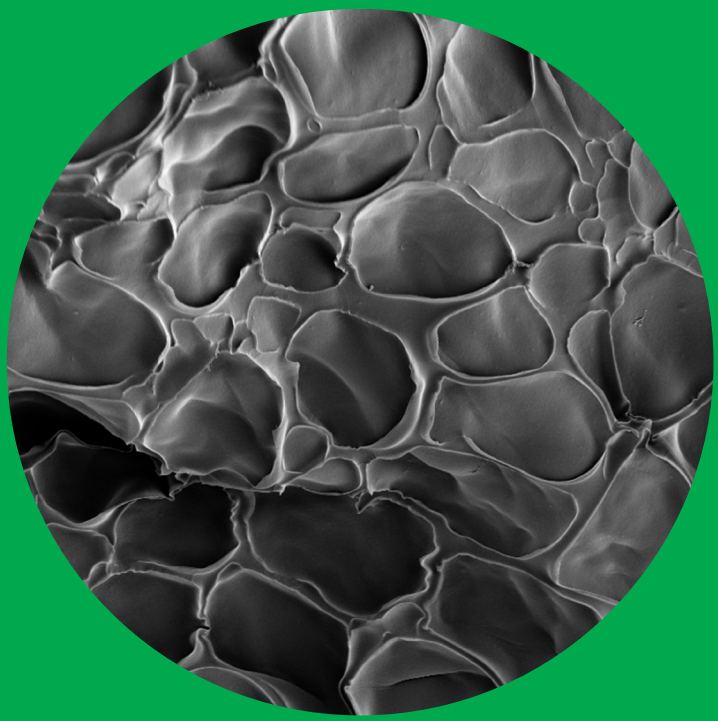
Department of Biotechnology and Chemical Technology

Functionalization of dextran, xylan and nanofibrillated cellulose using click-chemistry in aqueous media

Nikolaos Pahimanolis



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Functionalization of dextran, xylan and nanofibrillated cellulose using click-chemistry in aqueous media

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Aalto University
School of Chemical Technology
Department of Biotechnology and Chemical Technology
Polymer Technology

Supervising professor
Jukka Seppälä

Thesis advisor
Jukka Seppälä

Preliminary examiners
Professor Etienne Fleury, Université de Lyon, France
Professor Pedro Fardim, Åbo Akademi, Finland

Opponent
Professor Thomas Heinze, Friedrich Schiller University of Jena, Germany

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Abstract

Functionalization of dextran, xylan and nanofibrillated cellulose was performed by first introducing hydroxypropyl azide groups by etherification using glycidyl azide in alkaline aqueous media. The reaction efficiency and degree of functionalization was found to depend on the amounts of reactants, reaction temperature and concentration of the polysaccharide.

In the second step, the azide groups were used in copper-catalyzed azide-alkyne cycloaddition reactions for further derivatization. Dextran was grafted with polyethylene glycol and temperature-responsive xylan-based hydrogels were developed by crosslinking reactions using thermoresponsive poly(ethylene glycol)-b-poly(propylene glycol)-b-poly(ethylene glycol) block copolymers. In addition, the reaction was applied on surface functionalization of NFC, providing fluorescent 5-(dimethylamino)-N-(2-propyl)-1-naphthalenesulfonamide or pH responsive amine functionalized NFC.

Experiments of utilizing the developed amine functionalized NFC in graphene composites were conducted. The composites were found to exhibit good electrical and mechanical properties. In addition, all-cellulose NFC composites were developed. The modification of nanofibrillated cellulose was done by both physical and chemical means, using carboxymethyl cellulose and solid state epoxy chemistry. These composites were produced by tape-casting from aqueous NFC suspensions containing carboxymethyl cellulose. CMC was shown to alter the rheological properties of the suspensions by physical interactions, which allowed the production of anisotropic composites by shear-induced partial orientation of fibrils during tape casting. The CMC also provided carboxyl groups to the composite, which could be used to improve the wet strength of the material though ionic crosslinking using glycidyl trimethylammonium chloride.

Keywords chemical modification, polysaccharides, functionalization, nanocomposite

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Tekijä
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Väitöskirjan nimi
Dextraanin, xylaanin ja selluloosan nanokuitujen funktionalisointi click-kemialla vedessä

Julkaisija Kemian tekniikan korkeakoulu

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Tiivistelmä

Dekstraaniin, ksylaaniin ja selluloosan nanokuituihin (NFC) liitettiin hydroksipropyyliatsidiryhmiä käyttämällä reagenssina glysidyyliazidia alkalisessa vesiympäristössä. Reaktion tehokkuus ja funktionalisointiaste riippui reagenssien suhteista, reaktiolämpötilasta sekä polysakkaridien konsentraatiosta.

Valmistettuja atsidifunktionaalisia polysakkarideja käytettiin oksastus ja silloitusreaktioihin kuparikatalysoidulla atsidi-alkyyni sykloadditioreaktiolla (CuAAc). Tällä menetelmällä dekstraaniin oksastettiin polyetyleeniglykolia ja ksylaanipohjaista lämpöön reagoivaa hydrogeeliä valmistettiin ristisilloittamalla polyetyleeniglykoli-polypropyleeniglykoli-polyetyleeniglykoli-lohkopolymeerillä. Lisäksi CuAAc reaktiolla muokattiin selluloosan nanokuitujen pintaa amiiniryhmillä sekä fluoresoivalla 5-(dimetyyliamino)-N-(2-propyyli)-1-naftaleenisulfonamidilla.

Syntetisoituja amiinifunktionaalisia selluloosan nanokuituja käytettiin grafeenikomposiittien valmistuksessa. Komposiitit olivat sähköä johtavia ja niillä oli hyvät mekaaniset ominaisuudet. Lisäksi tutkittiin kokonaan selluloosasta valmistettuja NFC/karboksimetyyliselluloosa (CMC) komposiittien valmistusta, joissa hyödynnettiin sekä fysikaalista että kiinteän tilan kemiallista muokkausta. NFC/CMC komposiitit valmistettiin tape-casting menetelmällä, jolla tapahtui kuitujen orientoitumista leikkausvoimien ja CMC vaikutuksesta. Komposiittien märkälujuutta kasvatettiin hyödyntämällä CMC:n karboksyyliryhmiä sekä glysidyyli trimetyyliammoniumryhmien reaktiolla ja ionisella ristisilloituksella.

Avainsanat kemiallinen muokkaus, polysakkaridit, funktionalisointi, nanokomposiitit

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Preface

This work was carried out in the Polymer Technology Research Group at Aalto University School of Chemical Technology between 2009 and 2013 (Helsinki University of Technology until January 2010). The Graduate School for Biomass Refining (BIOREGS), TEKES (the Finnish Funding Agency for Technology and Innovation) project “Naseva”, the Academy of Finland and TES (Foundation for the promotion of technological advances) are gratefully acknowledged for financial support.

I would like to thank Prof. Jukka Seppälä, for the opportunity to work in his group and for his guidance during my studies and research around this interesting topic.

I wish to thank Dr. Jaana Rich, Dr. Ulla Hippi and Dr. Arja-Helena Vesterinen for all their help and guidance they have given me through the beginning of my research. I also wish to express my gratitude for the whole laboratory group for the good working atmosphere and all the help and invaluable discussions.

I am deeply grateful to all my co-authors for the fruitful collaboration. This research would not have been possible without their contribution.

Finally, I would like to thank my wife Paula, my parents, grandparents and parents-in-law, my sister and brothers and all my friends for their support and for giving me new perspectives on both science and life.

Espoo, April 9th, 2014
Nikolaos Pahimanolis

Contents

List of publications

This thesis consists of an overview and of the following publications, which are referred to in the text by their Roman numerals.

I Pahimanolis N., Vesterinen A-H., Rich J., Seppälä, J., Modification of dextran using click-chemistry approach in aqueous media, *Carbohydrate Polymers*, **82** (2010) 78-82.

II Pahimanolis N., Sorvari A., Luong N.D., Seppälä J., Thermoresponsive xylan hydrogels via copper-catalyzed azide-alkyne cycloaddition, *Carbohydrate Polymers*, 102 (2014) 637-644.

III Pahimanolis N., Hippi U., Johansson L-S., Saarinen T., Houbenov N., Ruokolainen J., Seppälä, J., Surface functionalization of nanofibrillated cellulose using click-chemistry approach in aqueous media, *Cellulose*, **18** (2011) 1201-1212.

IV Luong N.D., Pahimanolis N., Hippi U., Korhonen J.T., Ruokolainen J., Johansson L-S., Nam J-D., Seppälä J., Graphene/cellulose nanocomposite paper with high electrical and mechanical performances, *Journal of Materials Chemistry*, **21** (2011) 13991-13998.

V Pahimanolis N., Salminen A., Penttilä P.A., Korhonen J.T., Johansson L-S., Ruokolainen J., Serimaa R., Seppälä J., Nanofibrillated cellulose/carboxymethyl cellulose composite with improved wet strength, *Cellulose*, **20** (2013) 1459-1468.

The author's contribution to the appended publications

I Nikolaos Pahimanolis planned and carried out the experiments and wrote the manuscript with the assistance of the co-authors.

II Nikolaos Pahimanolis planned and carried out the experiments, excluding compressive tests and SEM experiments, and wrote the manuscript with the assistance of the co-authors.

III Nikolaos Pahimanolis planned and carried out the experiments, excluding rheological studies, XPS and AFM measurements and wrote the manuscript with the assistance of the co-authors.

IV Nikolaos Pahimanolis planned and carried out the etherification and cycloaddition reactions, including synthesis of the required reagents, and wrote the corresponding part of the manuscript.

V Nikolaos Pahimanolis planned and carried out the experiments, excluding XPS, XRD and SEM measurements, and wrote the manuscript with the assistance of the co-authors.

Abbreviations and symbols

AAc ascorbic acid
AEP 1-azido-2,3-epoxypropane
AFM atomic force microscopy
AGU anhydroglucose unit
A-NFC amino-nanofibrillated cellulose
ATR attenuated total reflectance
^{13}C-CP/MAS NMR carbon-13 cross-polarization/magic angle spinning nuclear magnetic resonance
AXU anhydroxylose unit
CMC carboxymethyl cellulose
CuAAc copper-catalyzed azide-alkyne cycloaddition
EDTA ethylenediaminetetraacetic acid tetrasodium salt
FT-IR Fourier transform infrared spectroscopy
GTMA glycidyltrimethylammonium chloride
DEPT distortionless enhancement by polarization transfer
DP degree of polymerization
NFC nanofibrillated cellulose
NMR nuclear magnetic resonance spectroscopy
PEG poly(ethylene glycol)
PEG2k poly(ethylene glycol) 2000 g/mol
PEG-MME poly(ethylene glycol) monomethyl ether
PEO-PPO-PEO Poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide)
PE6100 PEO–PPO–PEO triblock copolymers (BASF trade name Pluronic© PE6100), PPO block of 1750 g/mol and 10 % of PEO
PE6400 PEO–PPO–PEO triblock copolymers (BASF trade name Pluronic© PE6400), PPO block of 1750 g/mol and 40 % of PEO
SEM scanning electron microscopy
THF tetrahydrofuran
XPS X-ray photoelectron spectroscopy
XRD X-ray diffraction

1 Introduction

1.1 A general background of polysaccharides

Increasing environmental concern and the need for decreasing the dependence on fossil based raw materials have fueled research towards the possibilities of making use of renewable sources. Polysaccharides can be found in vast amounts in nature, cellulose being the most common organic polymer. High levels of sophistication have been formed during the evolution of biological systems, for instance, wood consists of cellulosic nanostructures having high strength [1, 2]. These biocompatible and biodegradable structural materials have drawn increasing attention in various research fields ranging from materials science to medical applications [3, 4].

