; Laine et al., 2013; Petzold, Günther, Kötteritzsch, & Heinze, 2008; Petzold, Schwikal, & Heinze, 2006; Ren, Sun, & Liu, 2007; Saghir, Iqbal, Hussain, Koschella, & Heinze, 2008; Saghir, Iqbal, Koschella, & Heinze, 2009; Schwikal & Heinze, 2007; Schwikal, Heinze, Ebringerová, & Petzold, 2006; Vincendon, 1998) and development of bifunctional derivatives using acrylamide (Ren, Peng, & Sun, 2008). In addition, esterification reactions typically employing anhydrides or activated carboxylic acids (Buchanan et al., 2003; Daus & Heinze, 2010; Fundador, Enomoto-Rogers, Takemura, & Iwata, 2012; Hansen & Plackett, 2011; Hesse, Liebert, & Heinze, 2006; Hettrich et al., 2006; Salam, Pawlak, Venditti, & El-tahlawy, 2011) and sulfating agents (Daus et al., 2011; Hettrich et al., 2006) have been used to alter the chemical properties and introduce new functionality to xylans. In order to tailor the properties of natural-based polymers, the copper catalyzed azide-alkyne cycloaddition (CuAAc), often referred as "click"-reaction, offers a straightforward and efficient way to build new molecular complexity (Kolb, Finn, & Sharpless, 2001; Rostovtsev, Green, Fokin, & Sharpless, 2002; Tornoe, Christensen, & Meldal, 2002). Characteristics for click-type reactions is that they can be performed at mild reaction conditions and have high tolerance toward functional groups, oxygen and water, which makes the use of this chemistry tempting for the modification of natural polymers. The CuAAC offers extensive possibilities to tailor polymer properties (Binder & Sachsenhofer, 2007, 2008; Fournier, Hoogenboom, & Schubert, 2007; Meldal, 2008; Lallana, Riguera, & Fernandez-Megia, 2011; Lallana, Fernandez-Trillo, Sousa-Herves, Riguera, & Fernandez-Megia, 2012; Lallana,

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Sousa-Herves, Fernandez-Trillo, Riguera, & Fernandez-Megia, 2012; Kempe, Krieg, Becer, & Schubert, 2012). The utilization of CuAAC on the modification of polysaccharides has been reported in several publications (Bernard, Save, Arathoon, & Charleux, 2008; De Geest et al., 2008a, 2008b; Eissa, Khosravi, & Cimecioglu, 2012; Elchinger, Montplaisir, & Zerrouki, 2012; Hafrén, Zou, & Córdova, 2006; Hasegawa et al., 2006; Koschella, Richter, & Heinze, 2010; Liebert, Hänsch, & Heinze, 2006; Pohl, Schaller, Meister, & Heinze, 2008; Ritter, Knudsen, Mondrzik, Branscheid, & Kolb, 2012; Schatz, Louguet, Le Meins, & Lecommandoux, 2009; Tankam, Müller, Mischnick, & Hopf, 2007; Xu, Zhang, & Kadla, 2012; Zhang, Xu, Wu, Zhang, & Zhuo, 2009). However, studies on the modification of xylan using the CuAAC-reaction is limited to a recent paper, where propargyl end-functionalized polylactide was grafted on azide-containing xylan (Enomoto-Rogers & Iwata, 2012).

Hydrogels, three-dimensional networks of hydrophilic polymers capable of retaining large amount of water, have a broad field of applications, and the research on such materials is an ongoing task. Applications such as drug-delivery systems, artificial muscles and sensors are being developed (Calvert, 2008; Coviello, Matricardi, Marianecci, & Alhaiqu, 2007; Klouda & Mikos, 2008). Polysaccharide based hydrogels have some advantages over synthetic polymers, since in addition to their abundant availability, they are biologically compatible and degradable (Coviello et al., 2007). Xylans are interesting candidates for such materials also due to their bioactive properties (Cipriani et al., 2008; Ebringerová & Heinze, 2000; Ebringerová, Kardosová, Hromádková, Malovíková, & Hribalová, 2002). For example, xylan based-hydrogels have been developed from methacrylated xylan and subsequent radical polymerization of hydroxyethyl methacrylate for drug release studies (Silva, Habibi, Colodette, & Lucia, 2011). In addition, xylan-rich hemicelluloses grafted with acrylic acid in the presence of N,N-methylene-bis-acrylamide crosslinker yielded hydrogels with multistimulus response properties (Peng, Ren, Zhong, Peng, & Sun, 2011). Allylated xylan derivatives were successfully crosslinked by UV induced radical crosslinking with and without N,N'-diallylaldardiamides yielding novel bio-based hydrogels (Pohjanlehto, Setälä, Kammiovirta, & Harlin, 2011). The xylan hydrogel formation has also been reported on enzymatically aided method (Chimphango, van Zyl, & Görgens, 2012), by physical (Hettrich & Fanter, 2010) or ionic interactions (Gabrielii & Gatenholm, 1998; Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000). The utilization of the CuAAC reaction for the synthesis of polysaccharide hydrogels has been reported for azide and alkyne derivatized hyaluronan and the obtained hydrogels were demonstrated to serve as drug reservoirs and scaffolds (Crescenzi, Cornelio, Di Meo, Nardecchia, & Lamanna, 2007, Huerta-Angeles et al., 2011; Huerta-Angeles et al., 2012). In addition, networks based on cellulose (Koschella, Hartlieb, & Heinze, 2011, Pierre-Antoine, Francois, & Rachida, 2012) and thermoresponsive cellulose/poly(N-isopropylacrylamide-co-hydroxyethyl methacrylate) hydrogels have been developed (Zhang et al., 2009).

