Department of Forest Products Technology

# Modification of nanofibrillated cellulose in aqueous media

**Karoliina Junka**

Aalto University

DOCTORAL
DISSERTATIONS

Aalto University publication series
**DOCTORAL DISSERTATIONS** 30/2014

# Modification of nanofibrillated cellulose in aqueous media

**Karoliina Junka**

Doctoral dissertation for the degree of Doctor of Science in Technology to be presented with due permission of the School of Chemical Technology for public examination and debate in Auditorium (Forest Products Building 2) at the Aalto University School of Chemical Technology (Espoo, Finland) on the 4th of April, 2014, at 12 noon.

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Aalto University publication series
**DOCTORAL DISSERTATIONS** 30/2014

ISBN 978-952-60-5595-4
ISBN 978-952-60-5596-1 (pdf)
ISSN-L 1799-4934
ISSN 1799-4934 (printed)
ISSN 1799-4942 (pdf)
http://urn.fi/URN:ISBN:978-952-60-5596-1

Unigrafia Oy
Helsinki 2014

Finland

NORDIC ECOLABEL
441 697
Printed matter

**Aalto University**

**Abstract**

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**Author**
Karoliina Junka

**Name of the doctoral dissertation**
Modification of nanofibrillated cellulose in aqueous media

**Publisher** School of Chemical Technology
**Unit** Department of Forest Products Technology
**Series** Aalto University publication series DOCTORAL DISSERTATIONS 30/2014
**Field of research** Forest Products Chemistry
**Manuscript submitted** 1 November 2013 **Date of the defence** 4 April 2014
**Permission to publish granted (date)** 11 February 2014 **Language** English
☐ **Monograph** ☒ **Article dissertation (summary + original articles)**

## Abstract

In this work, the modification of nanofibrillated cellulose (NFC) was conducted both indirectly via polysaccharide adsorption and directly by chemical modification in aqueous media. Developing modification methods for NFC in aqueous media is of importance because NFC has a tendency for surface passivation and aggregation in any other solvent than water. All the NFC modifications described herein were carried out using anionic carboxyl groups as anchor groups.

The adsorption and interactions between NFC and different water-soluble, linear polysaccharides were studied in a molecular level using cellulose model surfaces and surface sensitive techniques such as quartz crystal microbalance with dissipation monitoring (QCM-D), surface plasmon resonance (SPR), atomic force microscopy (AFM) and colloidal probe microscopy (CPM). The results pointed out that cellulose-like, linear polysaccharides can be irreversibly adsorbed on NFC surface which in turn allowed the use of polysaccharides as pre-modifiers of the NFC substrate. Indirect NFC modification via polysaccharide adsorption was demonstrated by using modified carboxymethyl cellulose (CMC). In detail, CMC was first functionalized (azide or alkyne) and then adsorbed on NFC surface. Subsequently click-chemistry reaction (Cu(I)-catalyzed azide-alkyne cycloaddition), was utilized for the covalent immobilization of different counterparts on NFC substrate. In addition, the use of CMC-adducts as lubricants in NFC system was explored by grafting CMC with polyethylene glycol and adsorbing the corresponding CMC-derivative (CMC-g-PEG ) on NFC.

Direct modification of NFC was carried out by using carboxymethylation as a pre-treatment. Next, carboxymethylated NFC (CM-NFC) substrates were subjected for carbodiimide-assisted conjugation chemistry (EDC/NHS) which allowed covalent attachment of luminescent carbon dots on CM-NFC model films and nanopaper. Furthermore, titrimetric methods for determining the surface and total charge of NFC in aqueous media were developed. The developed generic, mild and rather effortless modification methods of NFC in aqueous media are expected to open new venues for the larger scale applications of NFC .

**Keywords** nanofibrillated cellulose,polysaccharide,modification
**ISBN (printed)** 978-952-60-5595-4 **ISBN (pdf)** 978-952-60-5596-1
**ISSN-L** 1799-4934 **ISSN (printed)** 1799-4934 **ISSN (pdf)** 1799-4942
**Location of publisher** Helsinki **Location of printing** Helsinki **Year** 2014
**Pages** 184 **urn** http://urn.fi/URN:ISBN:978-952-60-5596-1

**Aalto-yliopisto**

# Tiivistelmä

**Aalto-yliopisto, PL 11000, 00076 Aalto** www.aalto.fi

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|---|---|
| **Tekijä**<br>Karoliina Junka | |
| **Väitöskirjan nimi**<br>Nanofibrillaarisen selluloosan muokkaus vedessä | |
| **Julkaisija** Kemian tekniikan korkeakoulu | |
| **Yksikkö** Puunjalostustekniikan laitos | |
| **Sarja** Aalto University publication series DOCTORAL DISSERTATIONS 30/2014 | |
| **Tutkimusala** Puunjalostuksen kemia | |
| **Käsikirjoituksen pvm** 01.11.2013 | **Väitöspäivä** 04.04.2014 |
| **Julkaisuluvan myöntämispäivä** 11.02.2014 | **Kieli** Englanti |
| ☐ **Monografia** | ☒ **Yhdistelmäväitöskirja (yhteenveto-osa + erillisartikkelit)** |

## Tiivistelmä

Nanofibrillaarista selluloosaa (NFC) muokattiin tässä työssä vedessä epäsuorasti polysakkaridiadsorption avulla sekä suoraan kemiallisesti muokkaamalla. Syy NFC:n vesipohjaisten muokkausmenetelmien kehittämiseen on NFC:n aggregoituminen ja pintapassivoituminen muissa liuottimissa kuin vedessä. Kaikissa käytetyissä NFC:n muokkausmenetelmissä hyödynnettiin karboksyylikemiaa joko suorasti tai epäsuorasti.

Vesiliukoisten ja lineaaristen polysakkaridien adsorptiota ja vuorovaikutusta NFC:n kanssa tutkittiin molekyylitasolla. Tämä pystyttiin tekemään hyödyntämällä kvartsikidemikrovaakaa (QCM-D), pintaplasmaresonanssia (SPR), atomivoimamikroskooppia (AFM) sekä pintavoimamittauksia (CPM). NFC:n epäsuora muokkaus demonstroitiin tutkimalla muokatun karboksimetyyliselluloosan (CMC) adsorptiota NFC-pinnoille, ja optimoimalla adsorptio-olosuhteet. Ensimmäiseksi, CMC:tä click-funktionalisoitiin (alkyyni tai azidi), adsorboitiin NFC pinnalle ja lopulta muokattiin käyttäen click-kemiaa. Toiseksi, CMC:tä muokattiin oksittamalla polyetyleeniglykoliketjuja (CMC-g-PEG) CMC:n runkoon. NFC systeemin kitkaa voitiin alentaa ja pintavuorovaikutuksia muokata adsorboimalla CMC-g-PEG NFC-pinnalle.

NFC:n suoraa kemiallista muokkausta tehtiin tässä työssä karboksimetyloimalla NFC:tä ja muokkaamalla sitä vedessä käyttäen karbodiimidi-kemiaa (EDC/NHS). Fluoresoivia hiilen nanopartikkeleita kiinnitettiin karboksimetyloituun NFC mallipintaan sekä nanopaperiin käyttäen NFC:n suoraa kemiallista muokkausta vedessä. NFC:n pinta- sekä kokonaisvarausmittausmenetelmät kehitettiin, jotta NFC:n varausominaisuuksia voitaisiin karakterisoida tehokkaasti ja luotettavasti.

NFC on uusiutuva nanomateriaali, jolla on potentiaalia sekä perinteisissä selluloosapohjaisissa käyttökohteissa sekä täysin uudenlaisissa sovelluksissa. Vesipohjaisia NFC-muokkausmenetelmiä kehitettiin tässä työssä, joilla voi tulevaisuudessa olla käyttöä uusien NFC:n sovelluksien kehityksessä.

**Avainsanat** nanofibrillaarinen selluloosa, polysakkaridi, muokkaus

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|---|---|---|
| **ISBN (painettu)** 978-952-60-5595-4 | **ISBN (pdf)** 978-952-60-5596-1 | |
| **ISSN-L** 1799-4934 | **ISSN (painettu)** 1799-4934 | **ISSN (pdf)** 1799-4942 |
| **Julkaisupaikka** Helsinki | **Painopaikka** Helsinki | **Vuosi** 2014 |
| **Sivumäärä** 184 | **urn** http://urn.fi/URN:ISBN:978-952-60-5596-1 | |

# Preface

The work presented in this dissertation was carried out in the Department of Forest Products Technology, Aalto University School of Chemical Technology. I am grateful for having the chance to be a part of the Graduate School for Biomass Refining (BIOREGS) during the years 2010-2013 funded by the Academy of Finland. In addition, this work was performed within "Naseva"-project funded by the National Agency for Technology and Innovation (TEKES) and industrial partners, and "FuBio"-project funded by the Finnish Bioeconomy Cluster (FIBIC) and industrial partners.

I would like to thank my professor Janne Laine for all the support. It has been a pleasure to work with you. I would also like to thank Dr. Ilari Filpponen, prof. Monika Österberg and prof. Orlando Rojas for all the help and comments regarding my scientific work. I am deeply grateful for all my co-authors for all the enthusiasm, comments and help I have received. In addition, I would like to thank Dr. Joe Campbell, Laura T. and Johanna for proof-reading the thesis.

I had the chance to work abroad twice during my doctoral studies; in Sweden at Innventia AB and in the U.S. at NC State. Thanks go to both prof. Tom Lindström (Innventia AB) and prof. Orlando Rojas (NC State) for giving me the opportunity to work as a part of your research groups.

The Department of Forest Products Technology is a great place to work for many reasons. The laboratory technicians (Risu, Marja, Anu, Aila, Rita, Mirja) are deeply acknowledged for their excellent assistance in the lab. I would also like to thank all my present and former colleagues for the memorable moments we had in conferences, courses, events, and at the department.

Finally, I would like to thank all my friends and family for the support during these years. Especially Äiti, Isä, Jonas, Kaisa, Juho and your families: Thank you for the love, caring and support.

In Otaniemi, Espoo, March 4th, 2014

Karoliina Junka

## List of publications

This thesis is based on the results presented in six scientific publications. The publications are referred as Roman numerals in the text. Some related unpublished results are also discussed.

**Paper I** Paula Eronen, Karoliina Junka, Janne Laine, Monika Österberg (2011), INTERACTION BETWEEN WATER SOLUBLE POLYSACCHARIDES AND NATIVE NANOFIBRILLAR CELLULOSE THIN FILMS, *Bioresources*, 6, 4200-4217

**Paper II** Karoliina Junka, Ola Sundman, Jani Salmi, Monika Österberg, Janne Laine (2014), MULTILAYERS OF CELLULOSE DERIVATIVES AND CHITOSAN ON NANOFIBRILLATED CELLULOSE, accepted to *Carbohydrate Polymers*

**Paper III** Anna Maria Olszewska, Karoliina Junka, Niklas Nordgren, Janne Laine, Mark Rutland, Monika Österberg (2013), NON-IONIC ASSEMBLY OF NANOFIBRILLATED CELLULOSE AND POLYETHYLENE GLYCOL GRAFTED CARBOXYMETHYL CELLULOSE AND THE EFFECT OF AQUEOUS LUBRICATION IN NANOCOMPOSITE FORMATION, *Soft Matter,* 9, 7448-7457

**Paper IV** Karoliina Junka, Ilari Filpponen, Leena-Sisko Johansson, Eero Kontturi, Orlando J. Rojas, Janne Laine (2014) A METHOD FOR THE HETEROGENEOUS MODIFICATION OF NANOFIBRILLAR CELLULOSE IN AQUEOUS MEDIA, *Carbohydrate Polymers*, 100, 107-115

**Paper V** Karoliina Junka, Ilari Filpponen, Tom Lindström, Janne Laine (2013), TITRIMETRIC METHODS FOR THE DETERMINATION OF SURFACE AND TOTAL CHARGE OF FUNCTIONALIZED NANOFIBRILLATED/MICROFIBRILLATED CELLULOSE (NFC/MFC), *Cellulose, 20, 2887-2895*

**Paper VI** Karoliina Junka, Jiaqi Guo, Ilari Filpponen, Janne Laine, Orlando J. Rojas (2014) MODIFICATION OF CELLULOSE NANOFIBRILS (CNF) WITH LUMINESCENT CARBON DOTS (CDs), accepted to *Biomacromolecules*

**Authors contribution in the joint publications:**

Paper I Karoliina Junka was responsible for the experimental design of the polysaccharide adsorption experiments, performed the polysaccharide adsorption and characterization measurements, analyzed the corresponding results, and wrote the manuscript together with the co-authors

Paper II Karoliina Junka was responsible for the experimental design together with the co-authors, performed the main part of the measurements, analyzed the results, and wrote the manuscript

Paper III Karoliina Junka was responsible for the experimental design together with the co-authors, conducted and designed the CMC-*g*-PEG synthesis and characterization, analyzed the corresponding results, and wrote the corresponding parts in the manuscript

Paper IV-VI Karoliina Junka was responsible for the experimental design, performed the main part of the measurements, analyzed the results, and wrote the manuscript

### List of main abbreviations

| | |
|---|---|
| AFM | atomic force microscopy |
| BSA | Bovine Serum Albumin |
| CD | carbon dot |
| CMC | carboxymethyl cellulose |
| CM-pulp | carboxymethylated pulp |
| CM-NFC | carboxymethylated NFC |
| CHI | chitosan |
| CPM | colloidal probe microscopy |
| CuAAC | copper(I)-catalyzed azide-alkyne cycloaddition reaction |
| DLVO | Derjaguin, Landeau, Verway and Overbeek |
| DPFM | digital pulsed force mode |
| DS | degree of substitution |
| EDC | 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide |
| GG | guar gum |
| GGM | galactoglucomannan |
| HECE | hydroxyethylcellulose ethoxylate |
| LBG | locust bean gum |
| MC | methyl cellulose |
| MFC | microfibrillated cellulose |
| Mw | molecular weight |
| NFC | nanofibrillated cellulose |
| NHS | N-hydroxyl succinimide |
| PDADMAC | poly(diallyldimethylammonium chloride) |
| PEG | polyethylene glycol |
| PEI | polyethylene imine |
| PEM | polyelectrolyte multilayer |
| QCM-D | quartz crystal microbalance with dissipation |
| SPR | surface plasmon resonance |
| TMSC | trimethylsilyl cellulose |
| XPS | x-ray photoelectron spectroscopy |
| XG | xyloglucan |

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# 1. Introduction and outline of the study

Forest products have always had a major role in the Finnish economy. In recent years the economical profitability of forest products, mainly pulp and paper, has been fluctuating and mills have been shut down. The created value to forest products only by traditional pulp, paper or board production does not meet today's demands, and novel, more value added products from forest raw materials are called for. The demand for using sustainable raw materials is also increasing as the oil resources in the world are depleted, which promotes the use of forest products in various application areas. All in all, nanocellulose products could be one important player in the future bioeconomy sector where new kinds of sustainable, value-added products are developed from biomass.

Nanomaterials have a lot of potential in different kinds of applications due to the possibility of making, e.g., light-weight structures with high strength by using these very small compounds with a huge specific surface area. Cellulosic nanomaterials can be roughly divided into the following three groups: nano-/microfibrillated cellulose (NFC/MFC), which is produced mainly by mechanical disintegration of pre-treated cellulosic fibres; nanocrystalline cellulose (NCC), which is produced by acid hydrolysis; and bacterial cellulose (BC), which is produced by bacteria. These different materials all mainly consist of cellulose, but they differ a lot in their application potential because of the different structural features and manufacturing methods. This thesis concentrates on NFC and its modification.

The manufacturing of NFC/MFC from wood pulp by mechanical disintegration was published for the first time in the early 1980´s (Turbak et al. 1983). A lot of research has been done within the last 30 years in order to make the production process more feasible on an industrial scale, especially in the last 10 years. The step of taking the production of NFC/MFC from laboratory scale to pilot/ semi-industrial scale has been taken. This gives promise for the discovery of the full potential of NFC/MFC in different applications. However, there are many challenges still to overcome within the next few years, in processability, in transportation and in the use of NFC/MFC. It takes time and motivation to create an industry out of this fascinating, sustainable nanomaterial, which is mainly built up of the most abundant polymer in the biosphere, namely cellulose.

This thesis starts with a fundamental study of polysaccharide adsorption on nanofibrillated cellulose (**Paper I**). The motivation to modify NFC in water came from the fact that cellulose nanofibrils have a tendency for irreversible surface passivation and aggregation in any other solvent than water. Water-soluble polysaccharide adsorption on cellulosic surfaces was thought a facile way of introducing functional groups to NFC in aqueous media. However, there was a lack of knowledge about the adsorption behavior of water-soluble polysaccharides on NFC, so the work had to be started with a fundamental and systematic, molecular scale study of polysaccharide adsorption on NFC. The mechanism and the effect of different solution conditions and structural features needed to be studied in order to use polysaccharide-cellulose interactions as tools for NFC modification. The polysaccharide adsorption work was followed by a study of polysaccharide multilayer build-up on NFC (**Paper II**). This was done in a systematic way so that the optimal conditions for multilayer build-up onto NFC could be distinguished. It was noticed that the polysaccharide multilayer build-up was not governed solely by electrostatic interactions as in the case of typical polyelectrolyte multilayer systems. In addition, valuable knowledge of the optimal adsorption conditions as well as the surface interaction of polysaccharide multilayers with NFC was obtained.

**Papers III and IV** took the indirect, polysaccharide adsorption –based modification route to the next step. The adsorption of modified or functionalized polysaccharides on NFC was studied in both cases. In **Paper III** carboxymethyl cellulose (CMC) was modified by grafting with polyethylene glycol (PEG). The adsorption of the modified polysaccharide, CMC-g-PEG, was studied on NFC. The idea for CMC-g-PEG attachment on NFC was to decrease the friction between cellulose fibrils which could help in developing advanced materials from NFC. **Paper IV** introduced a way to click-modify NFC via functionalized CMC adsorption followed by copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction in bulk conditions. The effect of click-functionalized CMC properties, e.g. degree of substitution (DS), were systematically studied in order to find the most optimal conditions for NFC modification in water using this indirect route.

It is known that NFC can be modified in aqueous media also directly via its natural functional groups (mainly hydroxyl groups) or by introduced functional groups, in this case carboxyl groups. **Paper V and VI** deal with direct functionalization of NFC in water. In this case NFC was carboxymethylated in order to be able to further functionalize it in aqueous media by ethylcarbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) conjugation chemistry. This procedure was used in order to systematically evaluate the applicability of titrimetric methods for charge characterization of alkyne-functionalized NFC (**Paper V**) and in order to attach luminescent carbon dots on NFC covalently (**Paper VI**). All the NFC modifications in this thesis are done indirectly or directly via anionic carboxyl groups.