Cellulose consists of beta-D-glucopyranose units linked together by acetal functions at C4 and C1 positions (Figure 1). The degree of polymerization (DP) varies according to the source and the pretreatment of the biopolymer, wood pulp having a typical DP of 300-1700 [5]. The cellulose molecules are assembled to elementary fibrils and fibrillar bundles called microfibrils, which are located in the plant cell wall constituting the framework of the cell along with other wall polymers such as hemicelluloses and pectins [2].

This nanostructure, often termed nanofibrillated cellulose [6], can be extracted by mechanical disintegration of pulp fibers [7-10] and is usually obtained as a water suspension with a solid content below 2 weight-%. The fibrils have an entangled network structure [11-13]. Characteristics of the obtained fibrils are a small diameter and high aspect ratio and because of its high strength [1], it is an interesting material as a reinforcing element in polymer composites for various applications.

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 (Figure 1) connected by β-(1→4)-linkages along with acetyl-, glucuronic acid and species dependent side groups [14]. Among other methods, xylan can be conveniently extracted from biomass by alkali extraction [15, 16], where the acetyl groups are also hydrolyzed rendering the backbone hydroxyl groups available for derivatization reactions. Xylans and

xylan derivatives have found applications in the paper and food industry as well as pharmaceutical applications [16, 17].

Dextran is a water soluble microbial polysaccharide predominantly consisting of linear alpha-1,6-linked glucopyranose units (Figure 1), and a small amount of branching at 1->2, 1->3 or 1->4 depending on the source [18]. Dextran is biodegradable and resists protein adsorption as well as cell adhesion, making it suitable for biomedical applications, such as plasma volume expansion and artificial tears [18, 19].

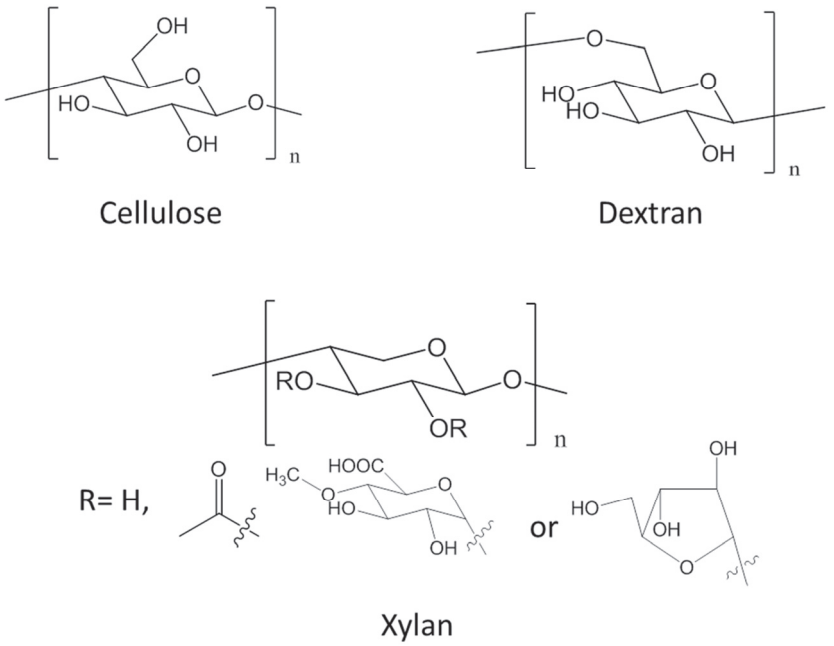

Figure 1. Structural units of cellulose, dextran and xylan. Typical xylan substituents are O-acetyl-, methylglucuronic acid and arabinose-residues.

Chemical derivatives of polysaccharides have a long history; cellulose nitrate, sulfate, acetate and carboxymethyl cellulose being the oldest cellulose derivatives having industrial significance. The reactions used for derivatization involve the numerous hydroxyl groups on the polysaccharide macromolecule, the hydroxyl group acting as a nucleophile towards an electron deficient carbon, forming the covalent bond [5, 20].

For example, modifications targeting the hydroxyl groups of xylan include etherification using epoxides [21-27] and alkyl halides [28-32] in alkaline media

and esterification reactions typically employing anhydrides [33-37] or activated carboxylic acids [38, 39] and sulfating agents [34, 40] have been used.

The tendency of polysaccharides to absorb water can become a problem when these natural polymers have to be chemically tailored, since water restricts the chemical reactions available for these modifications. Drying of the polysaccharides or using problematic solvents to do the chemical alteration is neither economical nor environmentally benign [20, 41]. For example, the use of NFC for making polymer composites usually requires removing the water though solvent-exchange procedures and by chemical modification of the surface of NFC fibrils to obtain better compatibility with polymers [13] and this may lead to destruction of the original nanostructure of the fibris [42].

1.2 Modification by Click chemistry

Copper catalyzed azide-alkyne cycloaddition (CuAAc) [43, 44] has gained much attention in chemical synthesis, popularized by click-chemistry concepts [45]. The concept was inspired by the way molecular diversity, such as proteins, is constructed in biological systems using rather simple building blocks. According to the click-concepts, the connection of building blocks have to happen via reactions that are selective, stereospecific, wide in scope and give high yields [45].

The by-products of these reactions should be non-toxic and easily removed from the reaction mixture by simple methods, for example by evaporation or filtration. The reaction should also preferably use non-toxic solvents such as water or, if possible, without a solvent. A good click reaction also should work in ambient temperatures and use readily available reactants [45].

These click-type reactions are irreversible and exothermic, typically including cycloadditions, nucleophilic additions to unsaturated carbon-carbon bonds and reactions of strained three membered ring systems, such as epoxides and aziridines. These reactions have gained increasing attention in synthesis of complex polymer architectures, such as block- and graft copolymers, branched, star-shaped and comb shaped polymers, to name a few [46, 47].

The use of these click reactions allows the usage of separately synthesized smaller molecules or polymers to be connected together in a convergent way. However, the functional groups necessary for the subsequent click reaction also need to be introduced to the molecules in a simple and efficient way, without tedious purification steps, otherwise the benefits of the click reaction may be lost [45-49].

Similarly to the thermal Huisgen 1,3-dipolar cycloaddition reaction, the CuAAc reaction involves an alkyl azide and a terminal alkyne, which are fused to a triazole ring (Figure 2) [43, 44, 50]. The copper (I) catalyst is commonly produced in situ by adding a reducing agent, such as ascorbic acid into a solution of copper (II) sulfate. This catalyst system works well in aqueous reaction media and has been shown to produce less side-reactions [43, 45, 51], such as alkyne-alkyne couplings [52, 53].

The popularity of the CuAAc reaction stems from its high conversion, selectivity and because water can be used as a reaction media [43]. The azide and alkyne groups necessary for the reaction usually tolerate other functional groups, don't interfere with other reactions and can be introduced rather easily on the target molecules [54-56], although that is not always the case [57, 58].

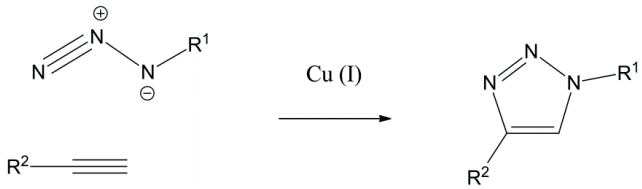

Figure 2. The copper-catalyzed azide-alkyne cycloaddition.

Since its discovery, the CuAAc has been applied in polymer synthesis widely [59-68] and has been demonstrated to work also on solid surfaces and particles [44, 69-73], further widening the scope of synthesis. An excess of either the azide or alkyne component is usually employed in order to drive the reaction to completion[62]. However, steric factors may slow down the reaction rate and reaction efficiency with increasing molecular weight, which may limit the applicability in some polymer syntheses [74-76].

1.2.1 Modification of polysaccharides using CuAAc

One of the first polysaccharides to be modified with CuAAc was cellulose, using heterogenous aqueous media for fluorescent labelling [70]. The reaction has also been applied to surface grafting of cellulose fibers [71, 73] and to cellulose nanocrystals [72, 77] but also to homogeneous derivatization reactions [76, 78-82]. A wide range of other polysaccharides have been modified with the CuAAc approach, such as, starch [83, 84], dextran [85-88], curdlan [89], xylan [90], chitosan [91] and hyaluronan [92].

A major drawback of the CuAAc procedure comes from the copper catalyst, which being a toxic heavy metal, may limit the applicability of synthesized materials and is also showed to participate in degradation of polysaccharides [93-96]. In addition, copper has been found to complexate with the hydroxyl groups of saccharide units, slowing down the reaction rate [97].

One major challenge of applying the CuAAc reaction to the modification of polysaccharides has been the difficulty of introducing the alkyne or azide group necessary for the subsequent click reaction, as it usually requires dry reaction conditions and protecting groups. One strategy has been to convert the hydroxyl group to a tosyl or a halide group and perform a nucleophilic substitution reaction with alkali metal azide [41, 98-100]. The halide group can also be introduced regiospecifically using triphenylphosphine and carbontetrahalides, leading to regioselective azide derivatization [41, 99, 101].

Alkyne groups can also be selected as the polysaccharide functionality instead of azide. This route typically involves esterification polysaccharide in organic solvents [70] or etherification in aqueous alkaline media [73, 83]. The alkyne group can be attached selectively to the reducing end of the polysaccharide [88, 97] or to the polysaccharide backbone through carboxyl groups [72, 92]. A drawback of alkyne functionalization can rise from a high proximity of alkyne groups in the polymer that induce formation of side reactions and slow reaction rates [46, 102].

1.2.2 Utilization of epoxides in functionalization

Epoxides have a long history in polymer synthesis and modification of polysaccharides [41, 103]. For example, hydroxypropyl- and hydroxyethyl

cellulose obtained from the reaction of propylene oxide and ethylene oxide, are used as thickeners, emulsifiers, lubricants, barrier film and coatings.