In our previous publication, we described a method for introducing azide-groups on the backbone of dextran using aqueous reaction media (Pahimanolis, Vesterinen, Rich, & Seppala, 2010). The azide functionalities provide a combinatorial approach to discover new materials, as a wide range of possible modifications via CuAAC become available. In this paper, the modification is applied to xylan. First, azide groups were introduced to the backbone of xylan using glycidyl azide under alkaline conditions. On the second step, the novel azide modified xylan was crosslinked with thermoresponsive alkyne end-functionalized polyethylene glycol/polypropylene glycol/polyethylene glycol (PEG-PPG-PEG) triblock copolymers using CuAAC, yielding temperature responsive hydrogels. Elemental analysis, NMR and FT-IR were used to confirm the chemical structure of the synthesized products. The temperature controlled swelling behavior of the developed hydrogels was evaluated in the range of 7–70 °C, which showed a reduction in water absorption with increasing temperature. The hydrogels could have applications as drug delivery systems, or work as part of separation, fractionation or self-cleaning membranes (Klouda & Mikos, 2008; Vermonden, Censi, & Hennink, 2012; Wandera, Wickramasinghe, & Husson, 2010).

2. Experimental

2.1. Materials

Birch wood xylan (xylose content ≥90%, degree of acetylation less than 4% determined with ^{1}H NMR), Mn = 11700 g/mol, PDI = 2.02 determined by GPC against PEG standards) was purchased from Sisco Research Laboratories Pvt. Ltd. and used as received.

Propargyl bromide (80 wt% in toluene) and HNO_3 (65%) were obtained from Fluka Chemicals. L-Ascorbic acid (99%), $CuSO_4{\cdot}5H_2O$ (99%), epichlorohydrin (99%), ethylenediaminetetraacetic acid tetrasodium salt dihydrate (EDTA, 99%), 2-propanol (99.8%), NaH (95%) and $NaNO_2$ (97%) were purchased from Sigma–Aldrich. NaN_3 (99%), acetic acid (99.8%) and NaOH (99%) were from Merck. All chemicals were used as received. Anhydrous grade tetrahydrofuran (THF) was purchased from VWR and stored over molecular sieves. Poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO–PPO–PEO) triblock copolymers were from BASF (trade name Pluronic© PE6100 and PE 6400 both having a central PPO block of 1750 g/mol and 10% or 40% of PEO in molecule respectively). Poly(ethylene glycol) 2000 g/mol was from Fluka. The polymers were vacuum-dried at 40 °C for 48 h before use.

2.2. Preparation of 1-azido-3-chloro-propanol

The synthesis of 1-azido-3-chloropropanol was done starting from epichlorohydrin. The ring-opening reaction of the epoxide with azide-ion was done according to a modified method (Fringuelli, Piermatti, Pizzo, & Vaccaro, 1999; Pahimanolis et al., 2010; Yang, Shao, Li, Wang, & Zhang, 2011). Isopropanol (109.0 ml) and acetic acid (7.2 ml, 125.8 mmol) were mixed with a solution of NaN_3 (8.177 g, 125.8 mmol) in 74.0 ml of water. Epichlorohydrin (6.6 ml, 84.2 mmol) was then added under stirring and the reaction was continued at 30 °C for 24 h, until ^{1}H NMR analysis showed complete consumption of the epoxide. A water solution of $NaNO_2$ (14.4 ml, 41.6 mmol) was then added, followed by the dropwise addition of HNO_3 (5.76 ml, 83.8 mmol) to eliminate any excess azide-ions. The stirring was continued for 24 h at room temperature, by which time the formation of nitrous oxides had ceased. To concentrate the solution, 12 g of NaCl was added and the separated propanol phase was collected. The aqueous phase was extracted once with 50 ml of diethyl ether and the organic phases were combined. This resulted in additional phase separation of water, which was discarded. The organic phase was further concentrated by removing diethyl ether by rotary evaporation. The obtained propanol solution of 1-azido-3-chloropropanol (58 ml, concentration 1.34 mmol/ml, yield 92% by ^{1}H NMR analysis, acetic acid content 0.61 mmol/ml) was stored in dark at room temperature and used without further purification.