# 2. Background

## 2.1 Nanofibrillated cellulose

Cellulose is the most abundant polysaccharide in the biosphere since it is the building material for most plants. It is a linear homopolymer that consists of glucan chains with repeating (1→4)-β-D-glucopyranose units linked together by O-glycosidic bonds (Klemm et al. 1998). The strong intra- and intermolecular hydrogen bonding of cellulose makes it a relatively stable polymer. It does not have a melting point and it does not dissolve in typical aqueous solvents. The structure of the cellulose polymer chain allows it to form different highly ordered crystalline domains (Klemm et al. 2005). The polymorphs of crystalline cellulose are called cellulose I, II, III and IV (Klemm et al. 1998). Cellulose I, which is the naturally produced crystalline structure of cellulose, has two polymorphs (triclinic Iα and monoclinic Iβ structure) and the proportions of these two vary depending on the source of cellulose (Atalla and Vanderhart 1984).

In the plant cell wall the polymer chains of cellulose are assembled in the form of elementary fibrils which are 3.5 nm in width (Brown Jr et al. 1996). These fibrils aggregate into larger macroscopic cellulosic fibres. Cellulose appears in the form of cellulose I and less ordered amorphous regions in native wood-based fibres. The cell wall also contains hemicelluloses, pectin and lignin as other constituents in addition to cellulose. The principle in the preparation of 3-20 nm wide and over 1 μm long cellulose nano- or microfibrils from plants and wood is to deconstruct the hierarchical structure of plant cell wall (Klemm et al. 2005). The difference in cellulosic fibres and nano-/microfibrils and the hierarchical structure of cellulose in plant cell wall is presented in Figure 1.

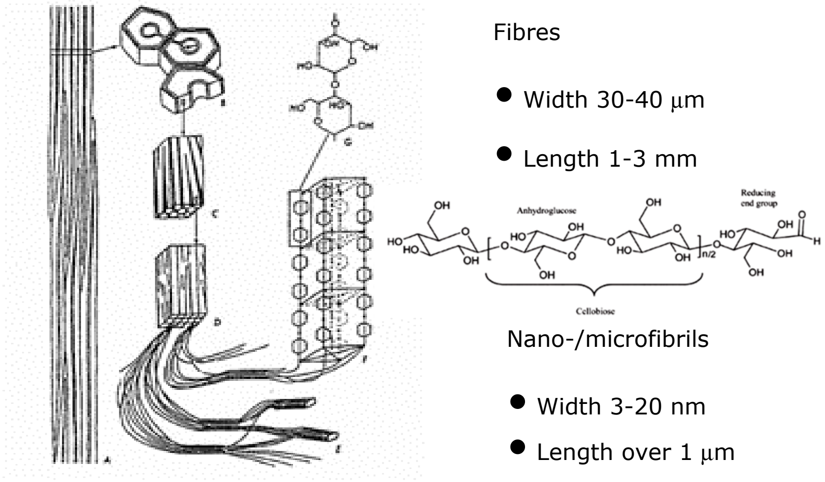


**Figure 1** The hierarchial structure of cellulose in plant cell wall (adapted from (Esau 1977)) and the difference between cellulosic fibres and nano-/microfibrils

Nanofibrillated cellulose (NFC) or microfibrillated cellulose (MFC) was first produced by ITT Rayonier in USA in the beginning of the 80s (Herrick et al. 1983, Turbak et al. 1983). The disintegration of nanofibrillated cellulose from source materials, e.g. wood and agricultural residues, is typically started by chemical pulping (Kraft or sulphite process) in order to obtain a suspension of cellulosic fibres. The next step can involve chemical modification of pulp by 2,2,6,6-tetramethylpiperidine-1-oxyl free radical mediated oxidation (TEMPO-oxidation) (Saito et al. 2007) or carboxymethylation (Wågberg et al. 2008). Both of these two chemical pre-treatments result in an increased amount of anionic, carboxyl groups on the nanofibril surface which prevents the aggregation of fibrils in water by electrostatic repulsion. Cationization of pulp has also been suggested as a pre-treatment for NFC production from cellulosic fibres (Aulin et al. 2010, Ho et al. 2011). Enzymatic hydrolysis (Pääkkö et al. 2007) can also be used in pre-treatment for pulp. The mild enzymatic hydrolysis can be done by an endoglucanase which is an cellulolytic enzyme that catalyzes the endohydrolysis of the cellulose chains. The aim of all the pre-treatments is to lower the energy consumption of the mechanical disintegration process and to gain more uniform and controlled nanofibril dimensions. The type of mechanical disintegration in addition to the type of possible pre-treatment as well as the source material properties affects the homogeneity and dimensions of the fibrils. The mechanical disintegration can be done by using either high-pressure fluidizers (Pääkkö et al. 2007), high-pressure homogenizers (Turbak et al. 1983), super-grinders (Taniguchi 1996) or cryo-crushing (Chakraborty et al. 2005).

### 2.1.1 Introduction of charged groups on cellulose

Pulp fibres contain always some anionic, charged groups. As a result of sulphite pulping the anionic groups are strong sulphonic acid groups whereas Kraft pulp contains weak carboxylic acid groups. The reason for introducing charged groups on cellulosic fibres as a pre-treatment for NFC production is that they result in electrostatic repulsion induced osmotic swelling pressure in the fibre cell wall (Scallan and Tigerström 1992). This enhances the fibre cell wall delamination. The main methods to increase the charge of pulp fibres are carboxymethylation, TEMPO-oxidation and cationization.

#### *Carboxymethylation of cellulose*

Carboxymethylation of cellulosic fibres to a low DS was first studied by Walecka (1956). As a pre-treatment for the NFC production this method was first used by Wågberg et al. (2008, 1987). Carboxymethylation of solvent-exchanged (ethanol) pulp is done in organic solvents (iso-propanol/methanol) in the presence of NaOH using monochloroacetic acid as a reagent at elevated temperature (boiling). After the reaction the pulp is extensively washed. In the case of NFC production the carboxyl groups on the pulp fibres are converted to their sodium form prior to fibrillation in order to enhance the effect of the charge induced fibre cell wall delamination (Wågberg et al. 2008). Figure 2 shows the chemical structure of carboxymethylated cellulose. The carboxyl groups can be either at C2, C3 or C6 position. A DS of 0.3 or more results in dissolution of the carboxymethylated cellulose chains in water (Liu et al. 1997). The carboxymethylation pre-treatment of pulp has been reported to lower the energy consumption of fibrillation down to ~ 2200 kWh/tonne for 2 % w/w suspension. Only one passage through a high pressure fluidizer is needed to form a NFC gel of carboxymethylated pulp suspension (0.515 mmol/g charge density) (Ankerfors 2012).

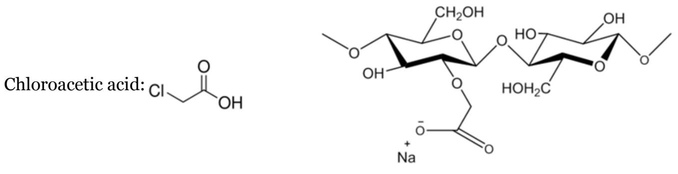


**Figure 2** The chemical structure of carboxymethylated cellulose (sodium form) and the monochloroacetic acid reagent

#### *TEMPO-mediated oxidation of cellulose*

TEMPO-mediated oxidation of cellulosic fibres has been extensively studied as a pre-treatment for NFC manufacturing (Isogai et al. 2011). The use of

TEMPO with NaClO as a primary oxidant and NaBr as an additional catalyst at pH of 10-11.44 results in carboxylate groups selectively at C6 position via C6-aldehyde groups (Saito and Isogai 2006). The reaction has also been developed to take place at neutral or slightly alkaline conditions (TEMPO/NaClO/$NaClO_2$ system) in order to avoid, e.g., the alkaline induced decrease in the degree of polymerization (DP) of cellulose during the reaction (Isogai et al. 2011). The reaction is presented in Figure 3. The energy consumption of TEMPO-NFC fibrillation process has been reported to be ~ 2000 kWh/tonne (1.5 mmol/g charge density) (Isogai et al. 2011).

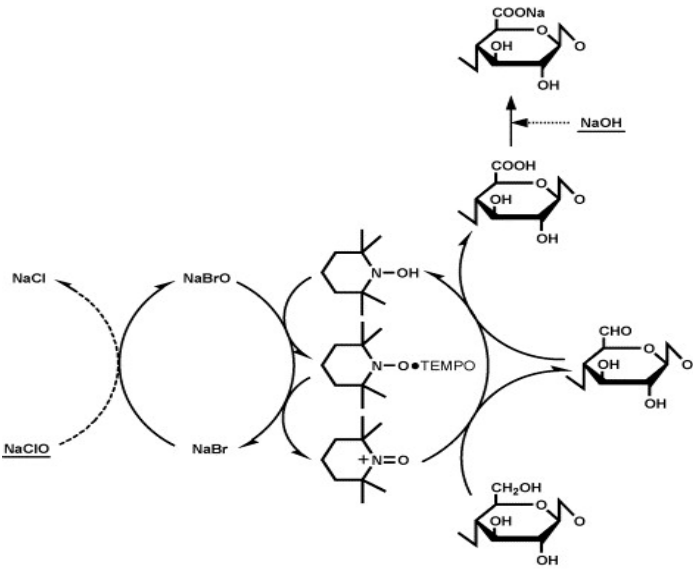


**Figure 3** TEMPO-mediated oxidation of cellulose (Saito and Isogai 2006)

### *Cationization of cellulose*

The introduction of cationic groups on cellulosic fibres can done by using N-(2-3-epoxypropyl) trimethylammonium chloride (EPTMAC) or chlorocholine chloride (ClChCl) as a reagent in an organic solvent in the presence of NaOH (Aulin et al. 2010, Ho et al. 2011, Olszewska et al. 2011). These both cationization procedures result in quaternary ammonium groups on cellulose. Usually, cationic NFC grades are amphoteric because the anionic groups in the originating pulps remain regardless of the cationization (Olszewska et al. 2011). The chemical structure of cationic cellulose produced by ClChCl or EPTMAC is presented in Figure 4. The effect of the cationization of pulp on the energy consumption of NFC manufacturing has not been reported in detail in the literature. A charge density of 0.354 mmol/g has been reported for cationic NFC (Olszewska et al. 2011).

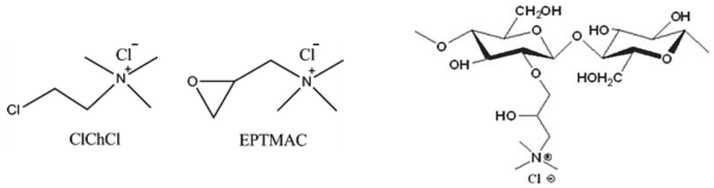


**Figure 4** The structure of cationic trimethylammonium cellulose and the two possible reagents ClChCl and EPTMAC

### 2.1.2 Properties of NFC

Nanofibrillated cellulose is a unique, biodegradable, light-weight, low density material which has a high aspect ratio (~100-300) and an extremely high specific surface area (Klemm et al. 2011). It has an excellent ability to form a strong weblike network or film, and because of its nano-scale dimensions it is transparent or translucent depending on the source material, pre-treatments and disintegration processes (Henriksson et al. 2008, Iwamoto et al. 2008, Nogi et al. 2009). Because of the low in-plane axial thermal expansion of the crystalline cellulose structure (cellulose I) combined with the random in-plain network structure, NFC has a low coefficient of thermal expansion (Nakagaito et al. 2010). This is an extremely important characteristic, e.g., in making displays of NFC-plastic composites.

NFC has very good strength properties and axial stiffness because of its structure (Eichorn et al. 2010, Moon et al. 2011). It is known that the DP of cellulose chains correlates with the tensile strength of NFC in such a way that a higher DP of cellulose in NFC gives a higher tensile strength for a NFC free-standing film (Henriksson et al. 2008). An interesting fact is that, in addition to strength, the NFC films have been shown to have very good oxygen barrier properties at low relative humidity conditions (RH<70) (Syverud and Stenius 2009). NFC is highly hygroscopic and this causes substantial water and moisture absorption and swelling in water, and a gel-like structure is formed already at low concentration (1-2 %) (Klemm et al. 2011, Zhang et al. 2013). In addition, it is not possible to redisperse NFC when it is once dried from water because of irreversible hornification of the fibrils (Hult et al. 2001). NFC can be used as a never-dried hydrogel, e.g., as a rheology modifier (Laukkanen et al. 2013), or as a dried material, e.g., strong aerogel (Pääkkö et al. 2008) or nanopaper (Henriksson et al. 2008, Österberg et al. 2013). There are several patent applications on a yearly basis about NFC in different application areas ranging from construction, packaging and chemical industry to food and biomedical applications (Aspler et al. 2013, Laukkanen et al. 2013).

## 2.2 Modification of NFC in water

Although NFC has interesting, unique properties, it has to be able to be modified in order to increase its application potential. It is well-known that some water-soluble linear cellulose-like polysaccharides have a tendency to irreversibly adsorb on cellulose. In this thesis the functionalization or modification of NFC in aqueous media has been done mainly by water-soluble polysaccharide adsorption. There are also other routes for indirect modification of NFC in aqueous media, e.g., by polyelectrolyte multilayers, but they usually involve oil-based polymers (Aulin et al. 2008, Eriksson et al. 2006, Wågberg et al. 2008). Engineered proteins can also be used in NFC modification in water (Varjonen et al. 2011).

In addition to indirect modifications, the modification of NFC has been done using a direct route of chemical functionalization of the NFC gel in this thesis. In this case carboxymethylation has been used as a pre-treatment followed by covalent modification of the NFC gel using EDC/NHS assisted coupling reaction in water. There are also other routes for direct chemical modification of NFC in aqueous media, e.g., by click-chemistry and free radical graft copolymerization reactions (Littunen et al. 2011, Pahimanolis et al. 2011, Stenstad et al. 2008).

### 2.2.1 Polysaccharide adsorption on cellulosic substrates

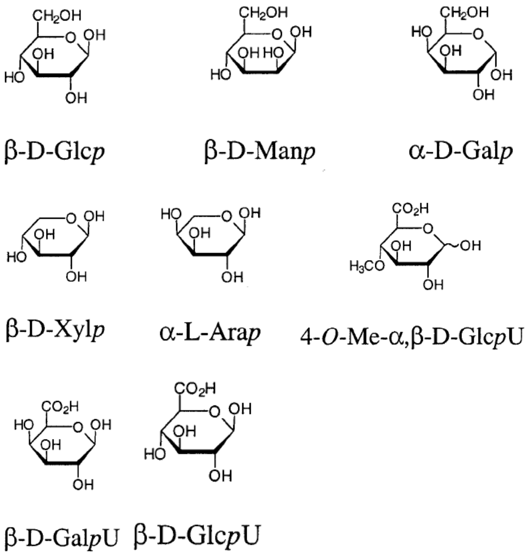
In order to adsorb, a polymer must diffuse towards the interface and meet the empty surface in such a way that it attaches. It also has to find the set of conformations which will minimize the free energy. The adsorption kinetics of a polymer adsorption process is typically dependent on molecular weight (Mw) of the polymer, concentration and solvent type. Temperature, chain length and structure all influence the polymer unfolding process (Fleer et al. 1993).

The available surface area of the adsorbent has a great effect on the possible amount of adsorption in the case of single layer adsorption (Fleer et al. 1993). The Langmuir isotherm can be used to model the single layer adsorption of a polymer on a surface. This means that polymers adsorb onto the surface as a monolayer in such a way that the molecules adsorb independently on well-defined sites on the surface.

Polymer adsorption is influenced by the Mw of the polymer so that the higher the Mw the stronger it is attached at the surface (Stenius 2000). The lower Mw fractions of polydisperse polymers adsorb more rapidly at the surface, but it is easier to remove from the surface by washing (Fleer et al. 1993). The conformation of the polymer must change before the adsorption process. A flat conformation causes a high bound fraction and the polymer chain will be well anchored at the interface (Fleer et al. 1993). The interaction parameters for adsorption of a non-ionic polymer are adsorption energy and the solvency parameter according to the Scheutjens and Fleer theory (Fleer et al. 1993). Charged polymers have, in addition, electrostatic interactions which are dependent on the surface charge density, polymer charge and ionic strength of the medium (Fleer et al. 1993).

The irreversibility of the adsorption process depends on the type of bonding and interaction between the polymer and the substrate. Basically the adsorption can be physisorption or chemisorption of the polymer on the substrate. In the case of physisorption the polymer does not bond covalently on the substrate but interacts with the substrate by weaker types of physical bonds, such as hydrogen bonds.

Polysaccharides are bio-based polymers which are mainly built up of D-glucose, D-xylose, D-mannose, D-galactose, L-arabinose, 4-O-methyl-D-glucuronic acid, D-galacturonic acid and D-glucuronic acid (Sjöström 1981) sugar residues. These sugar residues are presented in Figure 5.



β-D-Glc*p* β-D-Man*p* α-D-Gal*p*

β-D-Xyl*p* α-L-Ara*p* 4-*O*-Me-α,β-D-Glc*p*U

β-D-Gal*p*U β-D-Glc*p*U

**Figure 5** Main polysaccharide sugar residues (Alén and Stenius 2000)

Polysaccharides can be branched or linear, and the possible side chains can appear as regular intervals, blocks, or be distributed randomly along the backbone. They may also contain some functional groups, like carboxyl groups, strong acid groups or basic groups. The modification or grafting of polysaccharides can be done by different routes involving, e.g., partial hydrolysis and mild oxidation (Whistler and Smart 1953).

Polysaccharide-cellulose interactions have been studied thoroughly (Hannuksela et al. 2004, Ishimaru and Lindström 1984, Lindström et al. 2005, Zhou et al. 2007, Whistler 1959), and mainly xyloglucan (XG), galacto(gluco)mannans, xylan, chitosan (CHI) and some cellulose derivatives are known to adsorb irreversibly on cellulose. Although it is a well-known phenomenon, the mechanism is still under debate.

It is known that XGs have a high affinity to adsorb onto cellulosic substrates (Ahola et al. 2008a, Zhou et al. 2007). XG exists in plants, e.g., peas and the seeds of the tamarind tree. Also wood contains small amounts of XG and fucoxyloglucan (Alén and Stenius 2000). XGs have a β-D-(1→4) glucan backbone with α-linked xylopyranosyl residues at C-6, which may be substituted with galactopyranose, fucopyranose and arabinofuranose combinations (Fry et al. 1993). XGs are usually branched based on two different substitution patterns with regular substitution. The tamarind-based XG backbone has a flat and two-fold twisted structure in the solid state, similar to cellulose (Millane 1992, Taylor and Atkins 1985). The task of XG in plants is to cross-link cellulose microfibrils. The suggested reason for irreversible adsorption of XG on cellulosic substrates is strong hydrogen bonding (Zhou et al. 2007). It is known that the chemical structure, Mw and the conformation of XG affect its interaction with cellulosic substrates (Lima et al. 2004, Whitney et al. 2006, Zykwinska et al. 2008). Fucosylgalactose substitution has been claimed to be the key contributor for strong binding of substituted XG to cellulose since the self-association of less-substituted XG competes with the hydrogen bonding between XG and cellulose (Whitney et al. 2006).