The etherification reactions involve the hydroxyl groups on the polysaccharide macromolecule, acting as a nucleophile, which attacks an electron deficient carbon of the epoxide ring, forming the C-O-C ether linkage. Hydroxyl groups are weak nucleophiles and require activation for this reaction. Strong bases, such as alkali metal hydroxides are typically employed to catalyze the etherification reaction [20].

As noted previously, epoxide reactions can be seen as belonging to the family of click reactions. Due to their strained three-membered ring structure, epoxides react through S_n2 reaction with many nucleophiles, such as azide ions, rather easily at room temperature and aqueous media across a wide pH range [104], and thus have been used to obtain alkyl azides, creating the reactive species necessary for the subsequent CuAAc reaction [74]. Reactions involving hydroxyl groups usually require alkaline conditions in order to enhance the nucleophilicity, but the ring opening reaction can be facilitated by protic or Lewis-acid activation of the epoxide [105, 106].

1.3 Scope of the thesis

This thesis summarizes the results reported in five publications (I-V), supported with some unpublished data. The targets of this work were to develop aqueous phase modification of polysaccharides and to introduce novel functionality to these materials.

The methods included the covalent attachment of functional azide groups on to dextran (Publication I) and xylan backbone (Publication II) in homogeneous conditions and surface functionalization nanofibrillated cellulose (Publication III, IV) heterogeneously, utilizing epoxy chemistry in alkaline media.

The azide groups were used in copper-catalyzed azide-alkyne cycloaddition reactions for further functionalization. Dextran was grafted with polyethylene glycol (Publication I) and temperature-responsive xylan-based hydrogels were developed by crosslinking reactions using thermoresponsive poly(ethylene glycol)-*b*-poly(propylene glycol)-*b*-poly(ethylene glycol) block copolymers (Publication II). In addition, new functionalities were introduced on the surface of NFC by CuAAc, using propargyl amine and a fluorescent compound (Publication III).

In publication IV, research was conducted for utilizing the developed amine functionalized NFC (from III) in graphene composites for improved compatibility and mechanical and electrical properties.

In publication V, all-cellulose NFC composites were developed. The modification of nanofibrillated cellulose was done by both physical and chemical means, using carboxymethyl cellulose and solid state epoxy chemistry. These composites were produced by tape-casting from aqueous NFC suspensions containing carboxymethyl cellulose. CMC was shown to alter the rheological properties of the suspensions by physical interactions, which allowed the production of anisotropic composites by shear-induced partial orientation of fibrils during tape casting. The CMC also provided carboxyl groups to the composite, which could be used to improve the wet strength of the material though ionic crosslinking using glycidyl trimethylammonium chloride.

2 Experimental

2.1 Materials and methods

Nanofibrillated cellulose was obtained from The Finnish Centre for Nanocellulosic Technologies as a dilute hydrogel (xylan content 25 %). The sample was prepared by mechanical disintegration of bleached birch pulp by ten passes through a M7115 Fluidizer (Microfluidics Corp. Newton, MA, USA).

Dextran (average molecular weight 60000 g/mol) was obtained from Fluka Chemicals and used as received.

Poly(ethylene glycol) monomethyl ether (molecular weight 500 g/mol) was from Fluka Chemicals and it was dried under vacuum prior to use.

Sodium carboxymethyl cellulose (CMC, Sigma-aldrich, Mw = 250 kg/mol with degree of substitution 0.75 determined by titration) was dried in vacuum before use.

Birch wood xylan (xylose content ≥90%, degree of acetylation less than 4 %, Mn=11700 g/mol, PDI=2.02) was obtained from Sisco Research Laboratories Pvt. Ltd. and used as received.

Poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO–PPO–PEO) triblock copolymers were from BASF (trade name Pluronic® PE6100 (cloud point 23 °C) and PE 6400 (cloud point 60 °C) both having a central PPO block of 1750 g/mol and 10 % or 40 % of PEO). Poly(ethylene glycol) 2000 g/mol was from Fluka. The polymers were vacuum-dried at 40 °C for 48 h before use.

2.1.1 Azide functionalization of dextran, xylan and nanofibrillated cellulose [I, II, III, IV]

The synthesis of 1-azido-2,3-epoxypropane was performed starting with epichlorohydrin. The ring-opening reaction of the epoxide with azide-ion was done as follows: Isopropanol and acetic acid were mixed with a solution of NaN_3 in water. Epichlorohydrin was then added under stirring and the reaction was continued at 30 °C for 21 h, until ^{1}H- and ^{13}C-NMR analysis showed complete consumption of the epoxide. The excess azides were removed by adding a water solution of $NaNO_2$ and HNO_3. The obtained solution of 1-azido-3-chloropropanol was stored in dark at room temperature and used without further purification.

For the modification of xylan (Publication II) a concentrated solution was prepared by phase separation and evaporation. The conversion of 1-azido-3-chloropropanol to 1-azido-2,3-epoxypropane was done just prior to use, by adding NaOH solution and stirring the mixture for 5 minutes. The obtained epoxide-solution was immediately used for the azidation of the polysaccharides.

The introduction of azide groups onto the backbone of dextran, NFC or xylan was done as follows: The freshly prepared solution of 1-azido-2,3-epoxypropane was combined with the polysaccharide and NaOH in water. The obtained reaction mixture was stirred until ^{1}H-NMR analysis showed complete consumption of the epoxide.

The solution was then neutralized with acetic acid and the polysaccharide was purified twice by precipitation in ethanol [I] or acetone and ethanol [II]. In the case of nanofibrillated cellulose, the suspension was purified with deionized water by several centrifugation (20 000 gravities for 20 minutes) and redispersion steps until the pH of the suspension became neutral [III, IV].

2.1.2 Preparation of alkyne-end functionalized PEG and PEG-PPG-PEG copolymers [I, II]

The dried poly(ethylene glycol) monomethyl ether (PEG-MME), PEG2k, PE6400 or PE6100 were dissolved in dry THF. NaH was added to the solution and the mixture was stirred until the formation of hydrogen gas ceased. Propargyl bromide was then added dropwise and the reaction was

continued for 2 h, after which the reaction mixture was centrifuged to remove any formed salts and the polymers were purified by precipitation to cold hexane and dried in vacuum. [I, II]

2.1.3 Grafting of dextran with poly(ethylene glycol) using CuAAc [I]

The azidated dextran and an excess amount of the alkyne-end functionalized PEG were dissolved in water and the freshly prepared solution of $CuSO_4$ and ascorbic acid (AAc) was added and the reaction was carried out for 1 h at 30 °C [I].

2.1.4 Xylan hydrogel preparation using CuAAc [II]

The hydrogels were prepared by dissolving the azide functionalized xylan and the alkyne functionalized polymer in water in an ice-bath under stirring overnight at 7 °C. The stirring was continued in an ice bath for another 5 hours. To initiate the crosslinking, copper sulfate/ascorbic acid solution was added and the curing was allowed to take place in cylindrical silicone molds. The copper ions were afterwards removed by EDTA complexation and washings.

2.1.5 Labeling of azide functionalized NFC with 5-(dimethylamino)-N-(2-propyl)-1-naphthalenesulfonamide using CuAAc [III]

5-(dimethylamino)-N-(2-propyl)-1-naphthalenesulfonamide was prepared following a reported procedure [107] by reacting propargyl amine with dansyl chloride in dichloromethane. The fluorescence labeling of azido-NFC was done as follows: To an acetone solution of 5-(dimethylamino)-N-(2-propyl)-1-naphthalenesulfonamide, solution of $CuSO_4$ and ascorbic acid in water was added. A sample of a dried azido-NFC sheet was then immersed in the reaction mixture for 5 minutes, after which the sample was removed, rinsed with acetone and water, and dried at room temperature.

2.1.6 Introducing primary amino groups to NFC using CuAAc [III, IV]

The introduction of 1,2,3-triazole-4-methanamine groups was done as follows: Propargyl amine was added to the suspension of azido NFC and a freshly prepared water solution of $CuSO_4$ and ascorbic acid (AAc) was added. The reaction was carried out for 15 minutes at 30 °C, after which diethylenetriamine was added to the reaction mixture in order to complexate the copper ions. The suspension was then purified with deionized water by several centrifugation (20 000 gravities for 20 minutes) and redispersion steps, until the supernatant became neutral and no amines were detected with the ninhydrin test.

2.1.7 Preparation of reduced graphene oxide/amino-NFC composites [IV]

Graphite oxide was prepared by a modified Hummers method [108]. The graphene oxide/NFC composites were prepared using a variety of graphene oxide dispersions, which were added to the suspension of amine modified NFC to have mixtures with content of graphene from 0.1 -10 wt-% under stirring. The graphene oxide was reduced with hydrazine/amine at 95 °C. The composite films were prepared by filtration of the obtained suspension through a mixed cellulose ester membrane and washing with distilled water. The papers were finally peeled off from the filter paper and dried for 48h. NFC and A-NFC papers were also prepared by the filtration.

2.1.8 Preparation and functionalization of NFC/CMC composite films [V]

All nanofibrillated cellulose water dispersions were prepared as 25 g batches. Carboxymethyl cellulose solution (2 wt-%) was added to NFC suspensions to obtain desired CMC content and distilled water was added to adjust the NFC to 1.0 wt-%. The suspensions were mixed for 24 h with a magnetic stirrer at 1200 rpm and ultrasonicated at 20 kHz with a power of 20 W with 0.5 second active/passive pulses for 10 minutes under agitation at 1200 rpm. The mixing was continued for an additional hour and the suspensions were immediately tape-cast on polyimide supports. The films were dried at 22 °C and 40-60 % relative humidity and subsequently in oven at 75 °C for 24 h.

The GTMA treatment of films were done for dried films by immersing them into GTMA solution in ethanol/water mixture and washed and dried at 22 °C and 40-60 % relative humidity overnight and subsequently in oven at 75 °C for 24 h.

2.2 Characterization

^{1}H-, ^{13}C-NMR and DEPT-135 spectra were recorded on a Varian Gemini 2000 300 MHz [I] or a Bruker Avance III 400 MHz [II, III, IV, V] spectrometer. For quantitative ^{13}C-NMR, the decoupler was gated on only during acquisition, in order to suppress the nuclear Overhauser effect.

Carbon-13 cross-polarization/magic angle spinning nuclear magnetic resonance (^{13}C-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. [II]

Dynamic light scattering measurements were done using Zetasizer Nano ZS (Malvern Instruments). The samples were prepared to concentrations of 1 mg/ml and filtered through 0.45 μm filter. [I]

Elemental analysises was performed using a Perkin Elmer 2400 Series II CHNS [III, IV, V] or a Perkin Elmer 2400 CHN [II] equipment.

The infrared-spectra were recorded with a Nicolet Magna IR750 [I, III, IV] or a Unicam Mattson 3000 [II, V] FTIR spectrometer from KBr pellets (for azide containing samples) or using a diamond ATR accessory.