Warning! Low molecular weight organic azides are known to be potentially explosive. For this reason, handling highly concentrated solutions of these materials should be avoided.

^{1}H NMR (D_2O, ppm): δ = 3.36–3.54 (CH_2-Cl), 3.56–3.73 (CH_2-N_3).
^{13}C NMR (D_2O, ppm): δ = 70.50 (C-OH), 53.92 (C-N_3), 46.69 (C-Cl).

Table 1
The effect of different reaction conditions on the obtained degree of substitution (DS) in the etherification reaction of xylan with 1-azido-2,3-epoxypropane (AEP).

Entry	Time (h)	Temperature (°C)	Molar ratio NaOH/AXU	Molar ratio AEP/AXU	DS[a]	Reaction efficiency (%)
1	24	30	0.26	0.50	0.06	12
2	24	30	0.26	2.02	0.14	7
4	24	30	0.79	0.50	0.08	16
5	24	30	0.79	1.01	0.14	14
6	24	30	0.79	2.02	0.17	8
7	24	30	0.79	3.01	0.28	9
8	24	30	1.32	0.50	0.08	16
9	24	30	1.32	2.02	0.18	9
10	6	55	1.32	0.50	0.06	12
11	6	55	1.32	1.98	0.23	12
12	24	30	2.64	0.58	0.07	12
13	24	30	2.64	1.98	0.18	9

[a] Determined with elemental analysis.

2.3. Introducing azide groups to the backbone of xylan

The azide functionalization of xylan was done following a modified method (Pahimanolis et al., 2010; Yang et al., 2011): (Table 1, entry 11) To a water solution of xylan (0.500 g in 10.0 ml of water) 1.00 ml of 5 M NaOH solution was added and the mixture was stirred for 10 min at 55 °C. The 1-azido-2,3-epoxypropane needed for the etherification was prepared just prior to use, by adding 2.18 ml of 5 M NaOH solution to 5.60 ml (7.5 mmol) of 1-azido-3-chloropropanol and stirring for 5 min at room temperature. The obtained epoxide solution was combined with the xylan solution and the reaction was continued for 6 h at 55 °C, by which time ^{1}H NMR analysis showed complete consumption of the epoxide. The solution was neutralized with 0.30 g of acetic acid and precipitated in 60 ml of acetone, redissolved in a minimal amount of water and precipitated twice in 70 ml of ethanol. The obtained azide functionalized xylan was lyophilized, dried in vacuum at 40 °C overnight and stored in the dark at room temperature for further use.

2.4. Synthesis of alkyne end functionalized poly(ethylene glycol)-b-poly(propylene glycol)-b-poly(ethylene glycol)

Dry PEG-PPG-PEG (PE6400 2900 g/mol, 5.00 g, 1.7 mmol) was dissolved in 5 ml of dry THF under argon. NaH (0.120 g, 5.0 mmol) was added to the solution and the resulting dispersion was stirred for 2 h, until the formation of hydrogen gas ceased. Propargyl bromide (80% in toluene, 0.67 ml, 6.0 mmol) was added and the suspension was stirred for 2 h, after which 5 ml of THF was added. The salts were removed by centrifugation and the polymer was precipitated in cold (−21 °C) heptane. The centrifugation and precipitation was repeated two more times. The polymer was dried in vacuum at 30 °C for 48 h to obtain a yellow viscous liquid. The yield was 3.20 g (64%).

The alkyne-end functionalization of PE6100 was done using 0.168 g (7.0 mmol) of NaH and 0.89 ml (8.0 mmol) of propargyl bromide. The product was yellow viscous liquid and its yield was 1.24 g (25%).