Another polysaccharide that has affinity to adsorb on cellulose is galactomannan. Galactomannans exist in seeds and plants, e.g. guar plant seeds, and function as cell wall storage polysaccharides (Wielinga et al. 2000). Galactomannans are water-soluble and neutral polysaccharides. Guar gum (GG) and locust bean gum (LBG), which are the most typical commercial galactomannans, are built up of a (1→4)-linked β-D-mannopyranosyl unit backbone, with (1→6)-linked α-D-galactopyranosyl unit side groups (Whistler 1959). The main difference of these two gums is that LBG has a higher mannose-to-galactose ratio than GG (Whitney et al.

1998). Softwood contains a hemicellulose called galactoglucomannan (GGM), which has additional D-glucose units in its backbone (Wielinga et al. 2000). The galactose side units in galactomannans prevent self-adhesion of the mannan backbone. The mannose backbone has to be substituted by more than 12 w-% of galactose side units in order to be water-soluble. It is known that the chemical structure affects the adsorption of galactomannans on cellulosic substrates, and that a high mannose-to-galactose ratio promotes the adsorption of galactomannans on cellulosic substrates (Hannuksela et al. 2004, Ishimaru and Lindström 1984).

The xylan backbone typically contains β-(1→4)-linked xylose units with irregular 4-O-methyl-α-D-glucopyranosyluronic (MeGA) acid or α-L-arabinofuranosyl side chains which are linked with 1,2- and 1,3-glycosidic linkages to the backbone (Tenkanen 2004). The DS of xylan affects its water-solubility and interchain aggregation (Ebringerova and Heinze 2000). In case of anionic xylans there are electrostatic repulsion between xylan and anionic cellulosic substrates. The binding of xylans to cellulose microfibrils is suggested to be possible if the xylan has a low DS and a linear xylose backbone (Ebringerova and Heinze 2000). In addition, degraded xylans are suggested to be able to precipitate on the cellulose microfibril surfaces in more or less crystalline form (Linder et al. 2003, Yllner and Enström 1957). It has been shown that xylan can form complexes with cellulosic fibre cell wall components (Atalla et al. 1993). Van der Waals attraction and hydrogen bonding has been suggested to be the reason for adsorption of xylans on cellulosic substrates (Linder et al. 2003, Paananen et al. 2004).

CHI is a chitin derivative, prepared by hydrolysis of the N-acetyl groups. CHI is a linear carbohydrate with β(1→4)-linked 2-amino-2-deoxy D-glucose units. Because of the amine groups, CHI is a water-soluble, linear polycation in a dilute acidic solution (Li et al. 2004). CHI is known to adsorb on cellulosic substrates, and increase the wet-web strength of paper (Laleg and Pikulik 1991). Hydrogen bonding, van der Waals forces and electrostatic interaction are suggested to be the main mechanism in CHI-cellulose interactions (Li et al. 2004).

Other polysaccharides which are known to adsorb on cellulosic substrates are the linear, water-soluble cellulose derivatives methylcellulose (MC) and CMC. MC aggregates in high Mw solutions and at high temperature; therefore, MC is soluble only in cold water (Hirrien et al. 1996). MC is suggested to form hydrogen bonds with cellulose (Lindström et al. 2005).

CMC is an anionic cellulose derivative which is widely used in papermaking and other industrial applications, e.g., in food industry. CMC has a glucan backbone with carboxymethyl side groups at the C2, C3 or C6 position (Stelzer and Klug 1980). The functional properties of CMC depend on its DS and the backbone chain length. CMC is water-soluble at a DS over 0.3 (Liu et al. 1997). It is known that CMC can adsorb on cellulosic substrates under certain conditions, e.g. in high electrolyte concentration to screen the electrostatic repulsion (Laine et al. 2000). Co-crystallization between CMC and cellulose is suggested to be the reason for the irreversible adsorption of CMC on cellulose. A low DS of CMC promotes its adsorption on cellulosic substrates (Laine et al. 2000).

### *Interaction forces in polysaccharide-cellulose systems*

The interaction forces in the case of the polysaccharide and NFC systems can be partly described by DLVO theory (Derjaguin and Landau 1941, Verwey et al. 1948). DLVO theory explains the interaction forces arising from electrostatic and van der Waals forces. In addition to interaction forces described by DLVO theory there are some additional forces which exist in NFC-polysaccharide systems. These are hydration forces and steric forces which arise, respectively, from the effects of water molecules in the system and the structural features of the polysaccharides and NFC (Israelachvili and Wennerström 1996).

The first component in the DLVO theory, namely the van der Waals forces, are always present at colloidal systems. They are attractive, short-range forces which originate from permanent or temporary polarization of fluctuating electron clouds in atoms (Hamaker 1937, Lifshitz 1956). Electrostatic forces, in contrast, can be either repulsive or attractive. All the NFCs used in this work carry a negative charge in aqueous media. The electrostatic interactions between NFC and other charged polysaccharides can be controlled by the ionic strength and the pH of the aqueous media. The ionic strength of the media influences the thickness of the electric double layer (Debye length, $\kappa^{-1}$) which has great influence on the electrostatic interactions between the systems (Derjaguin et al. 1987). The lower the ionic strength, the stronger are the electrostatic interactions because of the larger Debye length.

Also the surface charge and the surface potential affect the electrostatic interaction forces in addition to ionic strength according to the DLVO theory. In case of NFC and other polysaccharides which contain weak carboxylic acid groups ($pK_a$ ~3-5) (Hoogendam et al. 1998), the pH affects the electrostatic interactions forces because the charged groups are known to be fully dissociated at alkaline conditions, approximately at pH = 8.5. On the other hand, the amine group containing CHI has a $pK_a$ of ~6.5 (Claesson and Ninham 1992) and it is not positively charged at alkaline or neutral conditions.

Friction forces in cellulose-polysaccharide systems are also studied in this thesis. The friction force between two substrates can be calculated by Amonton´s law (Equation 1).

$$F_f = \mu L \qquad (1),$$

where $F_f$ is the friction force, $\mu$ is the friction coefficient and $L$ the applied load. In case of friction measurements performed with an atomic force microscope (AFM), an additional parameter has to be added, namely $F_o$, which is independent of the normal force (Carpick et al. 1996, Lord 1955). Also the surface roughness has a marked effect on the friction between surfaces and has to be considered in case of friction forces in cellulose-polysaccharide systems.

### 2.2.2 Modification of cellulosic materials by polysaccharides

Polysaccharide adsorption has had growing interest in the modification of cellulosic materials. CMC-adsorption is known to increase the strength properties of paper (Laine et al. 2002). Also other polysaccharides, e.g. galactomannans, xylan and XG, are known to increase the strength of paper because they have a dispersing effect on cellulosic fibres resulting in an increase in bonding strength (Ahrenstedt et al. 2008, Lindström et al. 2005, Oksanen et al. 2011, Schönberg et al. 2001).

The polyelectrolyte multilayer (PEM) technique was first introduced by Decher et al. (1992) in the early 1990s. The technique is based on multilayer build-up of oppositely charged polyelectrolytes. It has been shown that the potential of multilayers range from biosensors and small electronic devices to membranes and molecular encapsulation (Haberska and Ruzgas 2009, Ho et al. 2000, Nolte and Fery 2006, Zhao et al. 2006). The PEM technique has been used in fundamental cellulose model surface modifications (Ahola et al. 2008a, Aulin et al. 2010, Eronen et al. 2012, Olszewska et al. 2013, Salmi et al. 2009, Wågberg et al. 2008) and in applied paper strength studies (Eriksson et al. 2006, Wågberg et al. 2002). Multilayered structures of non-ionic polysaccharides, e.g. XG, on cellulose have also been reported (Jean et al. 2009) which utilize the natural affinity of XG to adsorb irreversibly on cellulose instead of electrostatic interaction.

One of the first advanced modification routes for cellulose based on polysaccharide adsorption was the XG endotransglycosylase (XET)-technology. It utilizes the natural ability of XG and modified XGs (XG-R) to adsorb irreversibly on cellulosic substrates (Zhou et al. 2006). The schematic representation of XET-technology is presented in Figure 6.

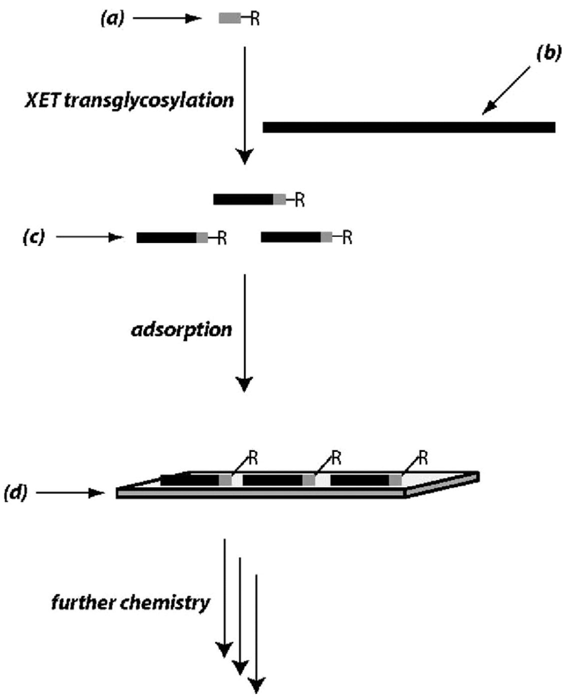


**Figure 6** The principle of XET-technology showing the a) $Glc_4Xyl_3$-based xylogluco-oligosaccharide bearing a specific molecule (R), b) high $M_r$ XG, c) chemically modified XG (XG-R) and d) cellulose surface with introduced specific molecule (R) (Zhou et al. 2006)

The use of functionalized polysaccharides and click-chemistry in cellulose modification has also recently been published (Filpponen et al. 2012, Xu et al. 2012). The "double-click" technology is based on the adsorption of CMC onto cellulose. CMC is first functionalized with alkyne or azide groups, adsorbed on cellulosic surface, and the activated surface is further modified by using click-chemistry. The schematic representation of the double-click technology is presented in Figure 7.

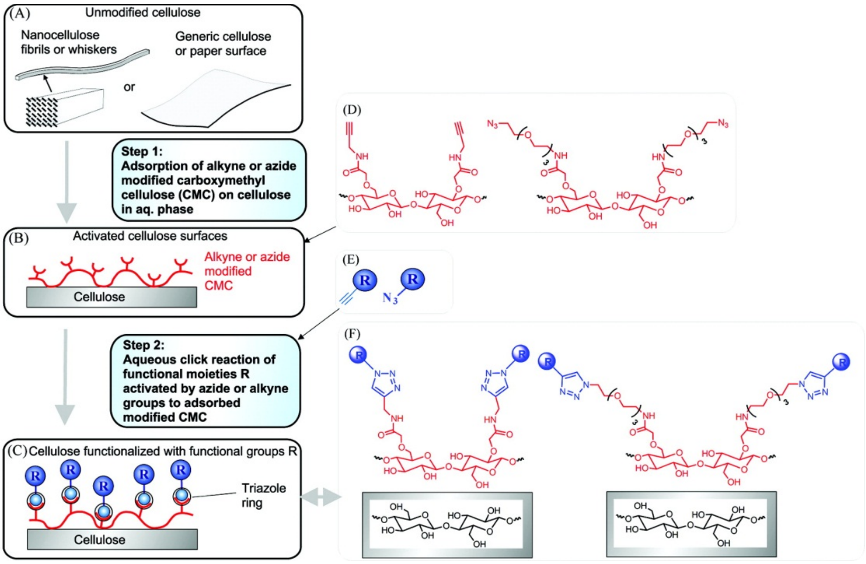


**Figure 7** The double-click technology. (A) Any unmodified cellulose surface is (B) exposed to a solution of CMC modified with alkyne or azide moieties (step 1) and (C) any molecule R with alkyne or azide moieties can be attached to the already adsorbed CMC by a click reaction (step 2). Chemical structures of (D) alkyne (left) and azide (right) modified CMC, (E) alkyne and azide modified functional groups, and (F) final surface modified product (Filpponen et al. 2012)

Another indirect modification method for cellulose based on polysaccharide adsorption and click-chemistry consists of galactose oxidase-assisted oxidation followed by reductive amination of galactosylated hetero-polysaccharides (XG and GGM) in order to introduce click-active groups (alkyne) on the polysaccharide, and their ability to adsorb on cellulose (Xu et al. 2012). The schematic representation of this polysaccharide adsorption-based click modification of cellulose is presented in Figure 8.

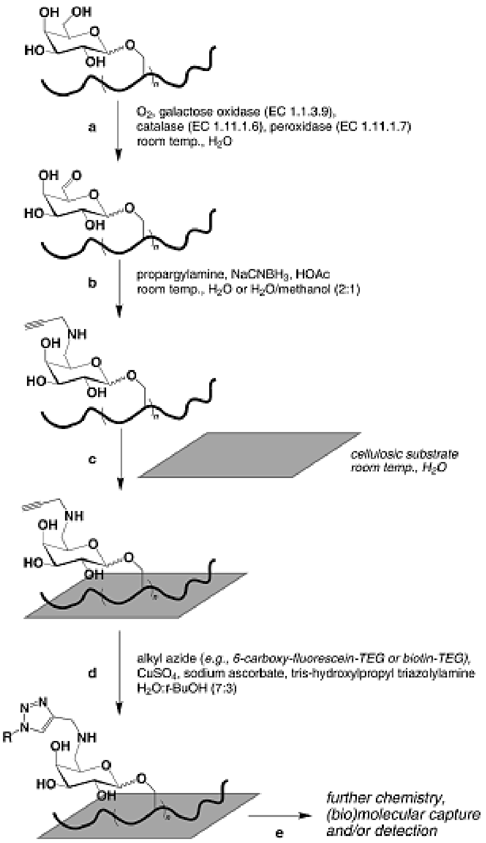


**Figure 8** General method for activation of cellulose surfaces with galactosylated hetero-polysaccharides. a) Oxidation of the polysaccharide with galactose oxidase, b) reductive amination, c) adsorption of the click-functionalized polysaccharide, d) *in-situ* click reaction, e) further chemistry (Xu et al. 2012)

### 2.2.3 EDC/NHS conjugation chemistry

Covalent modification of carboxyl group containing NFC or CMC can be done by EDC/NHS conjugation chemistry (Grabarek and Gergely 1990, Nakajima and Ikada 1995). The reaction can be done in aqueous media since 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) is a water-soluble activator for carboxyl groups. N-hydroxyl succinimide (NHS) is usually used together with EDC in order to make the conjugation reaction more efficient by preventing the rapid hydrolysis of the unstable *O*-acylisourea intermediate by creating a semi-stable amine reactive NHS-ester intermediate (Staros et al. 1986). The amine-reactive NHS-ester intermediate can in turn react with molecules containing amine groups in water and form stable amide bonds. Figure 9 shows the EDC/NHS conjugation reaction between a carboxylic acid containing molecule (1) and an amine containing molecule (2).

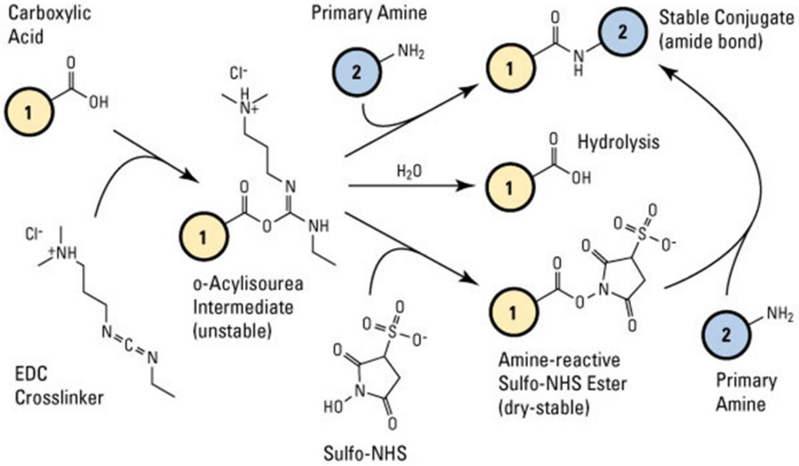


**Figure 9** EDC/NHS assisted coupling reaction (Hermanson 2008)

EDC/NHS conjugation has been used in cellulose modification, e.g., with nanocrystalline and nanofibrillated cellulose (Araki et al. 2001, Filpponen and Argyropoulos 2010, Karabulut et al. 2012). However, the cellulosic substrate has to be pre-treated by carboxymethylation, TEMPO-oxidation or CMC adsorption in order to introduce the carboxyl groups to the substrate prior to activation and covalent modification.

### 2.2.4 Click chemistry (Copper(I)-catalyzed azide-alkyne cycloaddition reaction)

Click chemistry is a term which has developed recently to describe a set of chemical reactions. The criteria to be called as a click reaction is that it is easy to perform, it has a high yields with minimum byproducts and a high tolerance to water and different kinds of chemical reaction conditions (Kolb et al. 2001). The copper(I)-catalyzed azide-alkyne cycloaddition reaction (CuAAC) is one of the click reactions, see Figure 10. The Huisgen´s 1,3-dipolar azide-alkyne cycloadditon reaction (Huisgen 1984) started to grow interest when it was discovered that by using copper(I) as a catalyst the reaction becomes more efficient and controllable (Iha et al. 2009, Rostovtsev et al. 2002, Tornøe et al. 2002). Specifically, the addition of the copper(I)-catalyst to this cycloaddition reaction results in regiospecifically united azide and terminal acetylenes producing only 1,4-disubstituted 1,2,3-triazoles (Rostovtsev et al. 2002). The reaction takes place in between an azide-group containing molecule (R2; Figure 10) and an alkyne-group containing molecule (R1; Figure 10) in the presence of Cu(I) as catalyst for the reaction. Water can be used as a solvent and the reaction can be performed at room temperature with a relatively high yield (Rostovtsev et al. 2002).

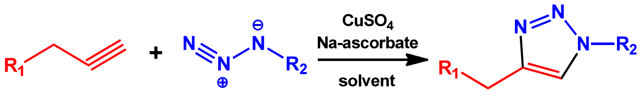


**Figure 10** Copper(I)-catalyzed azide-alkyne cycloaddition reaction (adapted from Filpponen et al. 2012)

Click chemistry has been used together with cellulosic materials and polysaccharides to some extent (Elchinger et al. 2011, Eyley and Thielemans 2011, Hafrén et al. 2006, Liebert et al. 2006). Also cellulose nanocrystals have been modified successfully with click chemistry (Filpponen and Argyropoulos 2010). Click-functionalized CMC adsorption (Filpponen et al. 2012) has been shown to be a relatively facile way to modify cellulosic materials to form a NFC template for further click-modification. In addition, clickable cellulose surfaces have been produced by using multivalent polysaccharide adsorption which was presented in the previous chapter (Xu et al. 2012). NFC can be also directly click-functionalized in aqueous media (Pahimanolis et al. 2011).