X-ray photoelectron spectroscopy (XPS) analysis was performed using a Kratos AXIS 165 electron spectrometer with monochromatic Al Kα radiation at 100 W. Elemental compositions were determined from low resolution survey scans combined with extended regional scans on trace elements (N, Na, and Cl). O 1s and C 1s regions were recorded in high resolution mode for a more detailed analysis of surface chemical compositions. [III, IV, V]

Acid-base titrations were done using a Philips PW9420 pH-meter equipped with a Hamilton electrochemical sensor (P/N 238000 Hamilton Bonaduz AG, Switzerland). [III, V]

Atom force microscopy (AFM) images were scanned in tapping mode using a Veeco Dimension 5000 scanning probe microscope with NanoScope V controller and silicon cantilevers. The samples were prepared by placing a drop of dilute NFC suspension (0.005 wt-%) on a silica wafer and dried at room temperature overnight. [III]

Scanning electron microscopy (SEM) images were obtained using a JEOL JSM-7500FA [V] or a Zeiss Sigma VP instrument [II]. For studying the morphology of NFC films, the sample was made conductive by Au/Pt sputtering [V]. The xylan hydrogel samples were prepared by preconditioning a sample (approximately 0.1 g) in water at the desired temperature, then frozen in liquid nitrogen and freeze-dried. The dried samples were cut with a sharp knife to expose fresh surface and made conductive by Au/Pt sputtering [II].

The tensile tests were performed using an Instron 4204 universal testing machine with a crosshead speed of 2 mm/min. At least 6 composite samples (approximately 15 x 0.010 x 5.6 mm3) were tested for each composite and the samples were conditioned at 23 °C and 50 % relative humidity for at least 24 h before testing. The wet strength measurements were done for samples immersed in distilled water for 24 h and the dimensions were measured from dry samples [V].

Two dimensional x-ray diffraction patterns were measured in perpendicular transmission geometry from 6 film pieces stacked on top of each other with uniform casting direction (φ=0°). The x-ray setup consisted of a Seifert ID 3003 x-ray generator (voltage 36 kV, current 25 mA) equipped with a Cu tube (wavelength 1.542 Å), a Montel multilayer monochromator and a MAR345 (Marresearch) image plate detector [V].

Rheological measurements were performed using a TA Instruments AR-G2 rheometer equipped with a vane-cup geometry operating at 25 °C (vane diameter 28 mm, vane length 42 mm, cup diameter 30 mm, gap 1 mm). The dynamic viscoelastic measurements were performed at the linear viscoelastic region. This was determined by strain sweep from 0.02 to 10000 % at 1 Hz, and a strain amplitude of 0.5 % was chosen. In order to

introduce equal shear history, a peak hold step at shear rate of 500 1/s for 30 minutes was done followed by a time sweep with 1 Hz with 0.5 % strain for 2 hours in order to recover the structure. The frequency sweeps were performed at 0.02-100 rad/s. Shear rates of 0.01 to 1000 1/s were used for shear viscosity studies. The samples were allowed to rest for 10 minutes between measurements [III, V].

The swelling behavior of hydrogels was studied by weighting approximately 100 mg of gel preconditioned in water at 7 °C. The samples were then placed in capped vials of water and 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 [II].

The compressive stress-strain measurements of hydrogels were performed using a TA Instruments AR-G2 rheometer equipped with a plate-plate geometry (diameter 20 mm). The hydrogel samples were conditioned in a water bath at 7 or 70 °C for 2 h 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 [II].

3 Results and discussion

3.1 Modification of dextran [I]

3.1.1 Azide functionalization of dextran in aqueous media

Dextran was used to study the etherification with glycidyl azide due to its relatively simple structure and good water solubility. The introduction of azide groups to the polysaccharide backbone was successfully done by combining traditional epoxide chemistry commonly used for hydroxyalkylation of polysaccharides, such as starch [41, 103]. The use of glycidyl azide in the etherification reaction has recently also been reported for cellulose [109].

The epoxide used in this study was 1-azido-2,3-epoxypropane, which could be obtained from a two-step reaction of NaN3 and epichlorohydrin in the presence of acetic acid [104] (Figure 3). Due to the low molecular weight of the synthesized azide, no concentration was attempted.

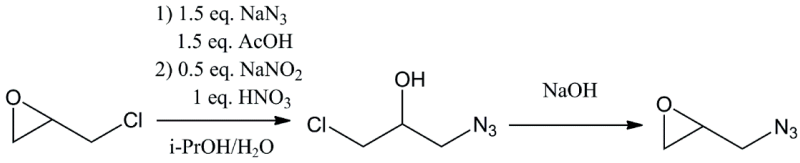

Figure 3. Synthesis of 1-azido-2,3-epoxypropane. [III]

The etherification of dextran was done by varying the amount of NaOH, the epoxide, the concentration of polysaccharide and the temperature (Figure 4, Table 1). The obtained azide functionalization of dextran was calculated using ^{13}C-NMR.

The etherification did not occur when low NaOH amounts were used, as there was insufficient amount of reactive alkoxides formed on the backbone of the polysaccharide that could participate in the ring-opening of the epoxide. However, adding more NaOH increased the functionalization to 0.17, until a decline in the value was observed with further NaOH increase.

There was an increasing proportion of hydrolysis of the epoxide with increasing amount of NaOH, which was seen as a drop in reaction efficiency, ranging from only 9-13 %. Using higher concentrations of dextran improved reaction efficiencies up to 30 %, as more dextran hydroxyl groups became available for the etherification.

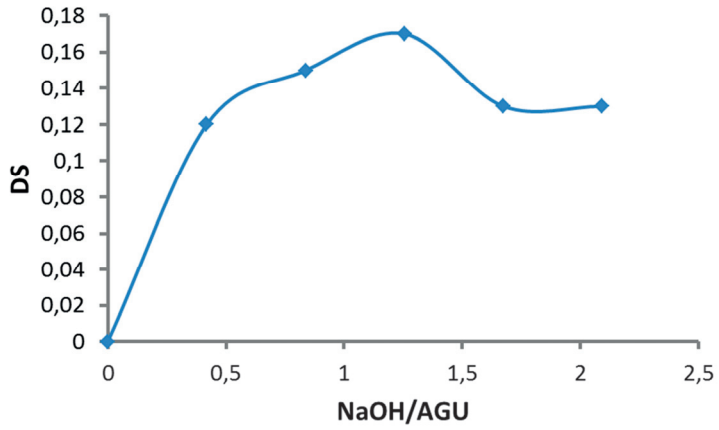

Figure 4. Effect of NaOH addition on the obtained degree of substitution on dextran. Reaction time 21h, 3.2 mmol of epoxide and 2.39 mmol of sugar units. [I]

Table 1. Effect of reaction conditions on azide functionalization of dextran. [I]

Entry [d]	Temp. (°C)	AEP (mmol)	Sugar-units (mmol)	DS theoretical	DS observed[e]	Reaction efficiency[f] (%)
C	30 [g]	3.2	2.4	1.34	0.17	14
G	30 [g]	6.4	2.4	2.67	0.16	6
H	30 [g]	6.4	6.4	1.00	0.20	20
I	55 [h]	3.2	2.4	1.34	0.10	8
J	70 [h]	3.2	2.4	1.34	0.12	9
K	70 [h]	3.2	6.4	0.50	0.07	14
L	55 [h]	6.4	6.4	1.00	0.17	17
M	70 [h]	6.4	6.4	1.00	0.16	16
N	55 [h]	3.2	6.4	0.50	0.07	14
O	55 [h]	3.2	12.8	0.25	0.08	32
P	55 [h]	6.4	12.8	0.50	0.13	26
Q	55 [h]	9.6	12.8	0.75	0.19	25

[d] 3 mmol of added NaOH in 20 ml of H_2O, pH=13.
[e] Calculated based on ^{13}C-NMR-analysis.
[f] 100% conversion of the epoxide based on ^{1}H-NMR-analysis.
[g] Reaction time 21 h
[h] Reaction time 4.5 h

The prepared 1-azido-2,3-epoxypropane solution, which could not be concentrated or purified for safety reasons, contained isopropanol, sodium acetate, NaCl and $NaNO_3$ salts from the preparation of 1-azido-2,3-epoxypropane, which might have an impact on the molecular conformation of dextran in the solution and thus affect the availability of hydroxyl groups for the reaction.

3.1.2 Grafting of dextran using CuAAc

The azide functional dextran was successfully used for grafting of alkyne-functionalized poly(ethylene glycol) monomethyl ether using CuAAc. The experiments were made with hydroxypropyl azide dextrans having DS values of 0.16 and 0.08. The triazole ring formed in the cycloaddition reaction can be identified in ^{1}H-NMR at around 8 ppm and in ^{13}C-NMR spectrum at 144 and 136 ppm (Figure 5). The dextran with the lowest degree of functionalization reacted quantitatively, however a considerable amount (38 %) of azides remained unreacted in the higher substituted dextran.

This may be attributed to steric factors, that have been observed on CuAAc reactions done with polymers [74, 75, 76]. The molecular conformation of dextran may have made the azide groups inaccessible for the catalyst-alkyne complex. Using prolonged reaction time (24 h) did not improve the grafting efficiency, but rather may have resulted to degradation of the polysaccharide by the copper catalyst [93, 94, 95].

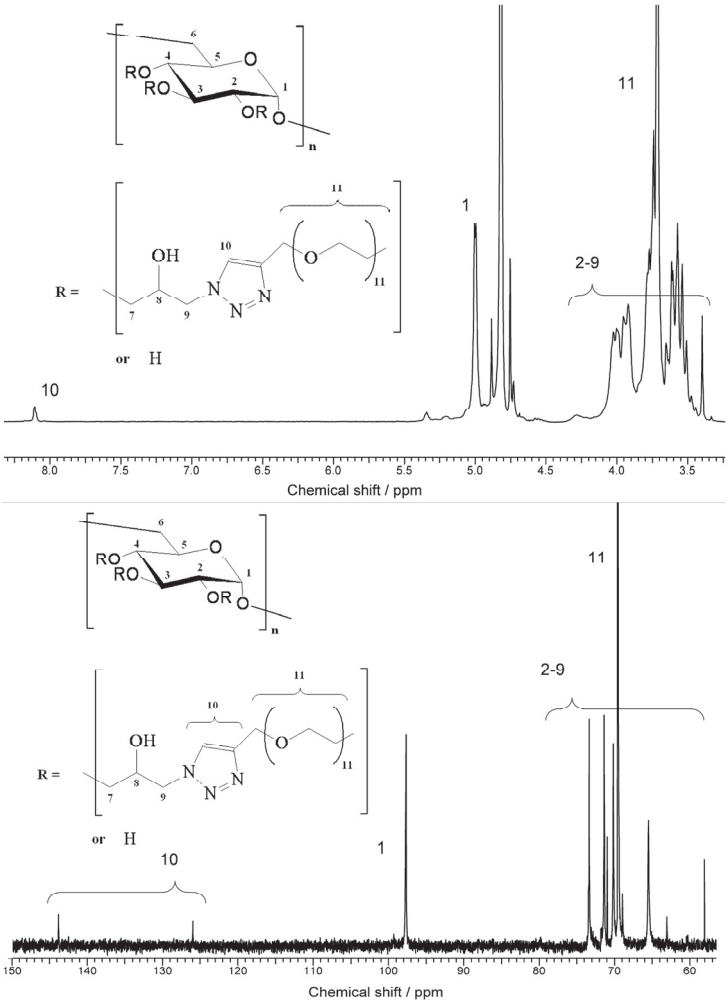

Figure 5. ^{1}H and ^{13}C-NMR spectra of dextran-graft-PEG. [I]

3.2 Modification of xylan [II]

3.2.1 Introduction of azide functionalities to the backbone of xylan

The etherification procedure for introducing azide functionalities on dextran (Paper I) was extended to the modification of birch wood xylan. The 1-azido-2,3-epoxypropane solution was prepared as described in publication III, however it was concentrated by phase separation and evaporation.