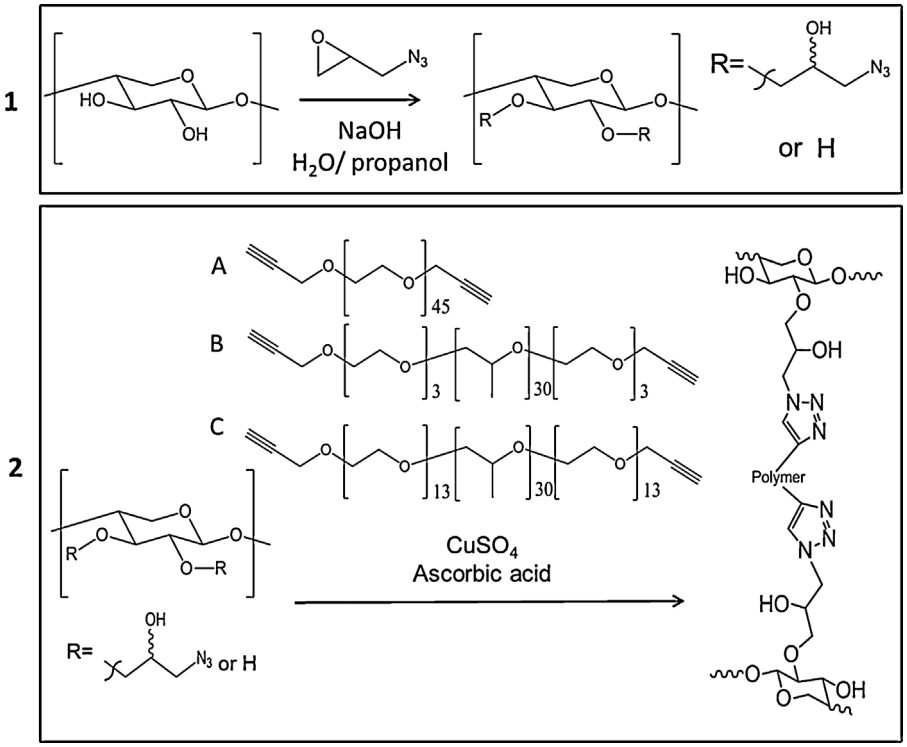

Fig. 1. Introducing azide groups on xylan (1), crosslinking of azide containing xylan with different polymers using CuAAC (2). PEG (A), PE6100 (B) and PE6400 (C).

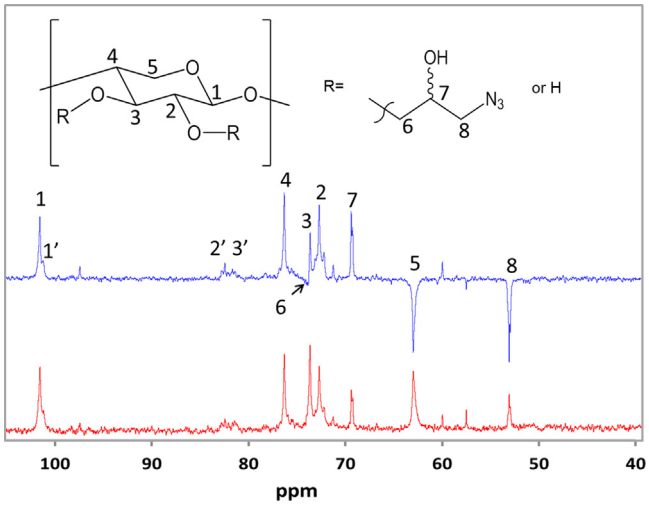

Fig. 2. DEPT-135 and ^{13}C NMR spectra of hydroxypropyl azide xylan.

^{1}H NMR ($CDCl_3$): (ppm) 1.09, 2.43, 3.20–3.80, 4.15.
^{13}C NMR ($CDCl_3$): (ppm) 17.4, 58.5, 68.5, 68.6, 69.0, 70.4, 70.5, 70.8, 72.9, 73.3, 74.6, 75.1, 75.3, 75.5, 77.4, 79.6.

The alkyne-end functionalization of PEG was done as above, except that 40 °C reaction temperature was used. The product was brown waxy solid, and its yield was 4.38 g (88%).

^{1}H NMR ($CDCl_3$): (ppm) 2.43, 3.40–3.80, 4.16.
^{13}C NMR ($CDCl_3$): (ppm) 58.4, 69.1, 70.4, 70.5, 74.7, 77.4, 79.6.

2.5. Hydrogel preparation using CuAAC

The hydrogels were prepared as follows: 0.100 g of azide functionalized xylan (Table 1, Entry 11, DS = 0.23) was dissolved in 4.00 ml of water. The polymer used for crosslinking (0.100 g) was added and allowed to dissolve for 5 h in an ice-bath under stirring and overnight at 7 °C. The stirring was continued in an ice bath for another 5 h. A freshly prepared water solution (0.63 ml) of copper sulfate (1 equiv. to alkyne groups used) and ascorbic acid (2 equiv.) was chilled in an ice bath and then added to the xylan/polymer solution and allowed to stir for 30 s. 1.1 ml of the solution was then pipetted on four cylindrical silicone molds (height 16.6 mm, diameter 16.0 mm) and allowed to gel at 7 °C for 4 h. The obtained gels were removed from the molds and submerged in 0.05 M EDTA solution for 48 h at 7 °C to complexate the copper ions. The solution was replaced with distilled water and changed daily for a period of at least a week.

2.6. Characterization

NMR spectra were recorded on a Bruker Avance-III 400 spectrometer in deuterium oxide (D_2O) or deuterated chloroform ($CDCl_3$), using standard ^{1}H, ^{13}C and DEPT 135 pulse sequences. Carbon-13 cross-polarization/magic angle spinning nuclear magnetic resonance (13C-CP/MAS NMR) spectra were recorded with a double resonance 4 mm probe. Samples were spun in zirconia rotors using a spinning rate of 10 kHz. A proton excitation pulse of 2.95 μs and a CP contact time of 2.0 ms were used applying a linear ascending variable amplitude ramp, the acquisition time being 33.9 ms under high power decoupling. 15 k scans were accumulated with a recycle delay of 5 s.