## 2.3 Summary

In this thesis, the adsorption of polysaccharides on NFC substrate was studied by using surface sensitive techniques, such as quartz crystal microbalance with dissipation (QCM-D) and surface plasmon resonance (SPR). This fundamental knowledge was then employed for the modifications of bulk NFC in aqueous media. The modification of bulk NFC was applied by using sequential adsorption of pre-functionalized CMCs (azido or propargyl) and click-chemistry with different counterpart molecules. In addition, compounds carrying amine functionality were grafted on to the carboxymethylated NFC via the carbodiimide-mediated (EDC/NHS) coupling, creating an amide linkage between the amine and carboxyl groups on the NFC substrate.

# 3. Experimental

## 3.1 Materials

### 3.1.1 Chemicals

All the chemicals used were of analytical grade. MilliQ-water (Millipore Synergy UV unit; Millipore S.A.S. Molsheim, France) was used for the dilutions. EDC was purchased from Fluka and NHS from Thermo Scientific. Methoxy polyethylene glycol azide (OMe-PEG azide, 20 kDa), Bovine Serum Albumin (BSA, 66 kDa) and $CuSO4 \cdot 5H2O$ (98+%) were purchased from Sigma-Aldrich. L-ascorbic acid (99%) was purchased from Acros Organics. Trimethylsilyl cellulose (TMSC) was synthesized from cellulose powder (spruce, Fluka), according to a previously published method (Kontturi et al. 2003). Methoxy polyethylene glycol amine (OMe-PEG-amine; 2 kDa; >0.4 mmol/g) was a product from Fluka. PEI was purchased from Polysciences Inc. All the buffers were prepared from known amount of salts. The anionic titration polymer was potassium polyvinylsulphate (KPVS, charge density $2.85 \cdot 10^{-7}$eq/mL) and the cationic titration polymer was poly(diallyldimethylammonium chloride) (PDADMAC, charge density 6.19 meq/g, Mw>500 000 g/mol). Water-dispersible carbon dots (CDs) were synthesized and purified according to Liu et al. (Liu et al. 2011a) except that the pyrolysis of glycerol was made in the presence of 2,2-(ethylenedioxy)-bis-(ethylamine) instead of 4,7,10-trioxa-1,13-tridecanediamine. The microwave assisted pyrolysis was carried out for 10 min at 750W.

### 3.1.2 Polysaccharides

The commercial polysaccharides used in Paper I were XG (tamarind seed, 35% xylose, 45% glucose, 15% galactose, 4% arabinose, Megazyme), CHI (low Mw, 448869, Sigma-Aldrich), GG (G4129, Sigma-Aldrich), LBG (G0753, Sigma-Aldrich), methylcellulose (M7140, Sigma-Aldrich), xylan (birch, Fluka 95588) and CMC (sodium salt, DS 0.52, CP Kelco). The properties of these polysaccharides are presented in Table 1.

**Table 1** Polysaccharide properties (Paper I)

| Polysaccharide | Abbr. | Reference for Mark-Houwink parameters | d*n*/d*c* (mL/g) | $M_V$ visc. (kDa) | $M_w$ SEC (kDa) | PDI * (SEC) ($M_w/M_n$) | $R_g$ (nm) |
|---|---|---|---|---|---|---|---|
| **Neutral** | | | | | | | |
| Xyloglucan | XG | (Picout 2003) | 0.146 ** | 190 | | | |
| Guar gum | GG | (Cheng et al. 2002) | 0.135 ● | 2110 | 2040 | 1.2 | 114 |
| Locust bean gum | LBG | (Cheng et al. 2002) | | 1960 | 1060 | 1.2 | 93 |
| Guar gum hydr. | $GG_{deg}$ | (Cheng et al. 2002) | | 250 | 460 | 11.8 | 66 |
| Methylcellulose | MC | (Hirrien et al. 1996) | | 20 | | | |
| **Anionic** | | | | | | | |
| Xylan | XYL | (Ebringerova and Heinze 2000) | | 9 | | | |
| Carboxy-methylcellulose | CMC | (Stelzer and Klug 1980) | | 370 | | | |
| **Cationic** | | | | | | | |
| Chitosan | CHI | (Mao 2004) | 0.181 ○ | 230 | | | |
| * the ratio of weight and number average molecular weights ($M_w$ and $M_n$, respectively) | | | | | | | |
| ** obtained from (Picout 2003) | | | | | | | |
| ● obtained from (Robinson et al. 1982) | | | | | | | |
| ○ obtained from (Harding 2005) | | | | | | | |

Two different CMCs (sodium form, 250 kDa), DS 0.7 and 1.2, were supplied by Sigma-Aldrich and purified prior to use by dialysis and freeze-dried (Papers II-IV). The polysaccharide solutions were prepared as described in Papers I-IV. In Paper II a cationic cellulose derivative, hydroxyethyl cellulose ethoxylate (HECE), was used. The charge density of HECE is 1.2 meq/g (the chemical structure of HECE is presented in Paper II).

### *Propargyl- and azido-CMCs (Paper IV)*

The click-functionalization of CMC was done according to Filpponen et al. (2012) in Paper IV. The two different CMCs (CMC1: DS 0.7 and CMC2: DS

1.2) were activated to two different levels. 100 mg of CMC was dissolved in water and mixed overnight. The CMC solution was diluted to 2 g/L with buffer (pH=4.5, I=10 mM) and the ionic strength was adjusted to 50 mM with NaCl. 1 mmol (CMC1) or 1.5 mmol (CMC2) of EDC, 4 mmol (CMC1) or 6 mmol (CMC2) of NHS and either 1.5 mmol (higher amount (a), CMC2), 1 mmol (higher amount (a), CMC1) or 0.8 mmol (lower amount (b), CMC2 and CMC1) of propargylamine (propargyl functionalization) or 11-Azido-3,6,9-trioxaundecan-1-amine (azido functionalization) were added. The solution was mixed in a beaker overnight protected from light at room temperature. The solution was dialyzed prior to freeze-drying.

#### *CMC-g-PEG (Paper III)*

CMC-g-PEG was synthesized using the procedure described by Araki et al. (2001) with slight modifications. 250 mg of CMC (DS 0.7) powder was dissolved in 10 mM buffer (pH=4.5) and mixed overnight. 479 mg of EDC, 1151 mg of NHS, and finally 250 mg of OMe-PEG-amine were mixed into the CMC solution. The reaction took place at room temperature (24 °C) for 24 h in a sealed beaker. Finally, the solution was dialyzed and freeze-dried.

### 3.1.3 NFC and modified NFCs

All the pulps used in this work were industrial, never-dried kraft pulp suspensions from Finnish pulp mills. The pulps were washed to sodium form prior to use (Swerin et al. 1990). Hardwood (birch) kraft pulp (73% glucose, 25% xylose, 1% mannose, 1% other carbohydrates) was used as the raw material for the unmodified NFC model surface studies (Papers I-III). It was fluidized for 20 passages through a high-pressure microfluidizer without any pre-treatments (Microfluidics Corp., USA). The charge density of the NFC was 65 μmol/g determined with conductometric titration.

The originating pulp for NFC bulk modifications (Paper IV) and carboxymethylated NFC (CM-NFC, Paper V and VI) was a birch kraft pulp from a Finnish pulp mill (71% glucose, 28% xylose, <1% mannose, <0.1% other carbohydrates). Prior to further processing the pulp was fractioned according to standard SCAN-M 6:69 in order to remove the fines (200 mesh). The carboxymethylation of the pulps was made according to Walecka using monochloroacetic acid (Walecka 1956). After carboxymethylation the pulps were washed to sodium form. The disintegration of the carboxymethylated pulps (CM-pulps) was done in a microfluidizer for 5 passages. After fluidization the CM-pulps (1-3) were called CM-NFC1-3. The total charge and surface charge of the CM-NFCs are presented in the results and discussion part of this thesis, determined with conductometric and polyelectrolyte titration (Paper V). The charge properties for the CM-pulps are presented in Table 2. AFM height images of the model NFC surface produced as well as the CM-NFC3 are presented in Figure 11.

**Table 2** Charge of the CM-pulps 1-3 (Paper V)

| Sample name | Reagent, (g)/pulp (g) | Charge density (µeq/g) | Surface charge (µeq/g) | Charge ratio<br>(surface charge/total charge) |
|---|---|---|---|---|
| CM-pulp3 | 0.06 | 315 ± 34 | 18 | 0.057 |
| CM-pulp2 | 0.035 | 236 ± 25 | 13 | 0.055 |
| CM-pulp1 | 0.015 | 129 ± 10 | 7 | 0.054 |

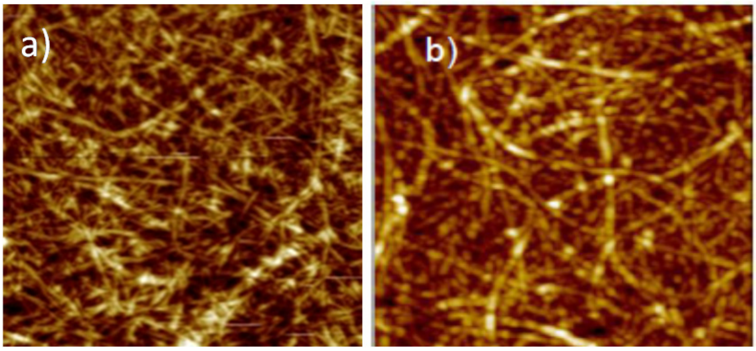


**Figure 11** AFM height images of the NFC model films, a) is the CM-NFC3 model film (1 µm$^2$) and b) is the unmodified NFC model film (4 µm$^2$)

### *Propargyl-NFC and -pulp (Paper V)*

The procedure of preparing propargyl-NFC and -pulp was presented in Paper V. CM-pulp3 (see Table 2) and CM-NFC3 were propargyl-functionalized using the EDC/NHS assisted coupling reaction according to Filpponen et al. (2012). The modifications were done at pH=4.5 with a concentration of 2 g/L for both the CM-NFC dispersion and the CM-pulp suspension. Three different amounts of EDC/NHS (see Table 3) were used for the activation of the carboxyl groups prior to the addition of propargylamine. The reaction took place overnight at room temperature. In the case of NFC dispersions, the excess of chemicals and salts were removed by dialysis and by filtration washing in the case of the pulp suspensions.

**Table 3** EDC/NHS activation amounts of CM-pulp3 and NFC3 (Paper V)

| m(sample) | COOH | EDC | NHS | Propargylamine |
|---|---|---|---|---|
| 1g | 0.32 mmol | 0.1 mmol | 0.4 mmol | 0.15 mmol |
| 1g | 0.32 mmol | 0.35 mmol | 1.4 mmol | 0.5 mmol |
| 1g | 0.32 mmol | 1 mmol | 4 mmol | 1.5 mmol |

### *CD-modified NFC (Paper VI)*

Carbon dots (CDs) were attached to CM-NFC by EDC/NHS assisted coupling reaction. The synthesis was done at pH=4.5 using a 0.2 % NFC dispersion. 119 mg/g of EDC and 460 mg/g of NHS was added to the NFC dispersion and finally 3 or 30 mg/g of the CD dispersion was added (the CD dispersion was sonicated for 10 min prior to addition). The reaction was conducted overnight at room temperature and protected from light. Finally, the NFC-CD dispersion was dialyzed.

### *Click-modified NFC (Paper IV)*

The procedure of preparing click-modified NFC was introduced in Paper IV. The modification of NFC was done by adsorption of azido-CMC (Paper IV) onto pulp fibres prior to fluidization followed by a CuAAC reaction with dansyl-alkyne. The azido-CMC (10 mg/g) was dissolved in water overnight, and slowly added to the 1% fibre suspension (pH=4.5, I=50 mM) while stirring. The fibre suspension was mixed for 2 h and washed in a Büchner funnel in order to remove any unbound azido-CMC. Finally, the azido-CMC modified fibre suspension was washed to sodium form (Swerin et al. 1990). The irreversibility of CMC adsorption was verified by conductometric titration of the pulp (Katz et al. 1984) prior to fluidization. After the functionalized pulp had been fluidized, it was diluted to a 1 g/L concentration with a total volume of 50 mL. 175 mg of L-ascorbic acid, 75 mg of $CuSO_4$ and 10 mg of dansyl-alkyne (pre-dissolved in a mixture of 0.5 mL of acetone and 0.5 mL of water) was added to the azido-functionalized NFC dispersion. The dispersion was mixed overnight in a sealed beaker protected from light. In order to remove any unbound dansyl-alkyne, the modified NFC was washed for several cycles with centrifugation (10,000 rpm); first with water (three additions of water, 30 min), then with acetone/water (15 min) and finally with water (two additions of water; 75 min) until the filtrate did not have any fluorescence. Three reference tests for the NFC modifications were performed by using unmodified pulp with Cu(I) catalyst, CMC-adsorbed pulp with Cu(I) catalyst and azido-CMC-adsorbed pulp where the CuAAC reaction was performed in the absence of Cu(I) catalyst.

## 3.2 Methods

### 3.2.1 Charge characterization of NFC

The charge of NFC is a highly important characteristic which affects its stability in water, its interaction and behavior with other substances. In Paper V a method for total charge and surface charge characterization of NFC/MFC was developed.

*Conductometric titration (Paper V)*

Conductometric titrations of all pulps were done by following the procedure by Katz et al. (1984). The titration of NFC was done as a back titration using HCl as follows: a known amount of NFC gel was diluted to water; 2 mL of 0.1 M NaOH was added; and the dispersion was mixed for 1h at 400 rpm at a total volume of 495 mL. After mixing the ionic strength of the dispersion was adjusted with NaCl (5 mL of 10 mM NaCl) and the sample was titrated with 0.1M HCl using an automatic titrator. Carbon dioxide was removed by bubbling $N_2$-gas before and during the titration. The total amount of carboxyl groups in the NFC sample was determined from the titration curve (conductivity as a function of the amount of $H^+$ added). A control sample without NFC was also titrated and taken into account in the calculations.

*Polyelectrolyte titration (Paper V)*

Polyelectrolyte (PE) titrations of the pulps in this work were done as described by Winter et al. (1986). The polyelectrolyte titrations were done with cationic polyelectrolyte PDADMAC (0.874 meq/L) using a Mütek particle charge detector (PCD 03; Mütek Analytic, GmbH, Herrsching, Germany) and an automatic titrator. The NFC gel was first dispersed (1 hour of mixing at 1 g/L) and diluted, and then the pH (8.5 or 4.5) and ionic strength were adjusted with $NaHCO_3$ and NaOH or with sodium acetate/acetic acid (NaOAc/HOAc) buffer. After this a known amount of dilute (0.1-0.5 g/L) NFC dispersion was titrated with PDADMAC (0.05 mL/30s). The surface charge of NFC was calculated based on the cationic demand.

### 3.2.2 NFC ultra thin films and nanopaper

The most common methods of preparing cellulose model films are Langmuir/Blodgett deposition (Tammelin et al. 2006) and spin-coating (Kontturi et al. 2003). Direct deposition of cellulose, with solvents such as N-methylmorpholine-N-oxide and dimethylacetamide with lithium chloride, usually results in cellulose model films which have rather high roughness. Spin-coated NFC model surfaces are mostly used in this work (Ahola et al. 2008b).

### *Cellulose model films*

The NFC model surfaces in Papers I-III were prepared by spin coating of a dilute nanofibrillated cellulose dispersion on $SiO_2$-crystals (QSX303, Q-Sense AB, Västra Frölunda, Sweden) according to a method first published by Ahola et al. (2008b) and later modified by Eronen et al. (2011). Prior to cellulose deposition the $SiO_2$-crystals were cleaned with 10 % NaOH, ultrapure water and a UV/ozonator (Bioforce Nanosciences, Ames, IA, USA); an anchoring layer of PEI was then adsorbed on the surface. A suspension of diluted, ultrasonificated and ultracentrifuged (10,400 rpm, 45 min) NFC dispersion was spin-coated (3,000 rpm, 1 min) on the crystal, carefully dried with $N_2$ -gas and finally heat-treated in an oven at 80 °C for 10 min.

The regenerated cellulose thin films for QCM-D experiments in Paper IV were prepared by spin-coating of a cellulose derivative, TMSC, onto $SiO_2$-coated sensor crystals (Q-Sense AB, Västra Frölunda, Sweden) and the regenerating it to cellulose by exposure to HCl vapor (Kontturi et al. 2003).

CM-NFC films in Paper VI were prepared using a slightly modified version of the method reported by Eronen at al. (2011). The CM-NFC gel was diluted in water to a final concentration of 1.25 g/L and sonicated for 5 min. Gold-coated QCM-D and SPR sensors were treated for 10 min in a UV/ozone oven and immersed for 15 min in 1 g/L PEI solution followed by rinsing with MilliQ water and drying with $N_2$-gas. The PEI-coated sensors were then spin-coated with diluted NFC dispersions (1.25 g/L, 1 min, 3,000 rpm). Finally, the surfaces were heat-treated (80 °C, 10 min) and placed in a desiccator. Prior to all the QCM-D and SPR measurements, the coated sensors were stabilized over-night in a buffer solution.

### *NFC nanopaper*

Membrane filtration was used to produce NFC nanopaper (Paper VI). The NFC gel was diluted to 0.1% concentration with deionized water and mixed for 1h followed by 5 min homogenization (Ultra-Turrax UTC, IKA-Werke GmbH & Co. KG, Staufen, Germany) and then an ultrasonic bath (2 min) to remove air bubbles from the gel. 800 mL of NFC was filtered on a glass filter funnel using two 0.65 µm DVPP filter membranes (EDM Millipore, Merck KGaA, Darmstadt, Germany) until approximately 790 mL water was removed from the film. The wet film was then pressed (standard paper press) for 15 min between filter membranes supported by blotting papers. Finally, the nanopaper was heat-treated overnight at 40°C.