To study the etherification reaction, the effect of NaOH and amount of epoxide was varied (Table 2). The degree of functionalization was determined by elemental analysis and the molecular structure of the xylan derivatives were accessed by NMR.

As was observed in the experiments made with dextran, a low degree of functionalization was obtained with low sodium hydroxide to anhydroxylose unit ratio (NaOH/AXU). The obtained degree of functionalization was between 0.06-0.28 and could be adjusted by the epoxide-to-anhydroxylose unit-ratio, however functionalization became increasingly difficult, giving lower reaction efficiencies when high degrees of functionalization was targeted. The obtained DS values were slightly higher and required lower amounts of NaOH than for dextran (Paper I), which might be due to the structural differences between the polysaccharides.

The structure of the xylan ethers were confirmed from DEPT135 spectra of modified xylan, given in Figure 6, where the hydroxypropyl azide group can be identified. The substitution of the hydroxyl group 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 [21, 23, 26]. However, attempts to determine the precise ether position on the xylose unit at either C2 or C3 were not conclusive.

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

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.

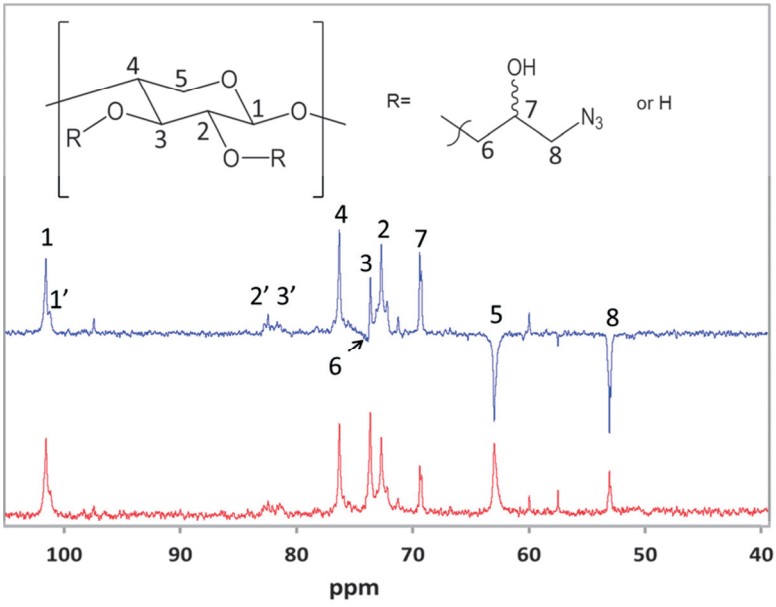

Figure 6. DEPT-135 and ^{13}C-NMR spectra of hydroxypropyl azide xylan. [II]

3.2.2 Thermoresponsive xylan hydrogels by CuAAc crosslinking

A series of xylan hydrogels were synthesized using azide functionalized xylan and different alkyne-end functionalized polymers according to Figure 7. The triblock copolymers used were of the type PEG-PPG-PEG, commercially known as Pluronics (BASF trade mark). These polymers have a critical micellization temperature (CMT) and critical micellization concentration (CMC) above which the hydrophobic PPG segments associate and phase separate from their water solution [110].

The CuAAc crosslinking was performed using the $CuSO_4$/ascorbic acid catalyst system. The reaction temperature was kept at 7 °C in order to keep the block copolymers dissolved and the crosslinking was allowed to proceed for 4 hours. Longer crosslinking times resulted in weakening of the gels, due to the degradation of the polysaccharide and/or polymer chain caused by copper [93-96]. To obtain stable hydrogels, the copper catalyst was removed by EDTA complexation after the reaction.

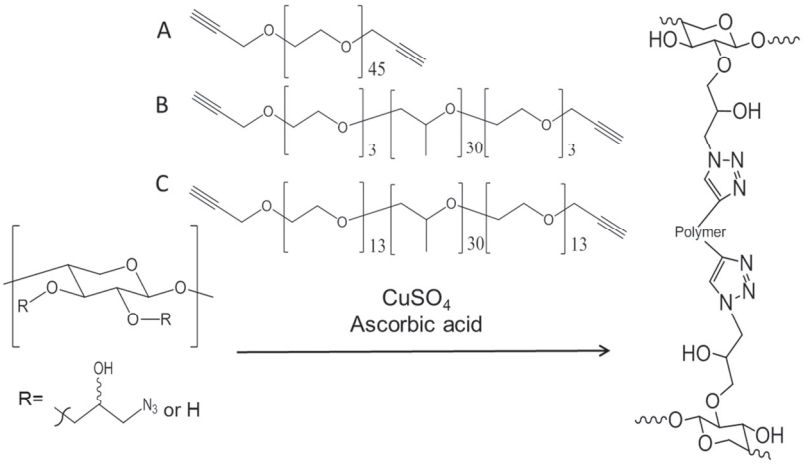

Figure 7. Crosslinking of azide containing xylan with different polymers using CuAAc (2). PEG (A), PE6100 (B) and PE6400 (C). [II]

FT-IR and solid state NMR were used to characterize the crosslinked xylan/polymer products. Figure 8 shows ^{13}C-CP/MAS spectra, where the peaks of both xylan and PEG-PPG-PEG along with unreacted excess azide groups (56 ppm) can be indentified. The triazole ring (148 and 128 ppm) formed during the CuAAc reaction is also visible.

The theoretical crosslinking density (xylose unit to polymer ratio) was 22 for PE6400 and 15 for the PEG2k and PE6100 crosslinkers. However, the formation of intra-chain loops and the presence of unreacted loose ends are possible in this type of bifunctional crosslinking system [111].

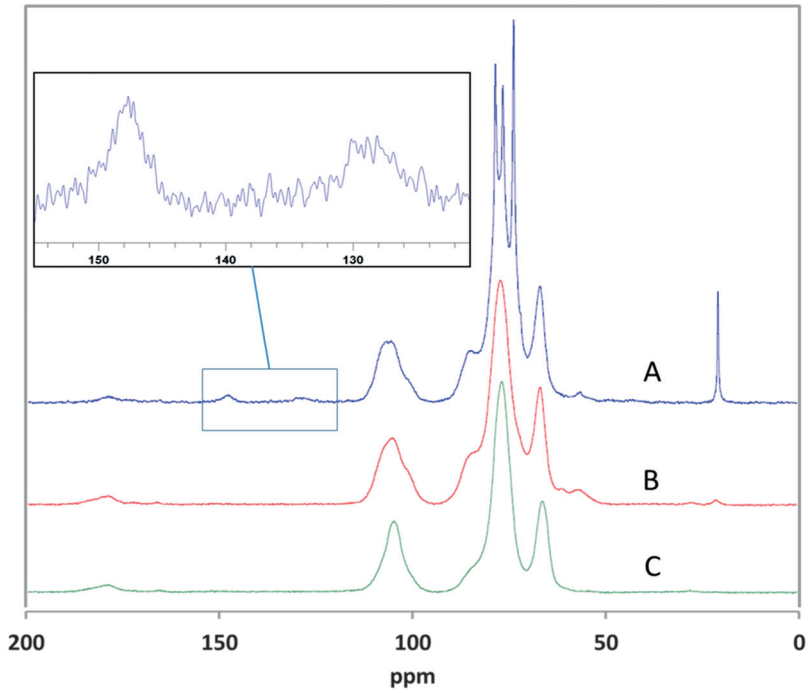

Figure 8. ^{13}C-CP/MAS spectra of crosslinked xylan/PE6100 (A), hydroxypropyl azide xylan (B) and unmodified xylan (C). [II]

The thermoresponsive properties of the pluronics used for crosslinking were transferred to the obtained xylan hydrogels and showed a considerable drop in water content when heated (Figure 9), whereas hydrogels obtained with PEG crosslinker showed only a small change in water content at the measured temperature range. The swelling/deswelling process was reversible, since the gels adopted their original hydrated weight when cooled.

The PPG/PEG ratio of the crosslinker clearly effects the temperature response range of the hydrogels. The PE6100 having a PPG/PEG ratio of 7.5

already deswells at 37 °C, whereas PE6400 with a PPG/PEG of 1.2 deswells to the same extent at 50 °C. The composition of the crosslinker affected also the compression moduli of the hydrogels, since the highest moduli at 7 °C was obtained with the PEG (31 kPa), compared to 15 kPa and 12 kPa for PE6400 and PE6100, respectively. At 70 °C, the gels crosslinked with PEG and PE6400 showed a 23 % and 19 % decrease in compressive moduli, whereas the modulus of PE6100, having the highest PPG content, increased 83 %, possibly due to decreased mobility of the polymer chain.

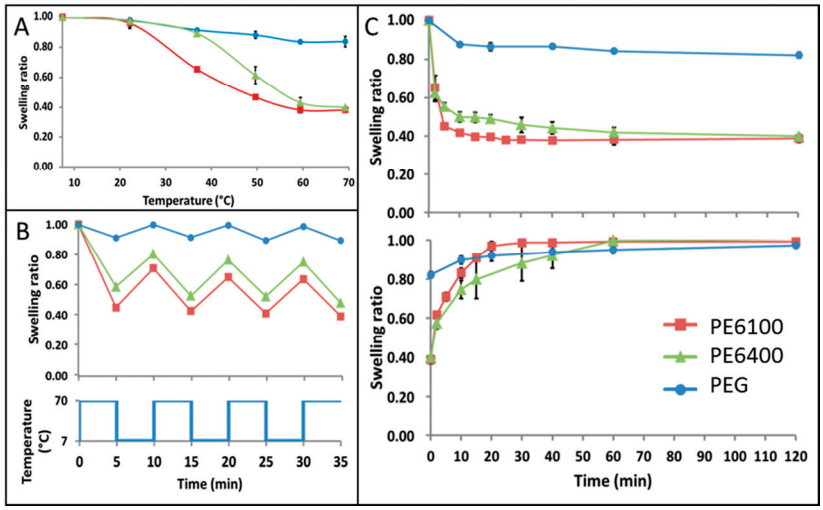

Figure 9. Swelling ratio of hydrogels as a function of temperature (A), swelling ratio over 5 minute temperature cycles between 7 and 70 °C (B). Swelling ratio as a function of time at 70 °C and 7 °C, for gel samples preconditioned at 7 °C and 70 °C respectively (C). The sample sizes were approximately 0.100 g. [II]

The morphology of the freeze-dried samples were studied by SEM (Figure 10), where the decrease in water content resulted 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. As expected, no notable changes in the morphology were seen when PEG was used for the crosslinking.