The degree of substitution (DS) was determined by elemental analysis using a Perkin Elmer 2400 CHN equipment.

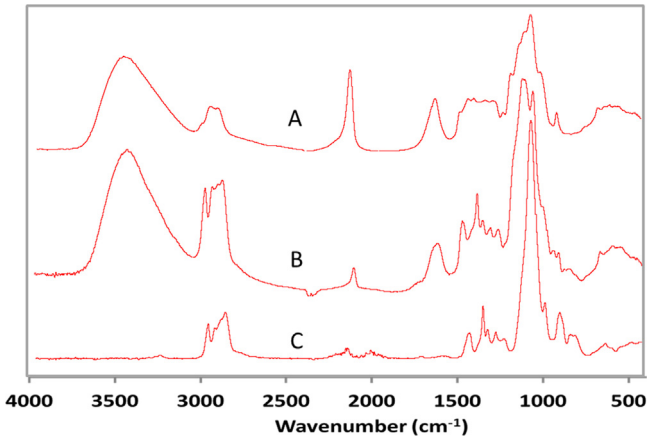

Fig. 3. FT-IR spectra of hydroxypropyl azide xylan (A), crosslinked xylan/PE6100 (B) and PE6100 (C).

The infrared-spectra were recorded with a Unicam Mattson 3000 FTIR spectrometer from KBr pellets (for azide containing samples) or using a diamond ATR accessory.

Scanning electron microscopy (SEM) images were obtained using a Zeiss Sigma VP instrument operating at 3 kV. Small gel samples (approximately 0.1 g) were conditioned in water baths at 7 °C or 70 °C for 2 h, then frozen in liquid nitrogen and freeze-dried for 48 h. The dried samples were cut with a sharp knife to expose fresh surface and made conductive by Au/Pt sputtering.

The compressive stress–strain measurements were performed using a TA Instruments AR-G2 rheometer equipped with a plate-plate geometry (diameter 20 mm). The synthesized cylindrical gel samples were conditioned in a water bath at 7 or 70 °C for 2 h. The dimensions of each sample were measured and then placed on the lower plate and compressed by the upper plate at a speed of 10 μm/s. The diameters of the samples were between 20.73 and 12.75 mm and the heights were between 5.09 and 7.40 mm. Compressive modulus was calculated for each sample from the slope of the stress-strain curve in the beginning of the linear region. For each material, two parallel samples were measured.

Swelling tests were done for three concurrent samples by weighting approximately 100 mg of gel preconditioned in water at 7 °C. The samples were then placed in capped vials of water and

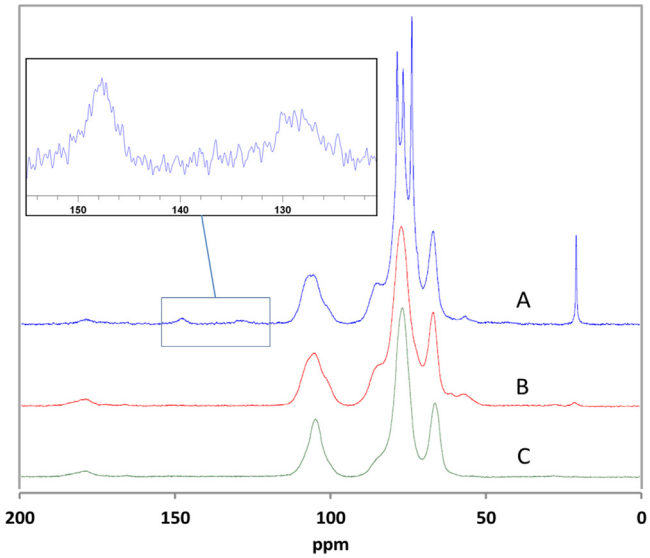

Fig. 4. ^{13}C-CP/MAS spectra of crosslinked xylan/PE6100 (A), hydroxypropyl azide xylan (B) and unmodified xylan (C).