### 3.2.3 QCM-D

The QCM-D measurements were performed using an E-4 instrument (Q-Sense AB, Västra Frölunda, Sweden) with controlled flow. The flow rate used in all experiments was 0.1 mL/min. The principle of QCM-D is to utilize the piezoelectric properties of quartz (Rodahl et al. 1995). The quartz crystal is placed between a pair of electrodes. When an alternating voltage is applied, the crystal starts to oscillate and the resonance frequency (f) of the oscillating crystal depends on the total oscillating mass, including water and other adsorbed molecules. The technique measures the change in the fundamental resonance frequency of the quartz crystal, 5 MHz, and its overtones (15, 25, 35, 45, 55, and 75 MHz). The frequency decreases linearly with the mass adsorbed on the crystal sensor according to the Sauerbrey equation, Equation 2 (Sauerbrey 1959):

$$\Delta m = -\frac{C \cdot \Delta f}{n} \qquad (2),$$

where $\Delta m$ represents the change in mass (mg/m$^2$), $C$ is the mass sensitivity (0.177 mg/Hz·m$^2$ for a 5 MHz quartz crystal), $n$ is the overtone number and $\Delta f$ is the change in frequency (Hz). In the case of polymer adsorption on cellulosic materials, the adsorbed film is far from rigid and the Sauerbrey relation is no longer strictly valid. The swelling of viscoelastic cellulose surfaces, and soft polymers, will not fully couple to the oscillation of the crystal, and in these systems the Sauerbrey relation underestimates the mass adsorbed at the surface.

In order to take into account the viscoelasticity of the system the Johannsmann´s model (Equation 3) for viscoelastic thin films can be applied when calculating the adsorbed amount (Johannsmann et al. 1992):

$$\hat{m}^* \approx m_0 (1 + \hat{J}(f) \frac{f^2 \rho d^2}{3}) \qquad (3),$$

where $J(f)$ is the shear compliance, $f$ is the resonance frequency in liquid, $d$ is the thickness of the film and $\rho$ is the density of the fluid. The true sensed mass ($m_o$) can be obtained from the intercept of the equivalent mass ($m^*$) and the square of the resonance frequency in liquid. QCM-D simultaneously measures the change in dissipation energy of the QCM-D sensor system. The definition of $D$ in Equation 4 is dependent on the dissipated (lost) energy per oscillation cycle, $E_{lost}$ $(J)$ and $E_{stored}$ $(J)$ is the energy stored in the oscillator.

$$D = \frac{E_{lost}}{2\pi E_{stored}} \qquad (4)$$

Both the cellulosic films used as well as adsorbing polysaccharides bind water and the changes in *Δf* and *ΔD* are due to both bound water and adsorbed polysaccharide. Up to 80-90 % of the polysaccharide mass adsorbed on cellulosic surfaces can be coupled water (Ahola et al. 2008b, Eronen et al. 2011).

### 3.2.4 SPR

Surface plasmon resonance (SPR) measurements were done with a SPR Navi 200 (Oy Bionavis Ltd., Tampere, Finland) or a Biacore 1000 instrument (GE Healthcare, Uppsala, Sweden). This technique was used in Paper I and Paper VI. A constant flow rate of 100 µL/min (Paper VI) or 5 µL/min (Paper I) was used during the measurements at a constant temperature of 25 °C. The SPR technique is based on the surface plasmon resonance phenomenon which takes place on a thin metal film (in this case gold). When a p-polarized light beam is directed through a prism to the metal film under conditions of total internal reflection, the photons interact with plasmons of the gold causing a SPR-wave through the thin gold film. The SPR-angle is the angle where the p-polarized light intensity changes and it is very sensitive to any changes in the refractive index of the gold film which can be modified by the adsorption of different molecules on the sensor (Homola et al. 1999). The change in the SPR-angle can be monitored as a function of time using a controlled flow of the sample or the buffer in the measurement chamber. Figure 12 presents the principle of SPR measurements.

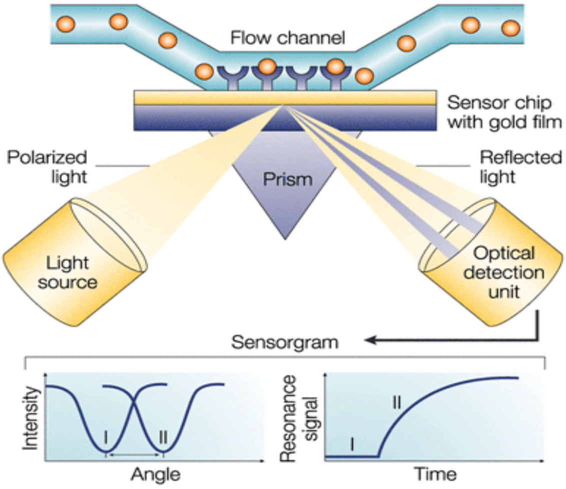


**Figure 12** The principle of SPR. The change in the refractive index of the gold-surface due to attachment of molecules results in a change in the SPR angle (adapted from Cooper 2002)

### 3.2.5 AFM, CPM and friction measurements

Atomic force microscopy using a Nanoscope IIIa MultiMode scanning probe microscope (Veeco, Digital Instruments, USA) or a Dimension 3000 AFM (Bruker, Santa Barbara, CA, USA) operating in tapping mode in air was used for imaging. The principle of AFM (Binnig et al. 1986, Butt et al. 2007) is presented in Figure 13. The AFM images in air were recorded using silicon cantilevers (NSC15/AIBS, MicroMasch, Tallinn Estonia) with a driving frequency of ~300-360 kHz. The radius of the tip according to the manufacturer was less than 10 nm. The scan size was 1$\mu m^2$, 4$\mu m^2$ or 25$\mu m^2$, and the images were scanned on at least three different areas of the sample. Flattening was the only image processing applied. Image analysis was performed using NanoScope software (version V6.13R1(R) Digital Instruments, Inc.)

AFM images in Paper I were taken from the QCM-D crystals after polysaccharide adsorption using the digital pulsed force mode (DPFM) in air with a WITec alpha 300R instrument (WITec GmbH, Ulm, Germany). The main advantage of using this imaging mode is that in addition to normal topographical images, also information about adhesion and stiffness can be acquired. The cantilevers in the DPFM measurements were Arrow FM (NanoWorld AG, Switzerland) cantilevers with nominal spring constant of 2.8 N/m. The scan size was 10x10 $\mu m^2$. More information about DPFM-measurements can be found from Schmidt et al. (2005). The amplitude set point was ~0.2 V, and a 1000 Hz frequency was applied.

In addition to imaging, AFM can be used in studying the molecular scale interaction forces between two surfaces either in air or liquid. This is called colloidal probe microscopy (CPM) (Ducker et al. 1991). The principle of the colloidal probe measurements is presented in Figure 13.

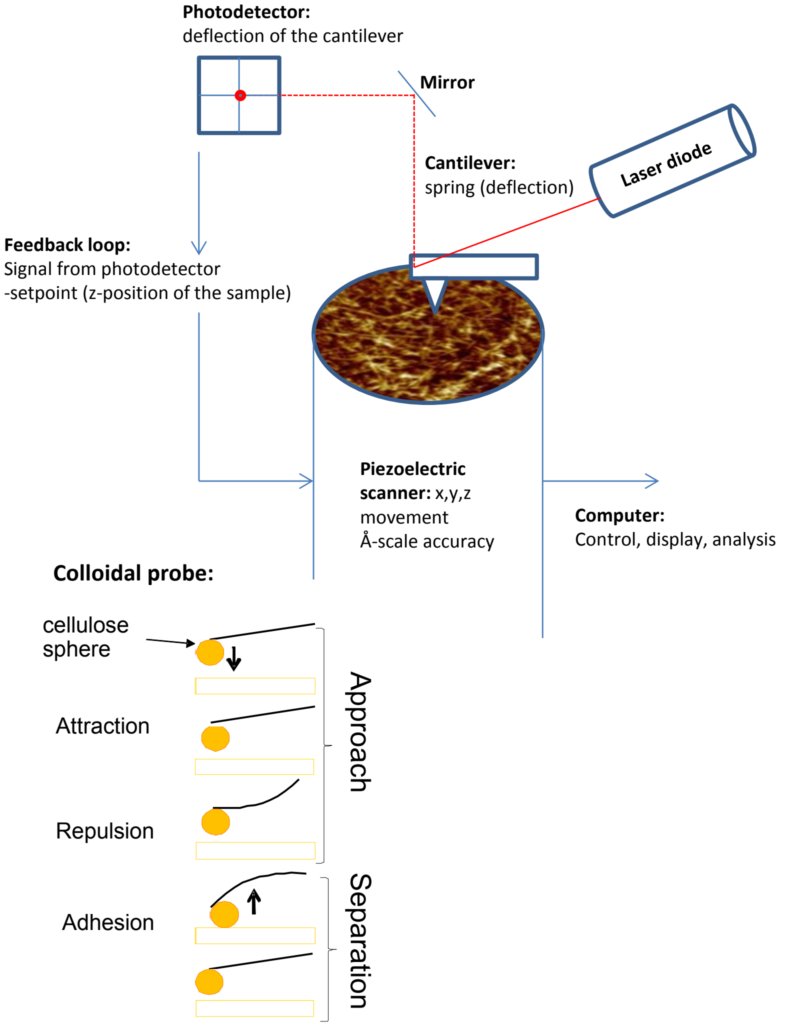


**Figure 13** The simplified principle of AFM and CPM

The surface forces in Paper II were studied with an AFM (NanoScope IIIa MultiMode, Digital Instruments, USA). Precipitated cellulose spheres (Kanebo Co., Japan), were used as colloidal probes. These spheres were 5–35% crystalline (cellulose II) and slightly negatively charged (Carambassis and Rutland 1999). The radius of the attached spheres was 18–30 µm, determined *in-situ* in electrolyte solution. A spin-coated NFC film on a silica wafer was used as the lower surface. The spring constant ($k$) of the cantilever was determined by the thermal noise method (Hutter and Bechhloefer 1993) prior to gluing the cellulose sphere on the tip. The surface force was calculated using Hooke's law ($F=k\cdot\Delta z$) and normalized using the radius of the sphere.

An AFM can be also used in studying the friction between surfaces. The colloidal probe and friction force measurements in Paper III were performed using a MultiMode Picoforce AFM with Nanoscope III controller (Digital Instruments, USA.) equipped with a closed loop scanner. The radii of the spheres were determined by optical imaging (Nikon Optiphot-100s) and they were between 6-10 μm in air. The cantilevers were calibrated using the AFM Tune It v 2.5 software (Force IT, Sweden), based on thermal noise with hydrodynamic damping. The lateral photodetector sensitivity was calibrated using a procedure of tilting the photodetector. The force measurements were performed at pH 4.5 and 7.3, with a ramp size of 1 μm at a rate of 2 μm/s on NFC surfaces. Friction was measured as a function of increasing and decreasing loads with a 2μm scan size and 1Hz scan rate.

### 3.2.6 Other techniques

*X-ray photoelectron microscopy (XPS)*

The surface chemical composition of the topmost ten nanometers of the modified NFC samples (Paper IV) was analyzed with XPS, using an AXIS 165 electron spectrometer (by Kratos Analytical) with monochromatic A1 Kα X-ray irradiation at 100W. Spectra were collected at three locations, using electron detection at 90° and from sample areas less than one mm in diameter. Elemental surface compositions were determined from low-resolution survey measurements (80 eV pass energy and 1 eV step); carbon surface chemistry was probed with high resolution regional scans (20 eV pass energy and 0.1 eV step); and for the analysis of trace amounts of nitrogen and sulphur, N 1s and S 2p regions were recorded using low resolution and long measurement times. The carbon C1s high-resolution spectra were curve fitted using parameters defined for cellulosic materials (Johansson and Campbell 2004), and all binding energies were referenced to the aliphatic carbon component of the C 1s signal at 285.0 eV (Beamson and Briggs 1992).

*Additional techniques*

The chemical analysis of the modified CMC and NFC samples (Paper IV) was made with an FTIR Bio-Rad FTS 6000 spectrometer (Cambridge, MA) with a MTEC 300 photoacoustic detector (Ames, IA) using a constant mirror velocity of 5 kHz, 8 cm-1 resolution and 1.2 kHz filter. The Mw of the polysaccharide samples (Paper I) was determined with either intrinsic viscosity measurements or size exclusion chromatography (SEC). Confocal microscopy was used to study the fluorescence of CD-modified nanopaper. The microscope used was a Leica TCS SP2 CLSM (Leica microsystems CMS GmbH, Manheim, Germany). The images (750 x 750 μm2) were obtained by using excitation and detection wavelengths of 488 nm and 500-530 nm, respectively. The intensity images (cross section and surface) were scanned using averaging mode and constant imaging conditions with laser powers of 700 and 650V during surface and cross-section imaging, respectively.

# 4. Results and discussion

The results of this thesis are divided into four parts. In the first part the physisorption results of different polysaccharides on NFC are presented. The second part deals with the adsorption of functionalized polysaccharides. The third part presents titrimetric methods for the determination of total and surface charges of CM-NFC. In the fourth part the NFC is modified in water either indirectly via modified polysaccharides or directly using EDC/NHS conjugation chemistry.

## 4.1 Physisorption of water-soluble polysaccharides on NFC

### 4.1.1 Polysaccharides

In order to use polysaccharide adsorption as a tool for NFC modification it is highly important to know what affects the adsorption of different polysaccharides on NFC. Therefore, different polysaccharides were chosen for comparison, i.e. with different backbones, degrees of substitution, Mw and charge, in order to see which properties affect the adsorption of polysaccharides on NFC at constant pH (pH=4.5), ionic strength (I=10 mM) and polysaccharide concentration (0.1 g/L).

#### *Comparison of the adsorption of different polysaccharides on NFC*

The adsorption of polysaccharides on NFC was studied using QCM-D because it gives the possibility to study the adsorption of polysaccharides (including water) onto NFC on a molecular level. Figure 14 presents a comparison of the adsorption of XG, GG, CMC and CHI on NFC.

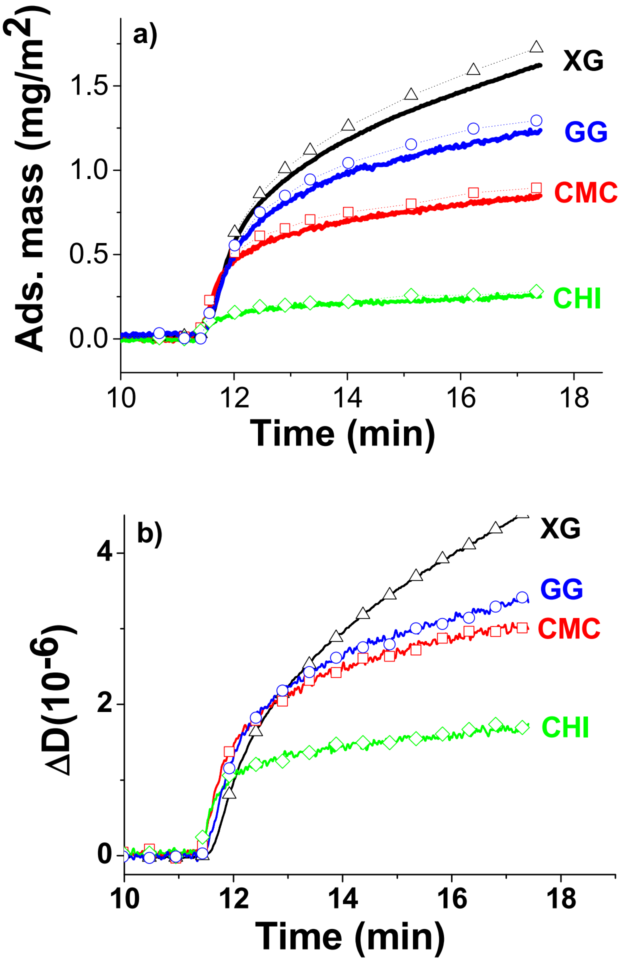


**Figure 14** The a) adsorbed mass and b) change in dissipation as a function of time for four different polysaccharides (XG, GG, CMC and CHI) on NFC (Paper I)

The adsorbed amounts ($mg/m^2$) were calculated by the Sauerbrey equation (Equation 2) (Figure 14a). The change in energy dissipation as a function of time of the corresponding measurements is shown in Figure 14b. The kinetics of the adsorption of these four different polysaccharides was compared within the first 8 min. It can be seen from the Figure 14 that XG has the fastest attachment rate and highest adsorbed amount in the initial stage of adsorption. GG and CMC show similar change in dissipation energy, but the adsorbed mass in comparison was higher for GG. Although the amount of CMC attachment was not as high compared to neutral polysaccharides, the anionic CMC adsorbed irreversibly at pH 4.5 on NFC. Adsorbed CMC was removed during rinsing when pH 8 was used in a previous study (Ahola et al. 2008a). The anionic charge of CMC explains both the slightly higher change in dissipation vs. change in mass values of

CMC adsorbed on NFC and the irreversible adsorption of CMC only at low pH conditions. CHI, which is cationic at pH 4.5, adsorbs on NFC, but the adsorbed mass is low compared to neutral or anionic polysaccharides. The change in dissipation as a function of the change in frequency is shown in Figure 15. These D-f-curves can be used in detecting differences in the adsorption behavior and layer structure of polysaccharides on NFC. Neutral, high Mw polysaccharides are shown in Figure 15a, and low Mw polysaccharides are shown in Figure 15b.

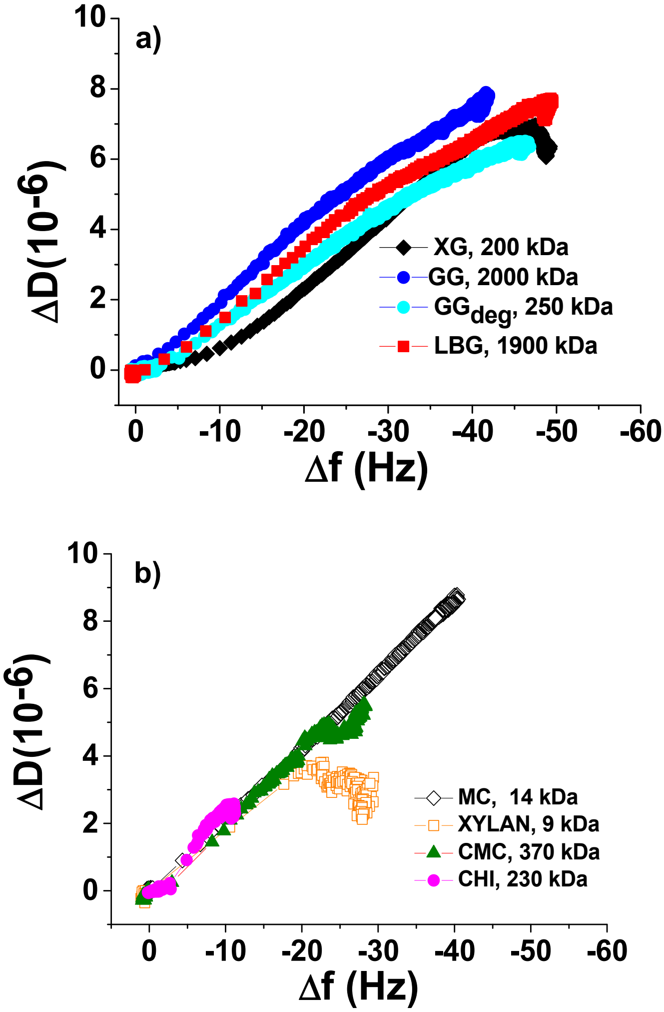


**Figure 15** The adsorption profiles (D-f – curves from QCM-D data) for a) neutral polysaccharides with high $M_w$ and b) polysaccharides with low $M_w$ (Paper I)

The adsorbed amounts of all the neutral polysaccharides on NFC were very similar. In case of galactomannans, a slightly higher amount of adsorption of LBG on NFC was observed compared to GG. This is most likely due to the higher mannose to galactose ratio of LBG compared to GG (Picout et al. 2002, Whitney et al. 1998). The persistence length and radius of gyration values for both galactomannans is 3-5 nm. For XG this value is very close to galactomannans (Picout et al. 2002, Picout 2003) and this can partly explain the similar amount of adsorption of neutral polysaccharides on NFC. GG has a dispersing effect on pulp fibres (Swanson 1950), and XG on NFC substrates (Ahola et al. 2008a), and this is essential in many applications, e.g, for paper strength. This can also be an advantage in using these neutral, readily available polysaccharides in the indirect modification of NFC. Figure 15b) presents the ΔD/Δf-curve for CMC, MC, CHI, and anionic xylan. CHI had the lowest amount of adsorption on NFC, although strong, non-electrostatic origin interactions between CHI and cellulose have been previously detected (Laleg and Pikulik 1991, Nordgren et al. 2009a).