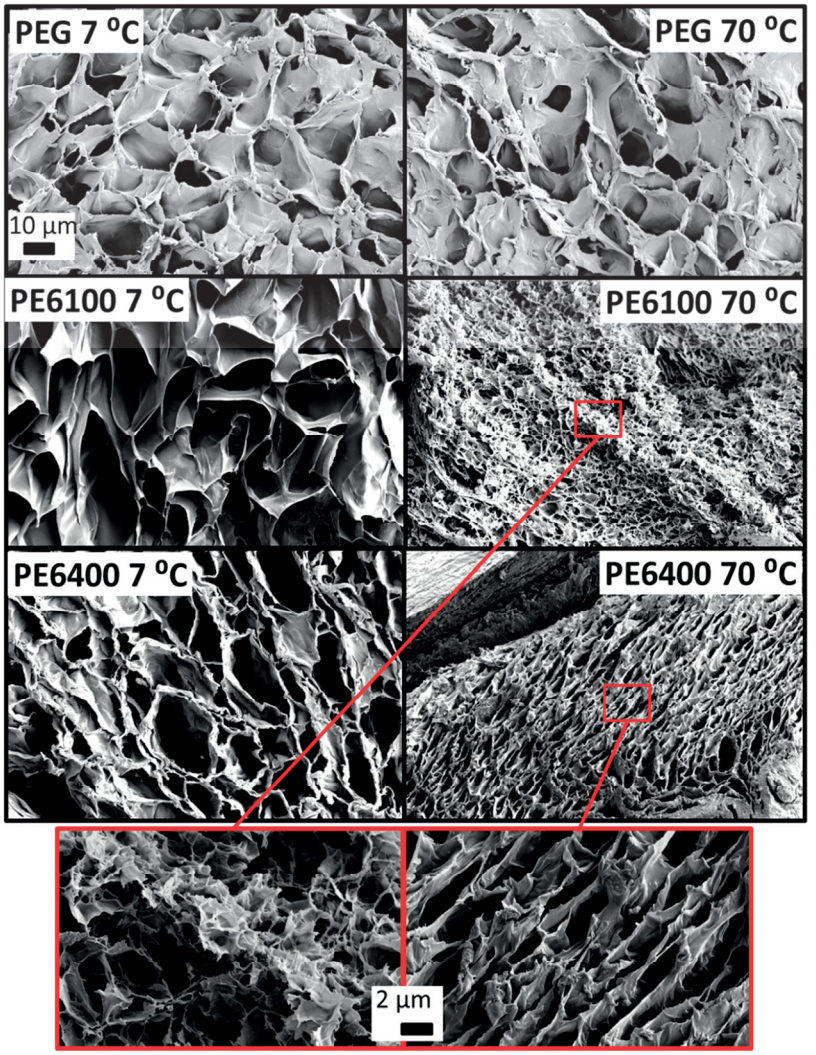

Figure 10. SEM images of freeze-dried hydrogels of xylans cosslinked with PEG, PE6100 and PE6400. The gel samples (0.100 g) were conditioned at 7 °C and 70 °C before freeze-drying [II].

3.3 Modification of nanofibrillated cellulose [III, IV, V]

3.3.1 Azide functionalization of nanofibrillated cellulose NFC [III, IV]

The developed hydroxyalkylation procedure to introduce azide groups on to dextran (Paper I) was extended to the modification of NFC. The reaction was performed by adding the synthesized epoxide to NFC suspension under alkaline conditions, a method similar to the cationization reaction of nanocrystalline cellulose [112]. The progress of the etherification was studied by FT-IR (Figure 11), where the peak at 2110 cm-1, belonging to the azide group could be identified. The degree of surface functionalization was obtained using XPS, while the overall atomic composition was determined with elemental analysis.

The overall degree of functionalization ranged from 0.01 to 0.08. Obtaining high functionalization values was difficult to achieve, since a large excess of the epoxide had to be used. This observation was similar to that of dextran (Paper I) and xylan (Paper II), where targeting a high degree of functionalization lowered reaction efficiency, suggesting that steric factors influence the accessibility of hydroxyl groups toward the etherification.

The low reaction efficiency can be explained also by the fact that the solid contents of the NFC suspension was only 1.66 % and only the xylan rich surface is accessible for derivatization. Thus, a low concentration of hydroxyl groups is available for etherification.

According to the XPS data, the surface nitrogen content of the azide functionalized samples were much lower than expected. This could be a result of adaptation of the surface upon drying, as non-cellulosic material accumulates on the NFC surface during sample drying, covering the nitrogen species [42, 113, 114].

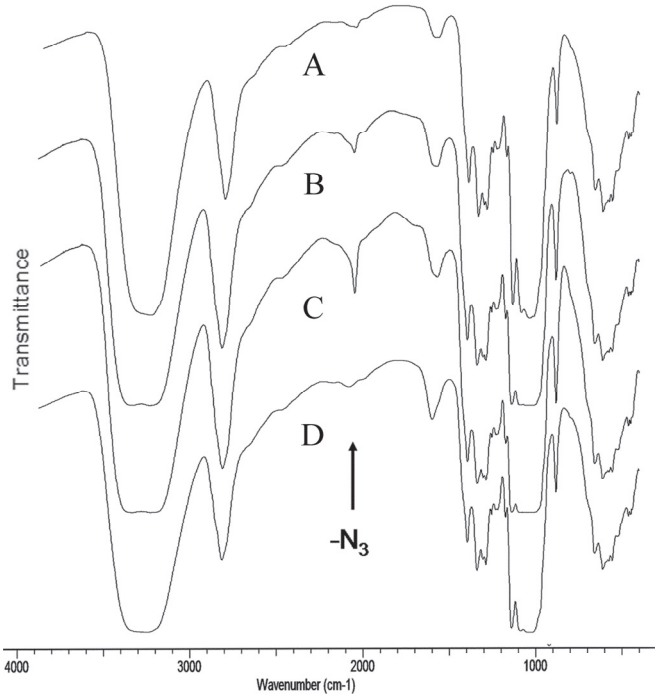

Figure 11. FT-IR spectra of samples taken from the etherification reaction of NFC with 1-azido-2,3-epoxypropane, after 1, 4 and 24h (A, B and C respectively) and after the CuAAC reaction with propargyl amine (spectrum D). [III]

3.3.2 Modification of NFC using CuAAc [III]

The azide-groups on NFC were used to introduce two different functionalities, a fluorescent probe and a 1,2,3-triazole-4-methanamine group (Figure 12) using the CuAAc reaction.

The alkyne-functionalized fluorescent probe 5-(dimethylamino)-N-(2-propyl)-1-naphthalenesulfonamide was reacted by simply dipping a dried azide functionalized NFC film in an acetone/water solution containing the probe and the copper sulfate/ascorbic acid catalyst, resulting in fluorescent NFC with excitation and emission maxima at around 400 nm and 460 nm, respectively (Figure 13).

No fluorescent NFC could be obtained without the copper catalyst or when unmodified NFC sheets where used, which is indirect evidence that the CuAAc-reaction is responsible for the fluorescence labeling [115, 116], and that the azide functionalities are accessible for reaction on the surface of the nanofibrils.

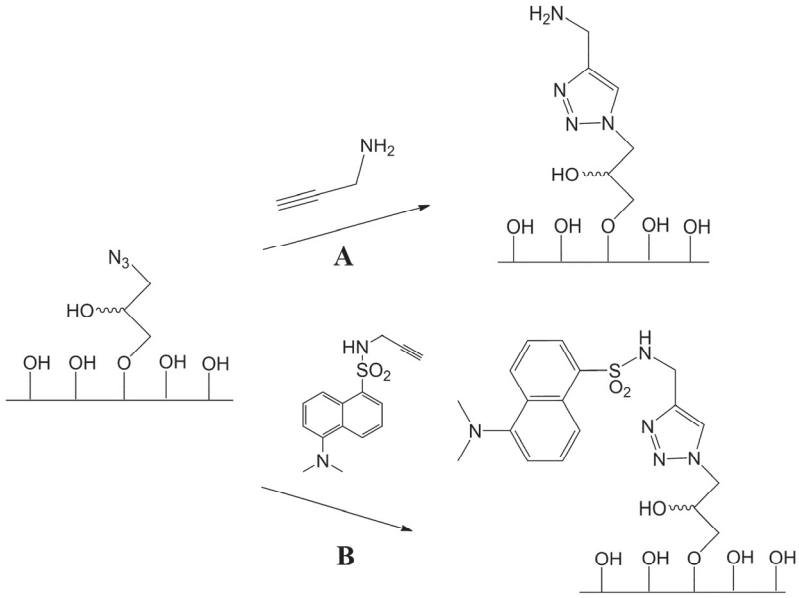

Figure 12. Schematic representation of the reaction of azide-functionalized NFC with A) propargyl amine, B) 5-(dimethylamino)-N-(2-propyl)-1-naphthalenesulfonamide. [III]

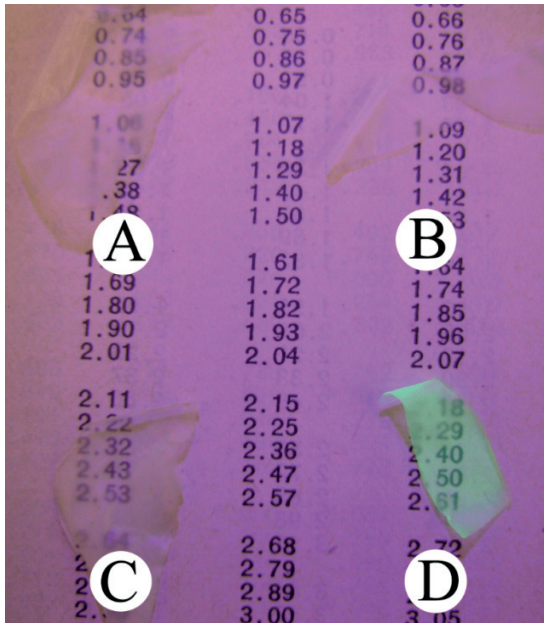

Figure 13. NFC samples under ultraviolet light after labelling of azide functionalized NFC with 5-(dimethylamino)-N-(2-propyl)-1-naphthalenesulfonamide via CuAAc. Unfunctionalized NFC (top samples), azide-functionalized NFC (bottom samples), without copper catalyst (A, C) and with catalyst (B,D).