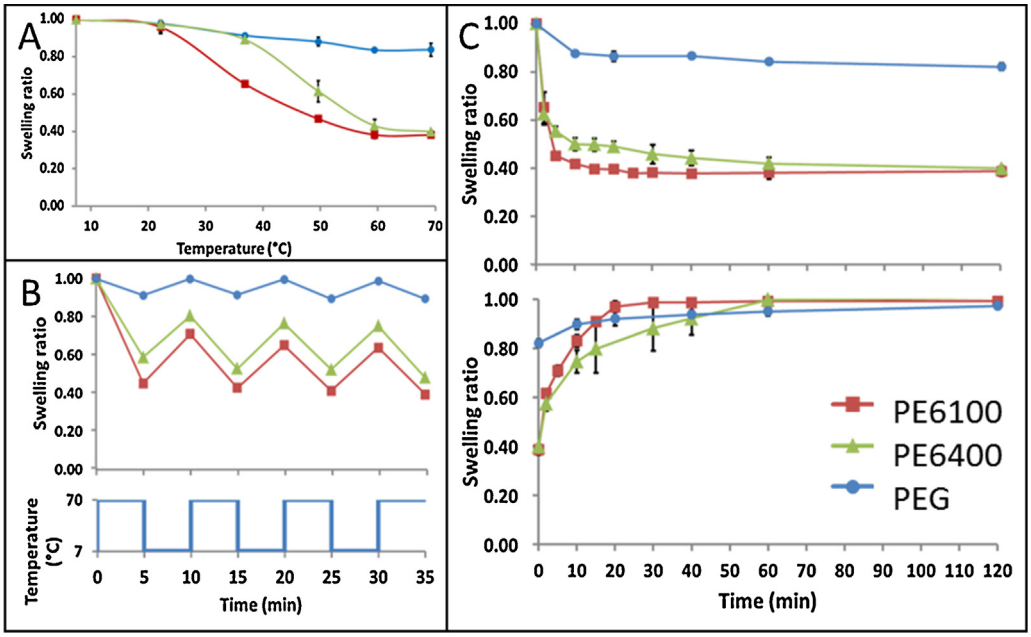

Fig. 5. Swelling ratio of hydrogels as a function of temperature (A) and swelling ratio over 5 min temperature cycles between 7 and 70 °C (B). Swelling ratio as a function of time at 70 °C and 7 °C, for gel samples preconditioned at 7 °C and 70 °C respectively (C).

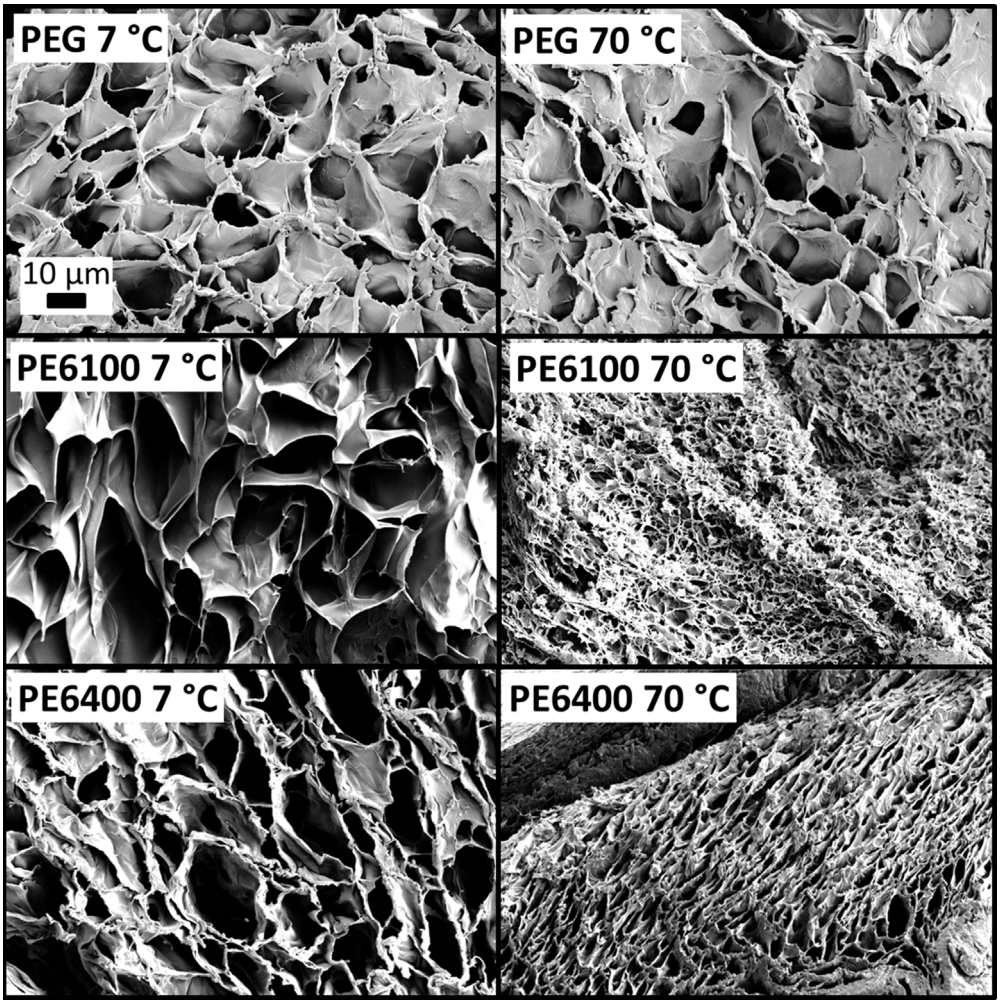

Fig. 6. SEM images of freeze-dried hydrogels of xylans crosslinked with PEG, PE6100 and PE6400. The gel samples were conditioned at 7 °C and 70 °C before freeze-drying.