*The effect of polysaccharide Mw on adsorption onto NFC*

The effect of Mw on amount of polysaccharide adsorption on NFC was investigated by decreasing the Mw of GG by mild acid hydrolysis. The GG D-f slope is slightly decreased when the Mw is decreased (Figure 15a). However, the adsorbed mass of the degraded GG was slightly higher compared to unmodified GG (Paper I). No marked difference due to the decrease in Mw could be observed based on the QCM-D data. This correlates well with previous studies on bleached softwood fibres (Hannuksela et al. 2002), where the polysaccharide structure (galactose to mannose ratio) had a more pronounced effect on the amount of adsorption than the Mw.

### 4.1.2 Polysaccharide multilayers

The next step was to study the multilayer build-up (Decher et al. 1992) of different polysaccharide systems on NFC in order to deepen the understanding of polysaccharide interactions with NFC and each other. The multilayer build-up of different polysaccharide systems in controlled solution conditions was monitored by using QCM-D. The polysaccharides chosen for comparison were CHI, HECE and CMC (DS 0.7, 250 kDa). Three different pHs (4.5, 7 and 8.5) and three ionic strengths (0.56 mM, 10 mM and 20 mM [$Na^+$]) were used. The polysaccharide concentration was kept constant throughout the measurements (0.1 g/L).

*The effect of pH and ionic strength on polysaccharide multilayer build-up*

The effect of pH, ionic strength and the properties of the polyelectrolytes were studied systematically using QCM-D. Figure 16 presents the calculated

(Equation 3) amount adsorbed (Figure 16a) and the change in dissipation (Figure 16b) for five polyelectrolyte layers on a NFC surface.

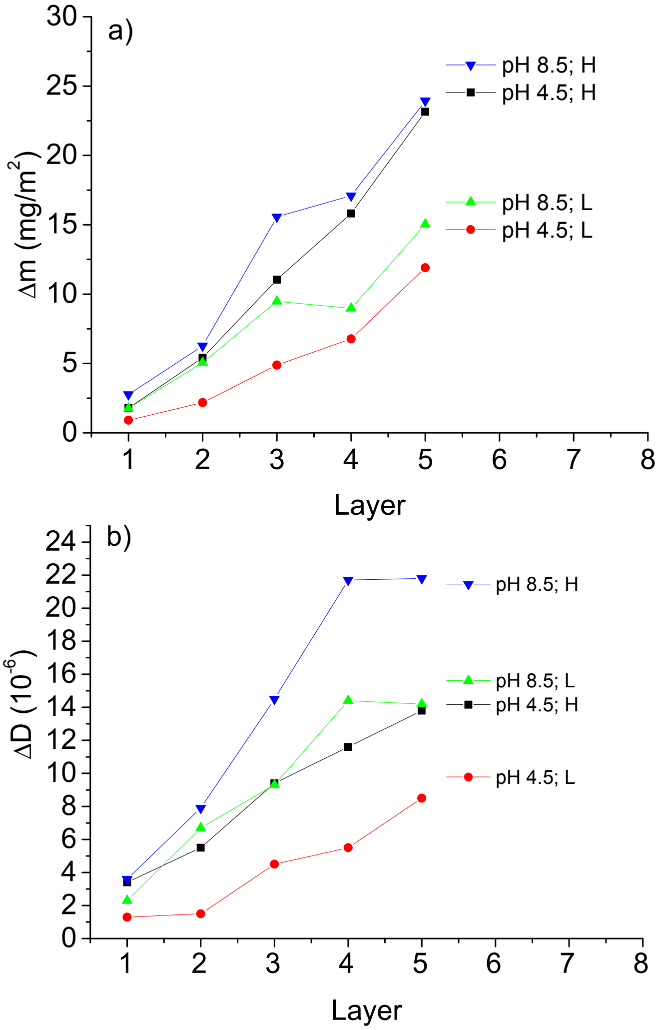
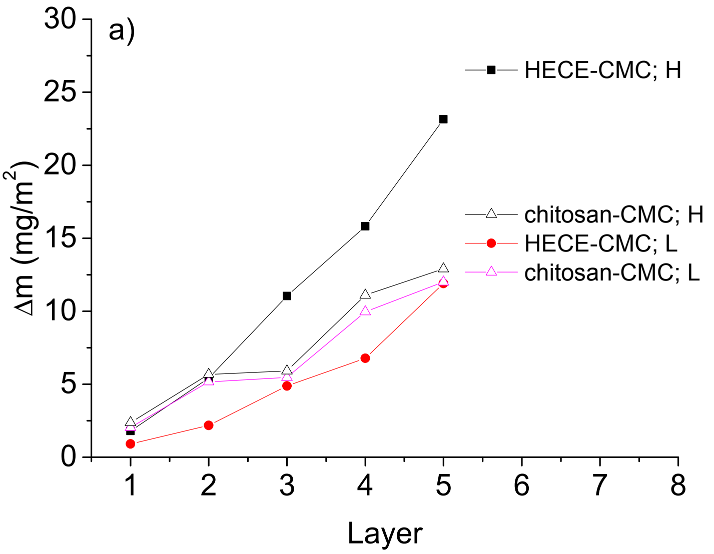


**Figure 16** The multilayer build-up of HECE and CMC (5 layers) on NFC model surface at two different ionic strengths (L: 0.56 mM and H: 20 mM) and pHs (4.5 and 8.5). The change in mass (a) and dissipation energy (b) of the system is presented as a function of the layer number (Paper II)

Both the adsorbed mass and dissipation clearly increase during the multilayer build-up (Figure 16a and 16b). A higher ionic strength (H) enhances the multilayer build-up both at high and low pH conditions (pH 8.5 and pH 4.5) which can be seen from Figure 16a. It is known that a coiled conformation of the polyelectrolytes in solution at higher electrolyte concentration result in more loops and tails when the polyelectrolyte is adsorbed on a surface in the case of polyelectrolyte multilayers (Antipov et al. 2003, Dubas and Schlenoff 1999). Also, the dissipation energy of the film (Figure 16b) increases with more layers. The system is clearly more dissipative at higher ionic strength and at higher pH, which indicates more swollen layers.

### *The effect of different cationic polysaccharides on multilayer build-up*

In order to investigate how the type of cationic polysaccharide affects the layer build-up, the experiments were also conducted with CHI as the cationic polyelectrolyte. CMC was adsorbed layer-by-layer with both CHI and HECE at both 20 mM and 0.56 mM ionic strength. Figure 17 shows the adsorbed mass and dissipation response for the CMC-CHI and CMC-HECE systems. This comparison between CHI and HECE were performed at pH 4.5 because CHI is only soluble and cationic at acidic conditions.



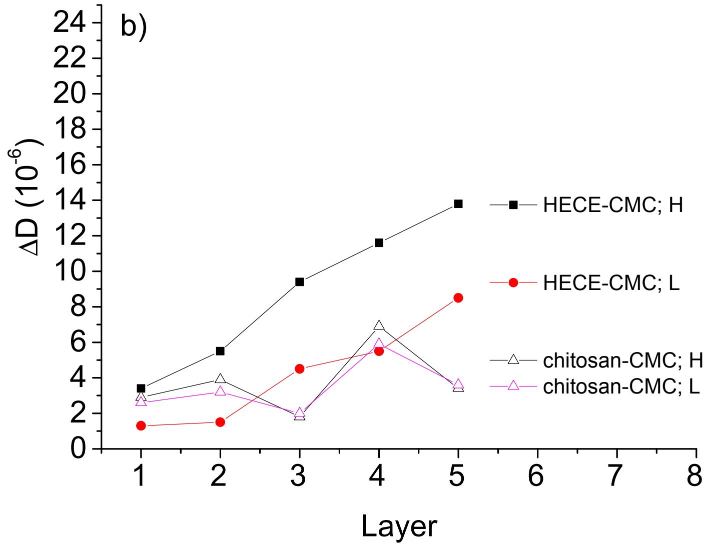


**Figure 17** The multilayer build-up of HECE and CMC and CHI and CMC (5 layers) on NFC model surfaces at two different ionic strengths (L: 0.56 mM and H: 20 mM) at pH 4.5. The change in mass (a) and dissipation energy (b) of the systems are presented as a function of the layer number (Paper II)

The effect of the cationic polysaccharide is marked based on the results in Figure 17. It seems that CHI (3rd and 5th layer) is able to densify the system and release bound water seen as decreased dissipation energy (Figure 17). Ionic strength did not influence the amount of adsorption in the CMC-CHI system. The effect of the DS (0.7 and 1.2) and $M_w$ (90 and 250 kDa) of CMC on the multilayer build-up of polysaccharides on cellulosic surfaces was studied with HECE as the cationic polysaccharide (see Paper II). The optimal conditions for CMC-HECE system on NFC was neutral pH, high ionic strength (20 mM) and low DS (0.7 for the CMC). Mw (90-250 kDa) did not have a marked effect on the CMC-HECE system multilayer build-up on NFC.

### *The effect of polysaccharide layers on NFC interaction forces*

The effect of polysaccharide layers (CMC-HECE, and CMC-CHI systems) on the interaction forces between cellulosic substrates were studied using CPM. The measurements were done at pH 4.5 and I=20 mM. For reference the forces between the bare cellulosic surfaces, the NFC film and cellulose sphere, were measured. In all, four polyelectrolyte layers were formed using a 0.1 g/L concentration and a 40 min adsorption time followed by rinsing with the buffer solution. The results from these CPM measurements are presented in Figures 18a and 18b.

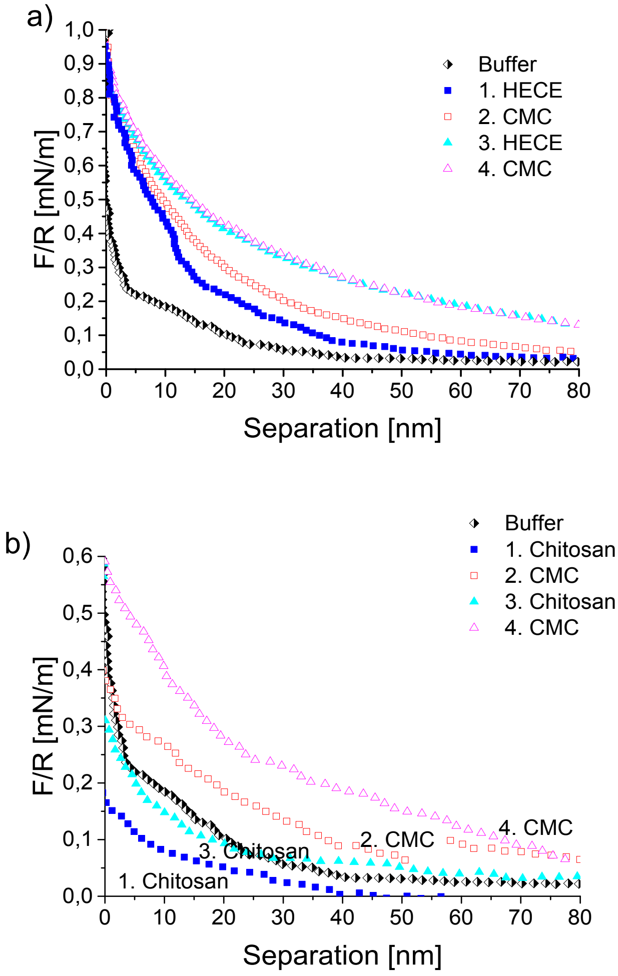


**Figure 18** The colloidal probe microscopy force curves (0-80 nm separation) for CMC-HECE (a) and CMC-CHI (b) system (Adapted from Paper II)

It can be clearly seen from Figures 18a and 18b that the forces between the two cellulose surfaces (NFC film and the cellulose sphere) in the buffer solution are repulsive. This is due to the electrostatic repulsion between the slightly negatively charged cellulosic surfaces and to the steric effect between surfaces (Holmberg et al. 1997). The following HECE and CMC (Figure 18a) layers substantially increased repulsion between the surfaces. Steric repulsion is the major cause for this increase in addition to electrostatic repulsion. In all, the CMC-HECE system was able to increase the range of repulsion between cellulosic surfaces at least in the case of three polysaccharide layers. CHI, on the other hand, decreased the range of repulsion between the cellulosic surfaces (Figure 18b). This could indicate that CHI adsorbs flat on the both surfaces and is able to densify the underlying layers. However, the CMC layers adsorbing on top of CHI increase the repulsion between the surfaces close to the same level as in the case of the CMC-HECE system. The maximum pull-off forces of the CPM measurements are presented in Table 4.

**Table 4** The effect of CMC-HECE and CMC-CHI layers on the pull-off force between the cellulose sphere and the surface at 20 mM ionic strength and pH 4.5 (Paper II)

| CHI-CMC system | Pull-off Force (mN/m) | HECE-CMC system | Pull-off Force (mN/m) |
|---|---|---|---|
| Cellulose-NFC | -0.08 ± 0.02 | Cellulose-NFC | -0.10 ± 0.05 |
| 1st CHI layer | -0.32 ± 0.11 | 1st HECE layer | -0.06 ± 0.03 |
| 2nd CMC layer | -0.08 ± 0.01 | 2nd CMC layer | -0.04 ± 0.03 |
| 3rd CHI layer | -0.11 ± 0.04 | 3rd HECE layer | -0.03 ± 0.01 |
| 4th CMC layer | -0.07 ± 0.01 | 4th CMC layer | -0.07 ± 0.02 |

The pull-off forces (Table 4) between two cellulosic surfaces are very low as has also been previously observed (Ahola et al. 2008b). The adsorption of cellulose derivatives, HECE and CMC, did not have an effect on the adhesion between the cellulosic surfaces. However, the adsorption of a CHI layer clearly increased the pull-off force between the cellulosic surfaces. The increase in pull-off force for the CHI-coated cellulosic surfaces is most probably due to the ability of CHI to decrease steric repulsion between the cellulosic surfaces. The second CHI layer has nearly the same pull-off force as the CMC layer, which could indicate overlapping of the layers. It can be concluded that the polysaccharide layers have a marked effect on the surface interaction of NFC systems.

## 4.2 Physisorption of functionalized polysaccharides on cellulose and NFC

### 4.2.1 Propargyl- and azido-CMC

The adsorption of the functionalized CMC samples on cellulose model surfaces (regenerated, amorphous cellulose thin film) was studied by using *in-situ* QCM-D monitoring. It was systematically studied how the functionalization type (either azido or propargyl), functionalization level (a, higher and b, lower), initial DS of CMC (CMC2: 1.2 and CMC1: 0.7) as well as the ionic strength (0 mM and 50 mM) affected CMC adsorption on cellulose. The results of the adsorption experiments for the unmodified (Figure 19a) and azido-functionalized CMC (Figure 19b) (0.2 g/L) on cellulose surfaces at two different ionic strengths can be seen in Figure 19.

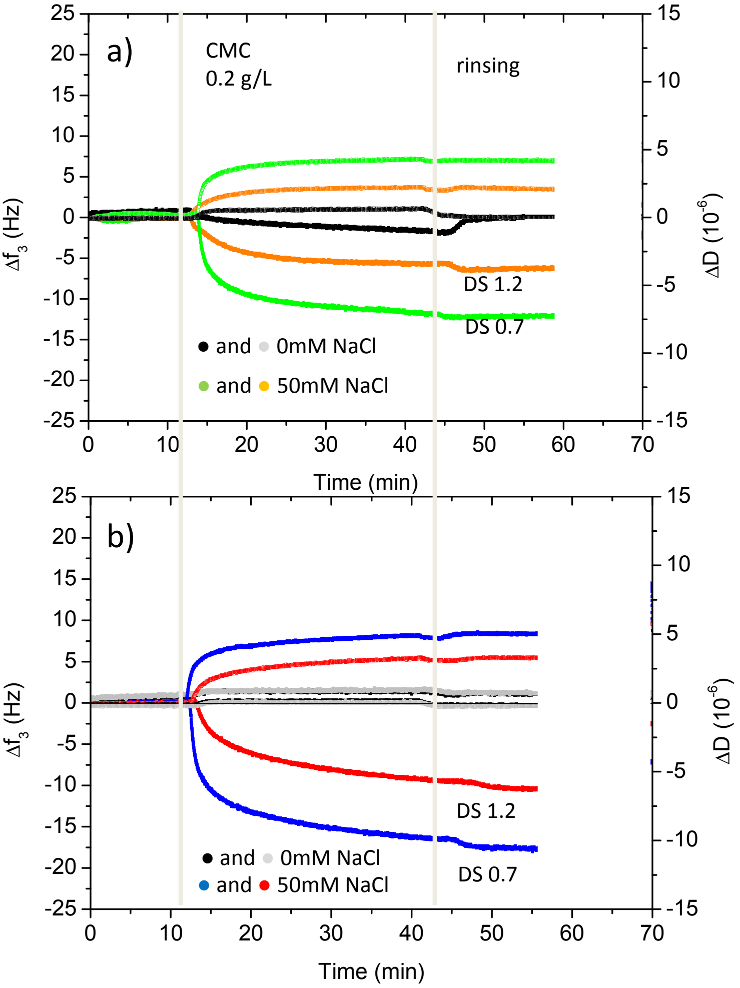


**Figure 19** The adsorption (charge in frequency and dissipation energy) of a) CMC1 (DS 0.7) and CMC2 (DS 1.2) and b) azido-CMC1a (DS 0.7) and azido-CMC2a (DS 1.2) on cellulose at two different ionic strengths (0 mM and 50 mM) (Adapted from Paper IV)

Azido-functionalized and unmodified CMCs adsorbed on the cellulose model surface in the presence of salt (I=50 mM) which is seen as a clear decrease in frequency (Figure 19). However, without an addition of salt, in this case NaCl, the CMC samples do not have affinity to cellulose. Electrostatic repulsion between CMC and cellulose can be screened by using a sufficient electrolyte concentration as has been previously observed (Laine et al. 2000, Liu et al. 2011b). It should be mentioned that the elemental analyses (see Paper IV) revealed the presence of unreacted carboxyl groups in the functionalized CMCs which explains the need to reduce the electrostatic repulsion between the functionalized CMCs and cellulose. However, the azido-and propargyl-functionalized CMCs have less charged groups due to the functionalization (azido or propargyl), which partially explains the slightly higher amount of adsorption compared to the unmodified CMCs at 50 mM ionic strength. No difference in the adsorption behavior of the propargyl-functionalized CMC (Supporting information in Paper IV) and the azido-functionalized CMC samples on cellulose could be detected. It is clear base on the QCM-D data that the azido- or propargyl-functionalization of CMC does not have a major influence on the interaction between CMC and cellulose (Table 5). However, the initial DS of the CMC does have an effect on the adsorption of functionalized CMC on cellulose: the higher the initial DS (CMC2 had an initial DS of 1.2 and CMC1 DS 0.7) the lower the amount adsorbed. The unsubstituted regions of the CMC backbone are able to interact and attach irreversibly on cellulose despite of the functionalization, since the functionalization affects only the regions that are already substituted with carboxymethyl groups.