The azide modified NFC was used to introduce amine groups using the CuAAc-reaction in a quantitative manner (Figure 12, A), confirmed by the disappearance of the azide peak from the FT-IR spectrum at 2110 cm^{-1} (Figure 11, D)and an increase in nitrogen content in both XPS and elemental analysis. The presence of amine groups could be confirmed by titration, as the modified NFC had a buffering effect in the pH region 7-11, typical for alkyl amines.

The transformation of the azide groups to 1,2,3-triazole-4-methanamine groups had also a notable effect on the rheological properties of the NFC suspension. All NFC suspensions had a decreasing viscosity with increasing shear rate and, at rest, a gel-like behavior with storage modulus G´ being higher than loss modulus G´´(Figure 14) [10, 117]. The azide functionalized NFC had the lowest storage and loss moduli and the lowest shear viscosity. However after the CuAAc reaction, the moduli and viscosity rise close to those of original NFC, possibly indicating some recovery of the original structure.

Due to the amine groups present on the surface of the NFC, the suspension rheology was pH responsive. Addition of acetic acid or NaOH to the suspension resulted in lower moduli as opposed to unmodified and azide modified NFC.

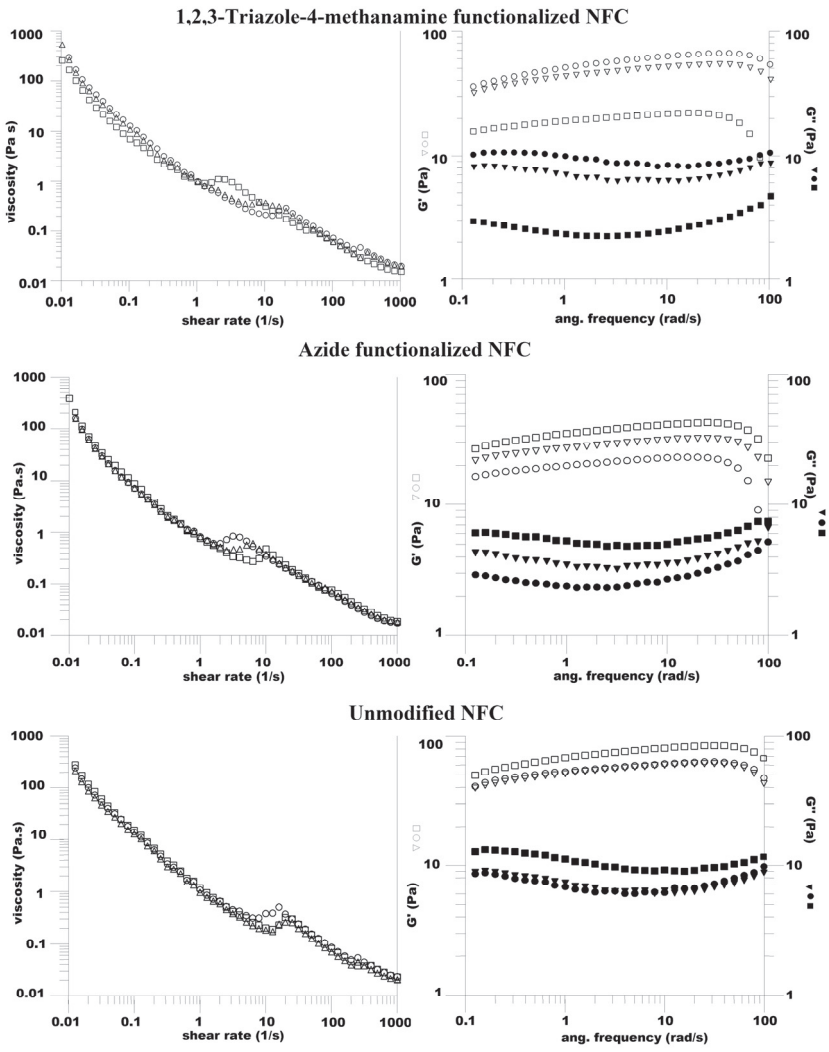

Figure 14. The effect of acetic acid or NaOH addition on the rheological properties of NFC. No acid or base (●○), AcOH 1.8 mmol/L (■□), NaOH 1.8 mmol/L (▼▽). [III]

3.3.3 Application of amine modified NFC in graphene / NFC composites [IV]

A series of graphene/NFC composites were produced from graphene oxide and 1,2,3-triazole-4-methanamine functional NFC (developed in publication III) with a graphene loadings of 0.1 -10 wt-%. The graphene oxide was subsequently reduced with hydrazine/ammonia. Graphene oxide sheets contain epoxy groups [118, 119, 120], which can be opened by amine

nucleophiles [121,122]. Therefore, amine functionalized NFC would participate in the ring-opening reaction, creating covalent bonding to graphene.

The produced graphene/NFC composites had a low percolation threshold of 0.3 wt-% ($4.79*10^{-4}$ S/m^{-1}) and good mechanical properties, which could be attributed to good dispersion between graphene sheets and amine functionalized NFC fibrils resulting in the formation of hydrogen bonds and possible covalent bonds between them.

Pure NFC and amine-modified NFC had tensile strengths of 194 MPa, 202 MPa, and modulus of 5.8 GPa, 5.4 GPa. However, with a graphene loading of 0.3 wt-% the tensile strength and molulus increased to 232 MPa and 6.4 GPa, respectively. Increasing the graphene content up to 5 wt-% increased the tensile strength further to 273 MPa. No improvements in tensile strength were observed for composites made using unmodified NFC.*

This in an indication that the presence of amine groups on the fibril surface results in strong interaction with graphene sheets, although there is no direct evidence of the ring-opening reaction between the epoxide groups of graphene oxide and the surface amines of NFC. Since reduced graphene oxide contains carboxylic acid groups [119, 121, 122], the interaction between 1,2,3-triazole-4-methanamine functional NFC and graphene sheets may well be of ionic nature.

* Unpublished results. Experiments conducted by Nguyen Dang Luong, Department of Biotechnology and Chemical Technology, Aalto University.

3.3.4 Preparation and modification of NFC/CMC composites [V]

The modification of cellulosic surfaces can be done by adsorption of polysaccharides, such as carboxymethyl cellulose [115, 116, 123-126], which is convenient since aqueous conditions can be used. This method has also been termed as being "physical click"[116], due to its robustness and environmentally friendly approach for modifying cellulosic surfaces and provides an alternative for the direct chemical modification.

Sodium carboxymethyl cellulose (CMC) 5-30 wt-% was used in NFC composites for introducing both carboxyl groups and to alter the rheology of the NFC suspension. Carboxymethyl cellulose has been shown to reduce friction of cellulosic surfaces and facilitate dispersion of nanofibrils [127-129].

The reduced friction between nanofibrils can be seen in rheological measurements (Figure 15), where addition of CMC to the NFC suspensions decreases both the storage and loss moduli due to disruption of the fibril network. The negatively charged carboxyl groups of CMC produce a lubricating effect between fibrils and facilitate breaking of fibril flocks [124, 127].

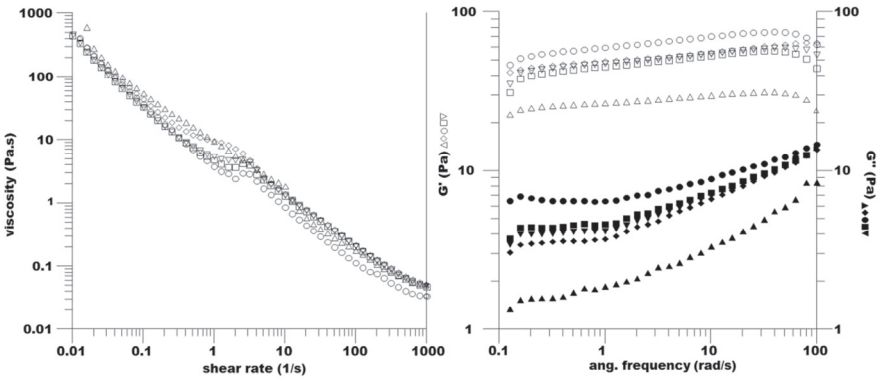

Figure 15. Viscosity as a function of shear rate, storage- and loss modulus as a function of angular frequency for 1.00 wt-% NFC suspension. CMC dry-contents: 0 wt-% (○●), 5 wt% (▽▼), 10 wt% (◇◆), 30 wt% (△▲). [V]

The NFC/CMC composite films were made by tape-casting of suspensions with different casting speeds, a method also used for producing ceramic substrates and composites with ordered structure [130-134]. The morphology of the obtained NFC/CMC composites were studied with SEM and wide-angle

x-ray scattering (Figure 16), which show increasing anisotropy with increasing CMC content and casting speed. The shear-forces created by tape casting induced partial alignment of NFC fibrils, however films without CMC remained isotropic. The anisotropy of the NFC/CMC composite could be visualized with the polarized light of a cellphone display and a polarizing lens, showing birefringence (Figure 16). Cellulose nano- [135 - 137] and microcrystals [138, 139], and microfibrillated cellulose [140] bearing surface charges, have been shown to orient during high shearing. In addition, electric [141, 142] and magnetic [143, 144, 145] fields, as well as mechanical stretching [146 - 148] have been used to induce ordering of cellulosic nano- and microstructures. However NFC fibrils are difficult to orientate without the addition of CMC, due to the strong interfibrillar interactions.

The amount of adsorption of carboxymethyl cellulose on NFC could not be determined directly with common methods used for pulp [123, 127, 149], since filtration or centrifugation (even at high speeds 19000 rpm, 60 min) of the suspensions were unsuccessful in removing all nanofibrils from the supernatant, which interfered with subsequent titration/gravimetric analysis of the CMC left in the solution. Previously published adsorption experiments have been done with quartz crystal microbalance measurements, using NFC films, which can give an estimate of adsorption values for two dimensional systems [129]. This method could not be used to calculate the extent of adsorption in NFC suspensions. However, the adsorption of CMC on cellulose surfaces is reported to be low and reversible when low electrolyte conditions are used [129, 149].

The orientation of cellulose nanostructures has been shown to be beneficial for the mechanical strength of composites along the direction of orientation [144-148]. However, the mechanical properties of the NFC/CMC composites produced in this study were not improved substantially, possible due to the low degree of orientation. The limited orientation may be due to insufficient shearing or wall slipping between the suspension and the glass surface of the tape-casting apparatus. In addition, relaxation phenomena during drying of the films may have affected the orientation, since it has been shown that relaxation after shearing depends on particle length [135].