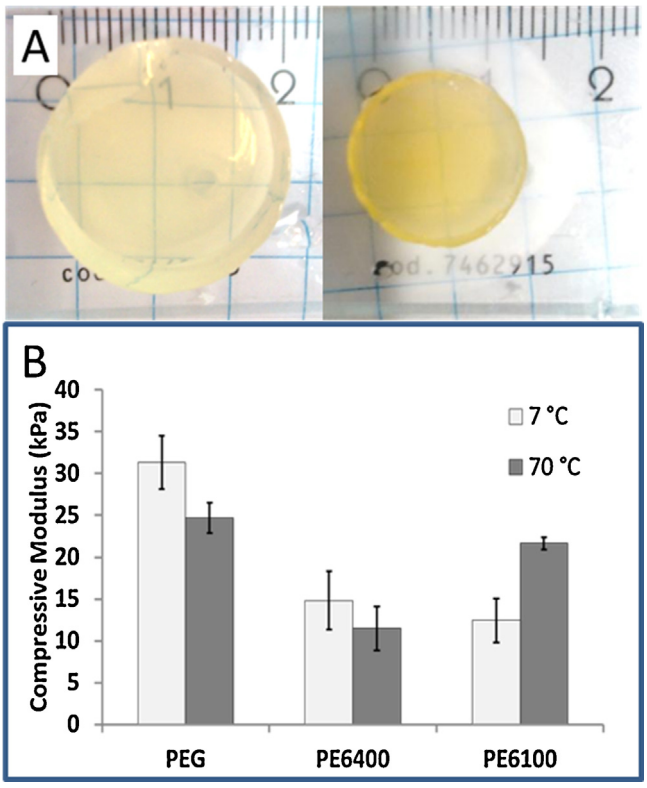

Fig. 7. Xylan hydrogel crosslinked with PE6100 conditioned at 7 °C and 70 °C (A). Compressive modulus of xylan hydrogels crosslinked with PEG, PE6100 or PE6400, conditioned at 7 °C and 70 °C (B).

heated to the desired temperature (7–70 °C). The samples were then weighted after careful removal of excess water. The gel weight was divided with the initial weight to obtain the relative swelling ratio.

3. Results and discussion

3.1. Introduction of azide functionalities to the backbone of xylan

The introduction of azide groups to the backbone of xylan was done following a method described for the functionalization of dextran and cellulose (Pahimanolis et al., 2010; Yang et al., 2011). The 1-azido-2,3-epoxypropanol was prepared in a one-pot synthesis procedure starting with the ring-opening of epichlorohydrin with azide-ion in the presence of acetic acid. The obtained 1-azido-3-chloropropanol was in turn converted to the epoxide-form with alkaline treatment in high yield (Fringuelli et al., 1999). Further, the etherification reaction of 1-azido-2,3-epoxypropane with the hydroxyl groups of xylan was carried out under alkaline conditions (Fig. 1), a method similar to the cationization and hydroxyalkylation of xylan and other polysaccharides using epoxides (Bigard et al., 2011; Ebringerová et al., 1994; Jain et al., 2001; Kataja-aho et al., 2011; Laine et al., 2013; Schwikal et al., 2006; Tomasik & Schilling, 2004). In this way, azide-groups necessary for the subsequent CuAAC-reaction were introduced in a simple one step reaction, without solvent-exchange or drying steps involved in the synthesis. Since low-molecular weight organic azides are potentially explosive substances, the obtained 1-azido-2,3-epoxypropane solution was used for the etherification reaction without further purification or concentration.

Table 1 shows the effect of the amount of added NaOH to the obtained degree of substitution (DS) for the azide groups in xylan. Low degree of functionalization is obtained with low sodium hydroxide to anhydroxylose unit ratio (NaOH/AXU), which may be due to insufficient activation of the xylan hydroxyl that undergo the ring-opening of the epoxide. The obtained functionalization values are generally similar to those previously reported for dextran, although lower amount of NaOH is needed for the reaction. This may be due to structural differences between the polysaccharides, or the fact that more concentrated epoxide solution was used in this study (Pahimanolis et al., 2010). Increasing the NaOH/AGU ratio to 0.79 yields slightly higher azide functionalization, however, a further increase of the amount of NaOH does not significantly affect the substitution values. The addition of higher amounts of epoxide increases the obtained DS value, although the functionalization becomes increasingly difficult as can be noted from the lower reaction efficiency. Raising the reaction temperature from 30 °C to 55 °C, results in a faster reaction, consuming all of the epoxide within 6 h, giving also a higher DS value with high epoxide feed. The modified xylans had increased water solubility, with the exception of the highest substituted xylan of DS = 0.28, which formed turbid solutions even when heated.