**Table 5** The adsorbed amounts of different functionalized CMC-samples at 50 mM ionic strength on cellulose calculated with Equation 2 (Paper IV)

| Sample name | $-\Delta f_3$ (Hz) in 50 mM NaCl | $\Delta m$ (mg/m$^2$) |
|---|---|---|
| **CMC1: DS 0.7** | 13 ± 4 | |
| Propargyl-CMC1a | 19 ± 4 | 3.4 ± 0.7 |
| Propargyl-CMC1b | 16 ± 3 | 2.9 ± 0.5 |
| Azido-CMC1a | 18 ± 4 | 3.1 ± 0.7 |
| Azido-CMC1b | 19 ± 4 | 3.3 ± 0.7 |
| **CMC2: DS 1.2** | 7 ± 3 | |
| Propargyl-CMC2a | 6 ± 3 | 1.0 ± 0.5 |
| Propargyl-CMC2b | 10 ± 3 | 1.9 ± 0.5 |
| Azido-CMC2a | 10 ± 3 | 1.9 ± 0.5 |
| Azido-CMC2b | 12 ± 3 | 2.1 ± 0.5 |

The indirectly functionalized (azido or propargyl) pure cellulose model surfaces were then subjected to CuAAC reaction with different clickable molecules. This reaction was done *in-situ* using QCM-D with clickable counterparts (OMe-PEG azide and BSA-alkyne). As can be observed in Figure 20, the reference trials (Figure 20, REF) in the absence of copper(I) catalyst did not show attachment of counterparts. It should be noted here that it is important to choose a counterpart that does not have any other type of affinity to the cellulose surface. The click-reaction was found to take place on the azido- and propargyl-functionalized surfaces, and the counterpart was not removed even after extensive washing (Figure 20).

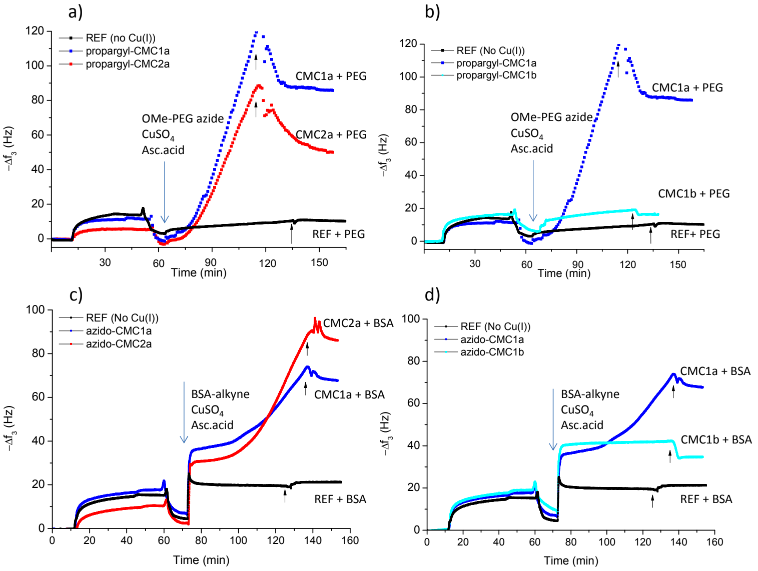


**Figure 20** Change in frequency as a function of time indicating the adsorption behavior of functionalized CMC on cellulose, and the sequential click-reaction with two different counterpart molecules. The effect of the amount of adsorption of propargyl functionalized CMC on the click-reaction with OMe-PEG-azide is shown (a) and the effect of the level of functionalization is displayed (b). The effect of the amount of adsorption of azido-modified CMC on the click-reaction with BSA-alkyne (c) and the effect of the level of functionalization (d) are shown. The starting point of the water rinsing in each system is indicated with a black arrow (Paper IV)

The highest amount of attachment in case of OMe-PEG-azide was observed when the cellulose surface was pre-treated with CMC1a (DS 0.7, high functionalization) (Figure 20a). The higher initial adsorption of CMC1a when compared to that of CMC2a clearly resulted in a higher amount of available alkyne groups on the surface. Interestingly, the attached amount of BSA-alkyne was slightly higher for cellulose pre-treated with CMC2a (initial DS 1.2) when compared to CMC1a (initial DS 0.7). The cellulose surface pre-treated with CMC2a is likely to contain lower amounts of available azide-groups due to the lower initial adsorbed amount of CMC2a when compared to that of CMC1a. However, the conformation of CMC2a on the cellulose surface may be more extended outwards from the surface which could promote the formation of interlocked structures in case of BSA-alkyne attachment.

The level of CMC functionalization (a and b) seems to have a clear effect on the counterpart attachment as can be observed in Figure 20b and 20d. In case of more functionalized CMCs (CMC1a and CMC2a) the subsequent click-reaction on the cellulose surface does not seem to level off before the water-rinsing. The films were in addition analyzed with AFM and XPS to confirm that the attachment of counterparts took place (Supporting information, Paper IV). The results point out that the attached amount of counterparts depends on the initial DS of CMC as well as the amount of propargyl- or azido-functional groups present on the surface.

### 4.2.2 CMC-g-PEG

CMC was modified by grafting with polyethylene glycol (PEG) (see Experimental section). The grafting was done so that the PEG was grafted only to the already substituted regions in the CMC backbone in order to maintain the natural affinity of low DS CMC to adsorb onto NFC in the presence of salt. In addition, the grafting density was kept low (~12%) in order to rule out major steric hindrance that would cause changes in the affinity of CMC to adsorb on NFC. The *in-situ* adsorption experiment was performed with QCM-D (Figure 21). The solution of CMC or CMC-g-PEG at pH 4.5 was injected in QCM-D. CMC and CMC-g-PEG were adsorbed at pH 4.5, 50 mM ionic strength and 0.1 g/L concentration. Figure 21a shows the change in frequency as a function of time and Figure 21b presents the change in dissipation vs. adsorbed mass.

Both CMC and CMC-g-PEG attached irreversibly onto NFC at these conditions (Figure 21a). Adsorption of CMC was slightly higher compared to CMC-g-PEG. Nevertheless, the adsorption kinetics (Figure 21a and 21b) and the change in dissipation was very similar for both samples.

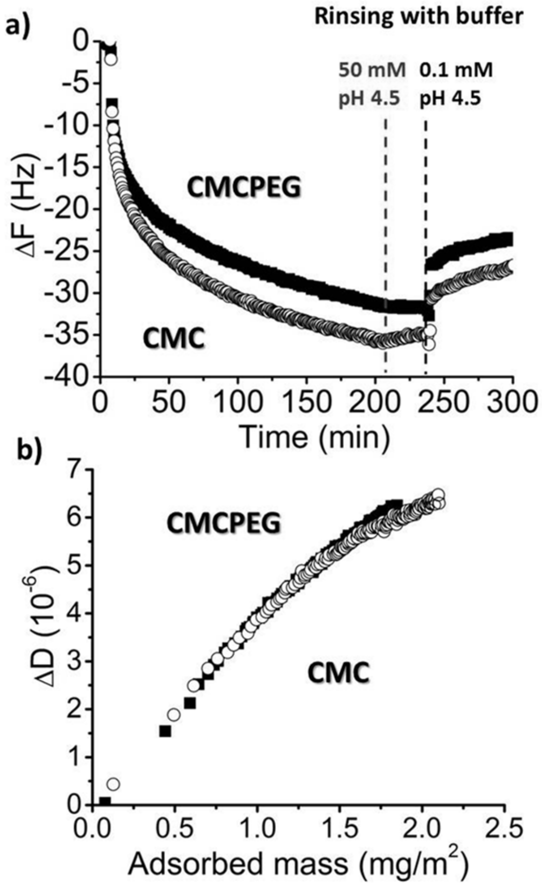


**Figure 21** The adsorption (a) of CMC and CMC-g-PEG on NFC, and b) the adsorption profile (D-m-curve) for the same systems (Paper III)

The slightly lower adsorbed mass of CMC-g-PEG onto NFC can be due to steric hindrance between the PEG grafted chains and NFC. For both systems the change in dissipation energy after the adsorption was approximately $\Delta D=6\cdot10^{-6}$. The increase in dissipation suggests that the layer formed is well hydrated, soft and viscous. In addition, the adsorption of CMC or CMC-g-PEG does not affect the morphology of a dried NFC film (see Paper III) and the RMS roughness was 4.2 and 4.3 nm for CMC and CMC-g-PEG respectively, when the roughness of an unmodified NFC film is approximately 4 nm.

## 4.3 Characterization of the charge of CM-NFC

The effects of the extent of carboxymethylation as well as propargyl-functionalization (see Experimental section) on the charge of NFC were systematically studied by means of polyelectrolyte and conductometric titrations. A procedure for the determination of both surface and total charge of the CM-NFC by titrimetric methods was developed (Paper V).

The conductometric titration of CM-NFC was done using a back titration method with hydrochloric acid (HCl). Within this method the time-consuming conversion of NFC from its sodium form to its H-form can be avoided. Figure 22 shows the amount of carboxyl groups in the samples as a function of sample mass. In order to determine the amount of NFC required for the consistent titration results, the observed amount of carboxyl groups (µmol) were plotted against the amount of titrated sample (g). The results for the three NFCs (titrated using HCl) and CM-pulps (titrated using NaOH) are shown in Figure 22.

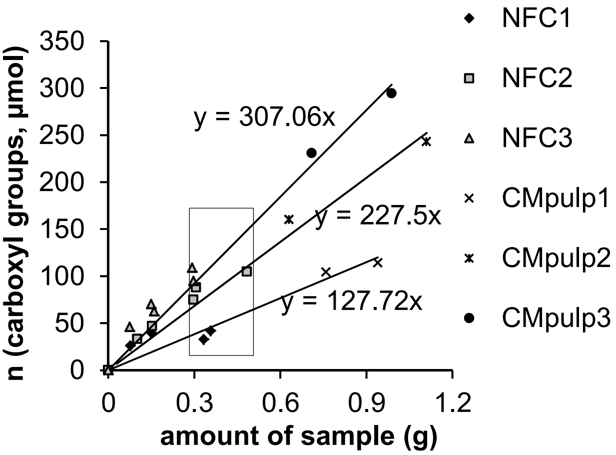


**Figure 22** The amount of carboxyl groups of the CM-pulps 1-3 and the CM-NFC samples (1-3), determined with conductometric titration as a function of the amount of sample. The NFCs were titrated with 0.1M HCl and CM-pulps with 0.1M NaOH. The slope shows the charge density of the samples (Paper V)

The conductometric titrations were performed with different NFC dosages (0.3-0.5 g to 500 mL). Based on the results (Figure 22) the disintegration of cellulosic fibres to corresponding fibrils does not change the total charge of the material.

The next step was to determine the surface charge of NFC samples by polyelectrolyte titration (see Experimental section). Figure 23 shows surface charge densities for all the three different CM-NFC samples as a function of ionic strength.

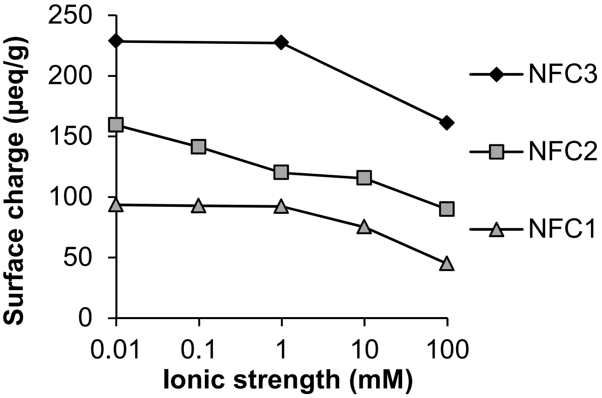


**Figure 23** The effect of ionic strength on the cationic demand of NFC1, NFC2 and NFC3. The NFC dispersions were titrated with PDADMAC at different ionic strengths (from 0.01mM to 100mM NaHCO3) at pH 8.5. The lines between the data points are a guide for the eye (Paper V)

As expected, an increase in ionic strength decreased the observed surface charge values of NFCs because of the changed charge stoichiometry (Figure 23). The higher the electrolyte concentration, the lower is the amount of polyelectrolyte adsorption due to the weaker electrostatic interactions. However, the polyelectrolyte titrations using the 0.01 mM $NaHCO_3$ buffer resulted in reasonable surface charge values which correlate to a fibrillation efficiency of 70-80 %.

## 4.4 The effect of the modifications on NFC properties

The final goal of this work was to modify NFC via polysaccharide adsorption or directly with EDC/NHS conjugation chemistry in aqueous media. The effects were demonstrated by studying the influence of polysaccharide adsorption on the NFC surface adhesion properties, by studying the effect of CMC-g-PEG on NFC friction properties, and by bulk modifications of NFC in aqueous media.

### 4.4.1 The effect of polysaccharide adsorption on NFC

To probe the influence of the polysaccharide layer on the properties of the NFC film, the AFM in DPFM-mode (see Experimental section) was used to investigate relative changes in the stiffness and adhesion properties due to polysaccharide adsorption (in this case LBG) on NFC films. The results are shown in Figure 24. The topographic images and stiffness values of the films are very similar but the adhesion between silicon nitride tip and film surface was clearly lower after LBG adsorption on the NFC film. The adsorption of a polysaccharide layer had a clear effect on the NFC film properties (Adhesion, Figure 24).

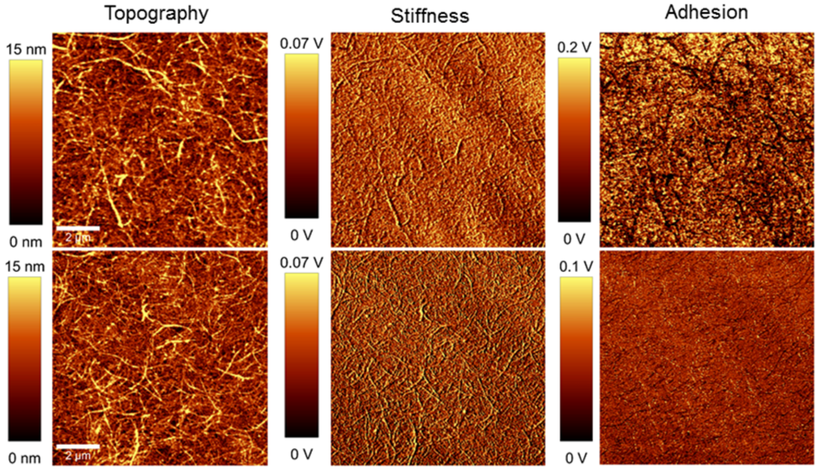


**Figure 24** The effect of the LBG layer (lower images) on the NFC surface topography, stiffness and adhesion based on DPFM measurements (Paper I)

### 4.4.2 The effect of CMC-g-PEG attachment on the friction of NFC

The effect of CMC-g-PEG adsorption on the NFC interaction forces and friction was studied. This was done because it is known that, when two surfaces bearing brush-like polymer chains slide past each other under compression in a good solvent, the inter-chain repulsion arising from increased osmotic pressure, together with the formation of cushion-like layer at the interface, leads to a substantial reduction in frictional force (Huang et al. 2001, Kampf et al. 2004, Theander et al. 2005). Figure 25a presents the lateral friction as a function of applied load between a cellulose sphere and a spin coated NFC film in 0.1 mM buffer solution at both at pH 4.5 and 7.3 in the presence and absence of CMC-g-PEG.

When the cellulose probe is moved into contact with the NFC film (Figure 25a, curves 1 and 2), as soon as contact is reached adhesive contact is made and the friction force jumps and the friction ($F_{Friction}$) starts to increase almost linearly with increasing load ($F_{Load}$). The effect is more pronounced at pH 7.3 (Figure 25, line 2). The reason for this pH dependence is the stronger adhesion at pH 7.3 (Supporting information, Paper III), since adhesion can be said to act as an additional load.