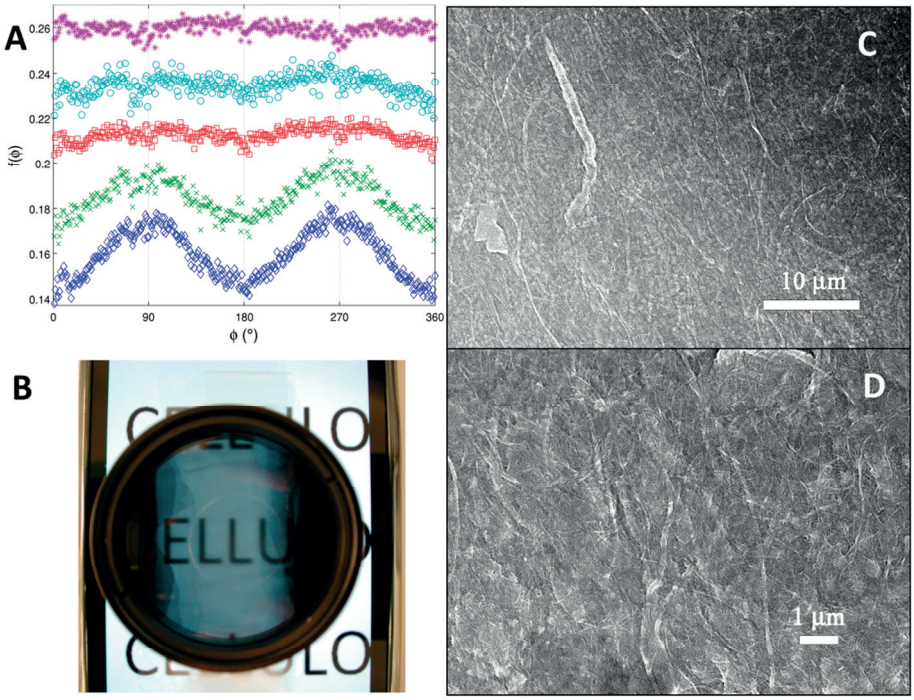

Figure 16. A) Azimuthal intensity distribution of cellulose 200 reflection from wide-angle x-ray scattering of tape cast films, shifted vertically for clarity: From top to bottom: CMC 0 wt-% speed 0.3 m/s, CMC 0 wt-% speed 1.9 m/s, CMC 10 wt-% speed 0.3 m/s, CMC 10 wt-% speed 1.9 m/s, CMC 15 wt-% speed 1.9 m/s, CMC 30 wt-% speed 1.9 m/s. B) Photograph of NFC/CMC 10 wt-% tape cast films when put between a cellphone OLED display and a cross polarizing lens, showing birefringence at 45° to the polarization axis. C,D) SEM images of NFC/CMC 10 wt-% films showing partial alignment of fibrils along casting direction. [V]

3.3.4.1 Wet strength improvement of NFC/CMC nanocomposite films

The carboxyl groups present in the NFC/CMC composites were used for further modification by ion exchange from sodium to ammonium groups by treatment with glycidyltrimethylammonium chloride (GTMA) (Figure 17). Previous studies on anionic cellulosic surfaces have shown that cationic surfactants can improve mechanical properties [150], increase conductivity [151] and surface hydrophobicity [152] and cationic polymers can increase the dry and wet strength of paper due to ionic crosslinking [153, 154].

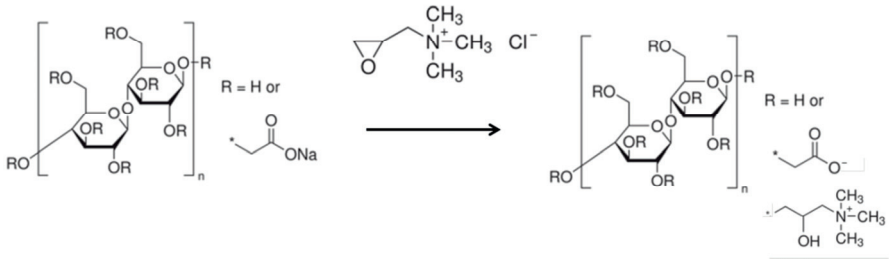

Figure 17. Schematic presentation of GTMA treatment of NFC/CMC composites. [V]

The GTMA treatment of the NFC/CMC composites could be evaluated by elemental analysis by the increase in nitrogen content in the films. XPS analysis of film surfaces showed the disappearance of sodium and the appearance of quaternary ammonium groups. No chlorine was detected in XPS, which suggests that the ammonium groups had carboxyl groups as their counter-ion. The amount of GTMA calculated from the nitrogen content in the NFC films containing 10 wt-% and 15 wt-% of CMC was 29 mg/g and 42 mg/g respectively. This corresponds to 74 % and 72 % of the theoretical CMC carboxyl groups in the composite, indicating that ion exchange was not complete.

Taking advantage of the spring loaded nature of the epoxide ring, the crosslinking reaction could be performed at elevated temperature, resulting in a small increase in dry strength of the composites. However, the wet strength improvement was more remarkable compared to neat NFC, which was too weak to be tested (Figure 18). Films with 10 or 15 wt-% CMC showed somewhat similar wet strength properties, whereas 5 wt-% CMC content provided weaker films. This increase in strength and flexibility in wet state can be attributed to ionic crosslinking.

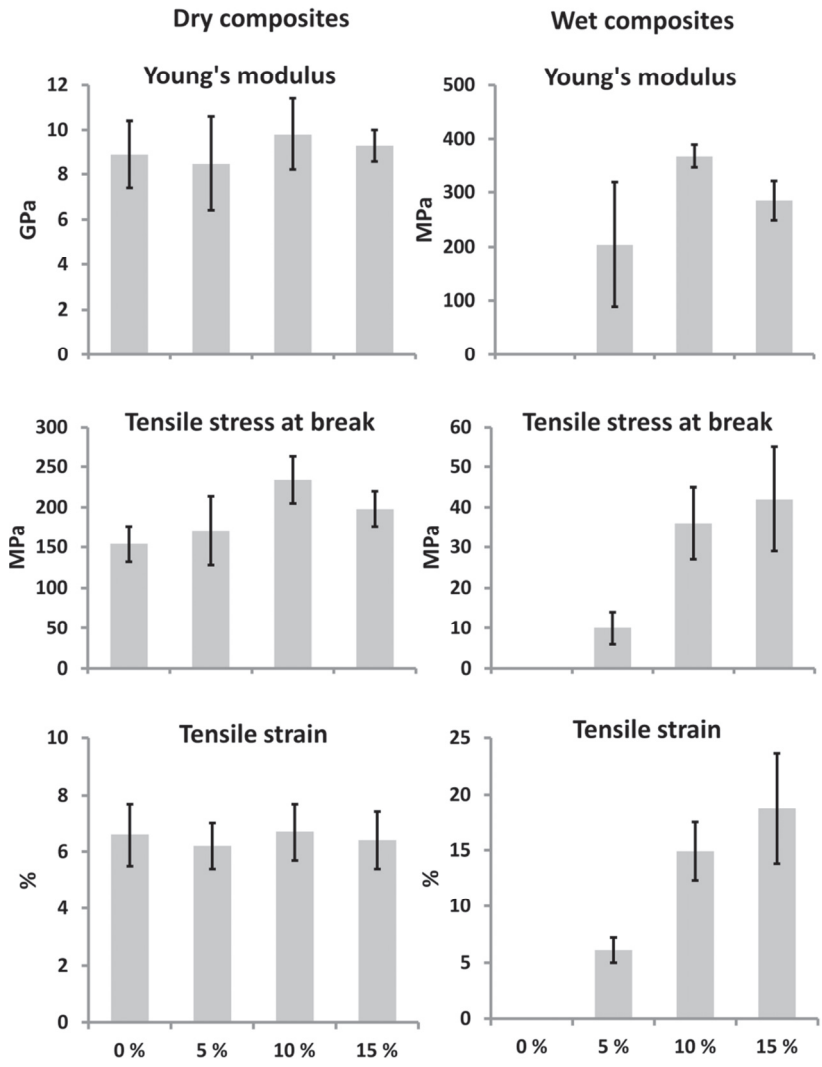

Figure 18. Mechanical properties of tape-cast NFC films with different CMC content. The films were oven dried at 75 °C for 24 h after GTMA treatment. The dry tests were done at 22 °C at 50 % relative humidity and the wet measurements were done for films soaked in distilled water. The wetted films without CMC were too weak to be tested. [V]

The GTMA treatment was done without alkali, but instead the reaction happened during heat drying of the treated films. The disappearance of epoxide was confirmed by ^{1}H-NMR of D_2O/NaCl extracted composite films. The epoxide ring opening may have been autocatalytic [155-157] or influenced by hydroxyl group activation of residual moisture or hydroxyl groups in cellulose and hemicellulose [158]. Carboxylic acid groups present in hemicellulose may have also been involved in the activation of the epoxide ring towards reaction with hydroxyl groups [159].

4 Conclusions

The aim of this research was to develop methods for aqueous phase modification of polysaccharides. The experiments were conducted on functionalization of dextran, xylan and nanofibrillated cellulose.

Hydroxypropyl azide groups were successfully introduced to the backbone of dextran and xylan and to the surface of NFC by etherification using glycidyl azide in alkaline aqueous media. The reaction efficiency and degree of functionalization was found to depend on the amounts of reactants, reaction temperature and concentration of the polysaccharide.

The materials were further used for further functionalization using copper-catalyzed azide-alkyne cycloaddition. The azide functionalized polysaccharides are valuable intermediates for broad modification possibilities via CuAAc, allowing convergent synthesis in creating new materials. The CuAAc reaction was demonstrated by grafting of poly(ethylene glycol) on to dextran. In addition, crosslinking reactions of xylan with poly(ethylene glycol) as well as PEG-PPG-PEG thermoresponsive block copolymers were performed, resulting in temperature responsive hydrogels. The reaction was also applied on surface functionalization of NFC, providing fluorescent and pH responsive amine functionalized NFC. Steric effects were found to influence the CuAAc reaction and degradation of the polysaccharide by the copper catalyst was found to take place.

Experiments of utilizing the developed amine functionalized NFC in graphene composites were conducted. The composites were found to exhibit good electrical mechanical properties, which are attributed to the good dispersion and compatibility between graphene sheets and the functionalized NFC fibrils.

In addition, experiments on all-cellulose composites of NFC and carboxymethyl cellulose were made, taking advantage of both physical and chemical modification. The physical interaction between NFC and CMC in aqueous media allowed control of fibril alignment during tape casting of the composite. Further, the carboxyl groups of CMC were utilized in ionic crosslinking in the dry phase, resulting in wet strength improvement of the composite.

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