The functionalization of xylan with azide groups can be seen from FT-IR spectra, showing the typical azide peak at 2110 cm^{-1} (Fig. 3A). Furthermore, the structure of the ethers can be confirmed from DEPT135 spectra of modified xylan, given in Fig. 2, where the hydroxypropyl azide group can be identified. The substitution of the hydroxyl groups at the positions 2 and/or 3, can be observed at around 82 ppm and an upfield shift of carbon 1 peak affected by the substitution, in accordance to previously reported xylan hydroxyalkyl ethers (Ebringerová et al., 1994; Kataja-aho et al., 2011; Schwikal et al., 2006).

3.2. Crosslinking of xylan using CuAAC

The azide functionalized xylan was used to obtain crosslinked hydrogels using poly(ethylene glycol) and thermo-responsive block copolymers of poly(ethylene glycol)/poly(propylene glycol) (Fig. 1). The reaction was performed at 7 °C in order to solubilize the polymers. The gelation started within minutes after addition of the copper catalyst, but it was allowed to take place for 4 h to ensure maximum crosslinking. We found that longer reaction time weakened the gels, eventually disintegrating and becoming solutions if the crosslinking time was prolonged for several days. This is likely because of the degradation of the polysaccharide and/or polymer chain caused by the copper catalyst, an unfortunate drawback of the CuAAC procedure (Lallana, Fernandez-Megia, & Riguera, 2009; Lallana, Fernandez-Trillo, et al., 2012; Lallana et al., 2011; Uchida & Kawakishi, 1986). For this reason, the copper catalyst was removed by EDTA complexation and washing after the reaction.

A typical FT-IR spectrum of the crosslinked product in Fig. 3B shows a reduction in the intensity of the azide peak at 2110 cm^{-1} and the characteristic peaks of xylan and PEG-PPG-PEG. Moreover, ^{13}C-CP/MAS NMR spectrum (Fig. 4) shows the typical peaks of both xylan and PEG-PPG-PEG along with unreacted excess azide groups (56 ppm) and in addition, peaks at around 148 and 128 ppm showing the presence of the triazole ring formed during the CuAAC reaction. It must be noted thought, that the formation of intra-chain loops and the presence of unreacted loose ends is possible in these types of bifunctional crosslinking systems (Ossipov & Hilborn, 2006).

The thermal response of the hydrogels was investigated by water absorption studies (Fig. 5). When PEG was used for the crosslinking, the water absorption was affected only by a small amount when the temperature was increased from 7 to 70 °C. On the contrary, the thermoresponsive PEG-PPG-PEG gels, showed a considerable drop in water content when heated. The hydrogel crosslinked with PE6100, also started losing water at lower

temperature, originating from the differences in cloud point, which shows that the thermo-responsive properties can be adjusted by the polymer used for crosslinking. The swelling/deswelling process is also reversible, since cooling the gel causes a rise in swelling ratio. The effect of temperature on the gels can be observed in the SEM images of freeze-dried samples (Fig. 6), where the decrease in water content results in a denser network structure as the pore size is reduced from approximately 10–20 μm at 7 °C to around 2 μm at 70 °C. No notable changes can be observed in the morphology when PEG was used for the crosslinking.

Fig. 7 shows the compressive moduli for the gels at 7 °C and 70 °C. At 7 °C, hydrogels crosslinked with PE6400 or PE6100 have lower compressive moduli than the hydrogel crosslinked with PEG. This shows that at 7 °C where PPG segments are water soluble, they decrease the stiffness of the gels. Instead, the difference between the PE6400 and PE6100 containing gels is not significant. The situation changes, when the temperature is increased to 70 °C. The stiffness of PEG and PE6400 crosslinked gels drops slightly but PE6100 crosslinked gel is clearly stiffer at 70 °C than at 7 °C. It seems that the higher amount of PPG, which aggregates at high temperature, makes the material stiffer. In addition, the length of the polymer chain is shorter and crosslinking density higher for PE6100, which may result in a decreased mobility of the network, thus increasing the compressive modulus of the material.

4. Conclusions

A simple, aqueous phase one-step synthesis route to prepare azide functionalized xylan is presented. The azide functionalized xylan is a valuable intermediate for broad modification possibilities via the copper catalyzed azide-alkyne cycloaddition (CuAAc). The azide groups were further reacted with alkyne bifunctional PEG or PEG-PPG-PEG, yielding novel tunable thermoresponsive hydrogels.

Liquid and solid state NMR, FT-IR and elemental analysis were used to characterize the obtained products. The hydrogels showed reversible swelling at low temperature and deswelling at high temperature. Depending on the length of the thermoresponsive PPG segment, the compressive modulus either decreased or increased at 70 °C compared to 7 °C. SEM images showed that the structure of the PPG containing hydrogels was denser at high temperature than without PPG or at low temperature.

The obtained material might be of interest in, for instance, drug release, particle separation or self-cleaning filtration applications.

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