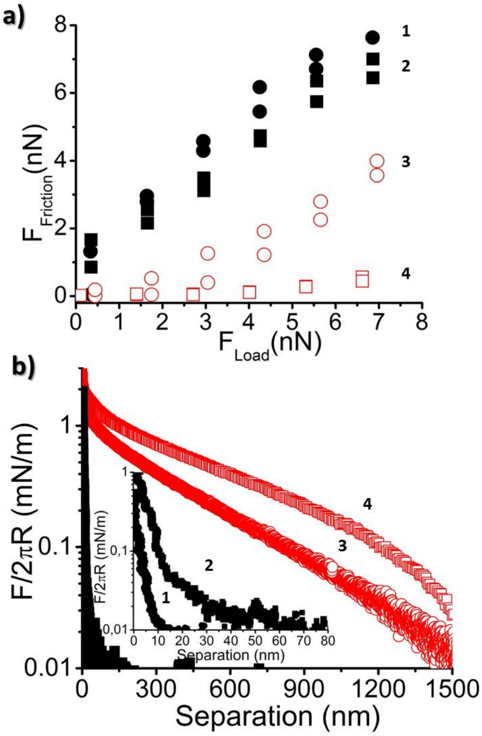


**Figure 25** a) Lateral friction as a function of load between NFC film and cellulose sphere before (1, 2) and after adsorption of CMC-g-PEG (3, 4). Measurements were conducted in aqueous 0.1 mM electrolyte solution at pH 4.5 (circles) and at pH 7.3 (squares) b) The corresponding force normalized to the radius of the cellulose sphere upon approach on a semilogarithmic scale. Inset shows the normal forces between NFC film and cellulose sphere at pH 4.5 (1) and pH 7.3 (2) (Paper III)

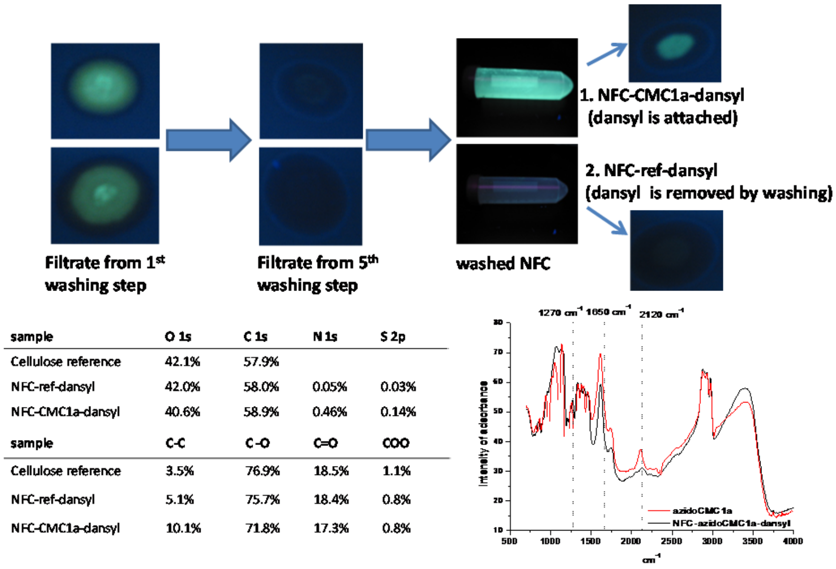
In all studied cases when both loading and unloading the frictional behaviour followed modified version of Amontons' law (Equation 1), which takes into account that adhesion acts as an effective load.

Only a slight change in the friction coefficient is observed for NFC at different pHs, $\mu$=1.00 ± 0.05 and $\mu$= 0.84 ± 0.08 were measured at pH 4.5 and 7.3, respectively. The calculated friction coefficients for unmodified NFC film correlate well with values previously observed for various cellulose surfaces (Dedinaite et al. 2010, Nordgren et al. 2009a, Nordgren et al. 2009b). The effect of CMC-g-PEG on the friction between the NFC film and cellulose sphere is strongly pH dependent. A drastic reduction of friction is observed at pH 7.3 (Figure 25a open squares) but only moderate change in friction is noted at the lower pH (Figure 25a open spheres).

Corresponding normal forces are presented in Figure 25b. The addition of CMC-g-PEG clearly increases the repulsion between the NFC film and the cellulose sphere at both pH conditions (Figure 25b). The increased steric repulsion is most likely the main reason for the significant reduction of the frictional forces in the presence of adsorbed CMC-g-PEG. For comparison the frictional response of an unmodified CMC layer adsorbed on NFC film was studied at both pH values. The total reduction in friction for the system where CMC was adsorbed was 13% and 22% when pH 4.5 and 7.3 were used, compared to the CMC-g-PEG system where the reduction was much higher, 65% and 88% for the respective pHs.

### 4.4.3 Modification of NFC in bulk conditions via azido-CMC using click-chemistry

After demonstrating the use of polysaccharide adsorption and subsequent click-reaction on smooth cellulose model surfaces (chapter 4.2.1) the focus was set on the bulk modifications of NFC substrate. First, azido-functionalized CMC was adsorbed onto cellulosic fibres prior to the disintegration of the pulp by microfluidization as described in the experimental section. Next, a fluorescent probe (dansyl-alkyne) was attached to the CMC-modified NFC by using CuAAC reaction. As can be seen in Figure 26, the fluorescence was detected only after the CuAAC reaction between the azido-CMC modified NFC and the dansyl-alkyne. In the case of all reference tests (see Experimental section), fluorescence tests resulted in negative response, i.e., the dansyl-alkyne was removed by washing. This indicates that dansyl-alkyne did not have any other type of affinity with NFC.



| sample | O 1s | C 1s | N 1s | S 2p |
|---|---|---|---|---|
| Cellulose reference | 42.1% | 57.9% | | |
| NFC-ref-dansyl | 42.0% | 58.0% | 0.05% | 0.03% |
| NFC-CMC1a-dansyl | 40.6% | 58.9% | 0.46% | 0.14% |

| sample | C-C | C-O | C=O | COO |
|---|---|---|---|---|
| Cellulose reference | 3.5% | 76.9% | 18.5% | 1.1% |
| NFC-ref-dansyl | 5.1% | 75.7% | 18.4% | 0.8% |
| NFC-CMC1a-dansyl | 10.1% | 71.8% | 17.3% | 0.8% |

**Figure 26** Fluorescent labeling of NFC. The modified NFC was characterized using UV-light (λ=254 nm), XPS and FTIR (red line is the azido-CMC1a and black line is NFC-CMC1a-dansyl). Filtrates from 1st washing step and the last washing step (5th) were characterized using UV-light. The NFC-ref-dansyl stands for the reference test where no copper(I) catalyst was used during the reaction. Characteristic bands for amides (1270 and 1650 cm$^{-1}$) and azide (2120 cm$^{-1}$) are shown as dotted lines in the FTIR spectrum (Paper IV)

The FTIR results (Figure 26) show that characteristic azide stretching band at 2120 1 $cm^{-1}$ has substantially decreased after the reaction between azido-modified NFC and dansyl-alkyne. This indicates that the CuAAC reaction took place between the azido-CMC-NFC and dansyl-alkyne. In addition, XPS revealed changes in the elemental composition of the NFC surface (Supporting information, Paper IV). While the carbon and oxygen signals remained similar in all samples, slight increases in sulfur and nitrogen contents of the NFC sample treated with dansyl-alkyne in the presence of copper(I) (in trace analysis, 0.14 at% and 0.46 at%, respectively) were observed. It should be mentioned here that the reference NFC (reaction with dansyl-alkyne without copper(I)) was not found to contain nitrogen or sulfur which indicates that the physically bound dansyl-alkyne was removed during extensive washing.

### 4.4.4 Direct modification of NFC in water by EDC/NHS conjugation

Direct modification of CM-NFC was done using EDC/NHS conjugation chemistry. CM-NFC was propargyl-functionalized using EDC/NHS conjugation chemistry (see Experimental section). The results for the total and surface charges of propargyl-functionalized CM-NFC and CM-pulp3 as a function of the propargylamine dosage are shown in Figure 27.

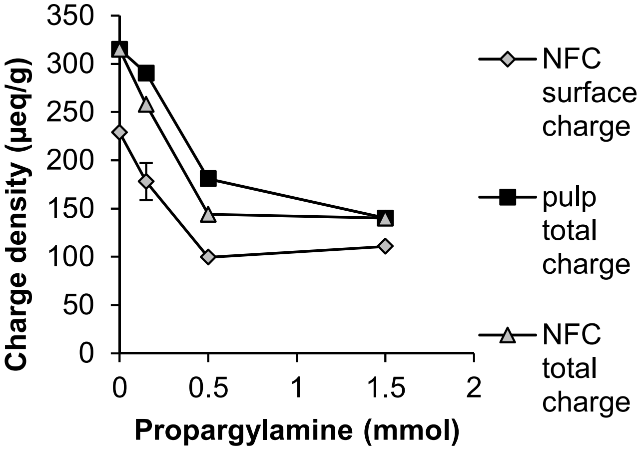


**Figure 27** Total and surface charges (at pH 8.5 and 0.1mM ionic strength) of NFC and CM-pulp as a function of the propargylamine dosage. The lines between data points are a guide for the eye (Paper V)

It can be clearly observed from Figure 27 that the total charges of propargyl-functionalized CM-NFC and CM-pulp decreased with the increased propargylamine dosages until reaching the plateau levels after which the total charge remained constant. Neither the surface nor total charge of modified CM-NFC and the CM-pulp reached zero charge densities which is indicative for the incomplete functionalization reactions. In fact, the degree of propargyl-functionalization in both the CM-NFC and CM-pulp with the highest propargyl-amine dosage was found to be approximately 60 % of the available carboxyl groups. It should be noted here that each anhydroglucose unit can theoretically contain 1, 2 or 3 carboxymethyl groups in which the propargylamine can react after the EDC/NHS activation. Therefore, it is logical to assume that steric hindrance is more pronounced with the more substituted (2 or 3 carboxymethyl groups) anhydroglucose units leading to the lowered reaction efficiencies.

In addition, elemental analysis was used as a complementary technique for the charge titration results. Figure 28 shows that the DS (carboxymethyl) determined using conductometric titration linearly decreases when the amount of nitrogen increases in the propargyl-modified NFC sample.

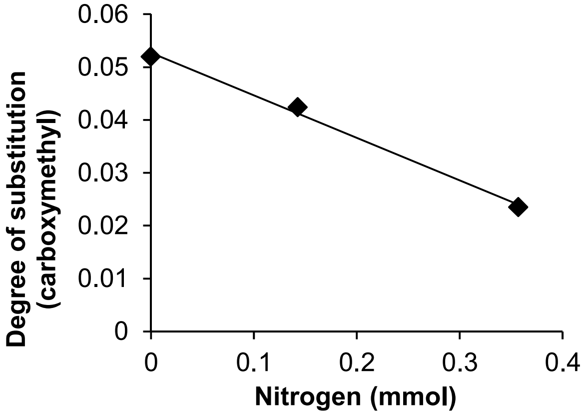


**Figure 28** The DS (carboxymethyl) of propargyl-functionalized NFCs based on conductometric titration results as a function of the amount of nitrogen (amide bonds) based on elemental analysis (Paper V)

The demonstration for the direct modification of CM-NFC was done also with luminescent CDs (Paper VI). The attachment of CDs on spin-coated CM-NFC surface was monitored *in-situ* with SPR and QCM-D (Figure 29) at pH 4.5, 10 mM ionic strength and 0.1 g/L concentration.

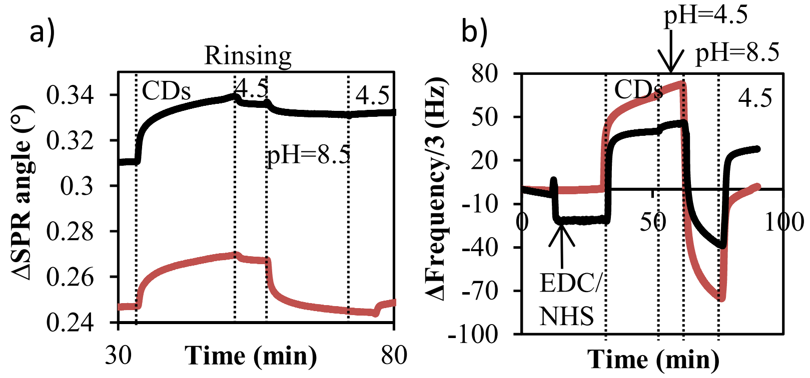


**Figure 29** Attachment of CDs (pH 4.5; 10 mM ionic strength) on EDC/NHS activated (black line) and non-activated (red line) CM-NFC films as a function of time measured with a) SPR and b) QCM-D. The ionic strength was kept constant (10 mM) throughout the measurements (Adapted from Paper VI)

As expected, the SPR angle increases (adsorbed mass increases) when CDs are introduced onto the CM-NFC surface at pH 4.5 (Figure 29a). This can be explained by the electrostatic interactions between the negatively charged CM-NFC surface and the cationic CDs. However, when the pH is increased to 8.5, the CDs become deprotonated (neutral), which decreases the electrostatic interactions between the CDs and CM-NFC. This leads to a removal of CDs from the CM-NFC surface which can be observed as a decrease in the SPR angle (Figure 29a, red profile). However, the EDC/NHS activation of carboxyl groups of the CM-NFC film in prior to CD injection produced more stable binding of CDs (Figure 29a, black profile) and they remained on the surface even after rinsing at pH 8.5.

The introduction of CDs at pH 4.5 clearly increased the resonance frequency of the CM-NFC coated sensor (QCM-D data, Figure 29b). This indicates that CDs can displace bound water from the CM-NFC film upon attachment. However, a substantial swelling of the film is observed (decrease in the frequency and an increase in the dissipation energy (data not shown)) when the film is rinsed with pH 8.5 buffer. This is likely due to the dissociation of carboxyl groups in the CM-NFC film. Furthermore, no change in total mass of the film (compared to the starting condition) was observed after changing back to the pH 4.5 buffer. This clearly indicates that the CDs were removed from the CM-CNF surface during the high pH rinsing, in a similar manner as was observed in SPR experiments.

However, the attachment of CDs onto EDC/NHS-activated CM-NFC film seems to result in permanent removal of bound water from the film seen as an increase in the frequency (compared to the starting condition) after the final pH 4.5 buffer rinsing. This suggests that the CDs were covalently attached onto the activated CM-NFC film. Moreover, the activated film swelled less at high pH which is likely due to the activation of the carboxyl groups and the attachment of CDs on the surface. The films were imaged with AFM before and after the QCM-D measurements and the results are presented in Figure 30.

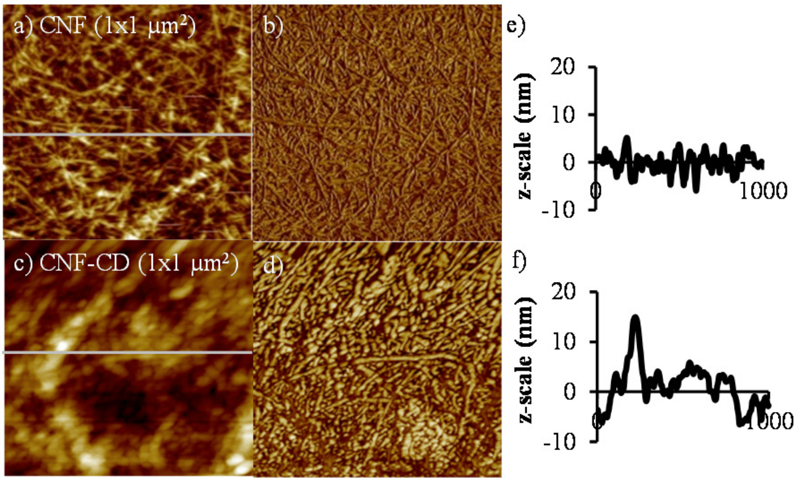


**Figure 30** CM-NFC imaged with an AFM (1x1 μm²) before (a, height and b, phase image) and after the CD attachment (c, height and d, phase image). Graph e) is the z-scale (nm) of the surface prior to the attachment of CDs and graph f) is the z-scale (nm) of the surface after the attachment of CDs (Paper VI)

The morphology of the CM-NFC film clearly changed due to the irreversible attachment of CDs (Figure 30). In addition, it seems that the attachment of CDs onto the surface of CM-NFC film may prevent the aggregation of the CDs at their dry state (no evidence of aggregated CDs on the film surfaces).

The next step was to modify the CM-NFC in bulk conditions (see Experimental section). Nanopaper was made of the CD-modified NFC gel and the CM-NFC. The filtration time during nanopaper manufacture was decreased by 20% as a result of CD modification. Confocal microscope and digital camera images of the films are presented in Figure 31.

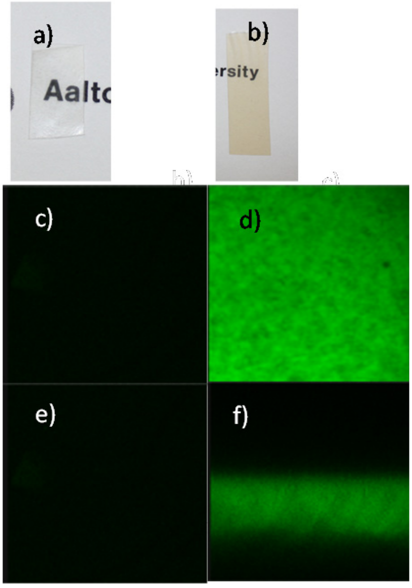


**Figure 31** Digital camera images of a) a CM-NFC film and b) a NFC-CD film. The confocal microscopy images were taken from the surface (c and d) and the cross-section (e and f). Images c) and e) are the CM-NFC film, d) and f) are the NFC-CD film images (Adapted from Paper VI)

CM-NFC nanopaper was found to be translucent and it did not demonstrate fluorescence (see confocal microscopy and digital camera images in Figure 31a, 31c and 31e). Nanopaper was also prepared from the modified NFC-CD gel. Also this film was found to be translucent while a slight brownish color was observed (Figure 31b). Moreover, fluorescence was detected both from the surface and the cross-section of the film (Figure 31d and 31f) which indicates that the CDs were evenly distributed in the nanopaper matrix.

# 5. Concluding remarks

In order to widen the application potential of NFC there have to be facile routes for NFC modification, without losing its unique properties. NFC has a tendency for surface passivation and aggregation in any other solvent than water. Therefore, modifications of NFC were done in aqueous media in this thesis. It was shown that NFC can be modified both indirectly via polysaccharide adsorption and directly by chemical modification in aqueous media.

Prior to being able to use natural polysaccharide adsorption as a tool for NFC modification, the factors which affect the interaction between polysaccharides and NFC have to be clear. In this thesis a systematic study of polysaccharide adsorption on NFC was done as a basis for using polysaccharide adsorption as an indirect route for NFC modification. The quartz crystal microbalance with dissipation monitoring and other surface sensitive methods were utilized in this work to get molecular level information of polysaccharide physisorption onto NFC substrates. The adsorption of polysaccharides on cellulose seems to be driven by the co-crystallization tendency of linear, cellulose-like polysaccharides to cellulose. Thus it is important that the functionalized polysaccharides are water-soluble, and that the DS is optimized so that the polysaccharides can adsorb irreversibly onto cellulose.

It was demonstrated that polysaccharides can be used for modifying the surface interaction, adhesion and friction of NFC. In addition, polysaccharide adsorption can be used for indirect functionalization of NFC to build a NFC template for further chemical click-modification in aqueous media with virtually any counterpart molecule. In addition, a characterization technique for the charge properties of NFC by titrations was developed. In this thesis the titration methods developed were utilized in the surface and total charge characterization of modified and unmodified NFC grades. NFC properties, in this case fluorescence, were adjusted in a molecular level by modification of NFC in aqueous media both indirectly by using azido-CMC adsorption followed by click-chemistry, and directly by using luminescent carbon dot attachment by the EDC/NHS assisted coupling reaction. The development of NFC modification methods shows promise for novel value-added applications of NFC, which need molecular scale modification of NFC material without drastic changes in its unique properties.

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Aalto-DD 30/2014

ISBN 978-952-60-5595-4
ISBN 978-952-60-5596-1 (pdf)
ISSN-L 1799-4934
ISSN 1799-4934
ISSN 1799-4942 (pdf)

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