

Refined cellulose derivatives for high-value biomedical applications (ReCell)

FINAL REPORT

Title of the research project	Refined cellulose derivatives for high-value biomedical applications
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Coordinator of the project	Monica Ek (Sigbritt Karlsson until 2010 06 30)
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BASIC PROJECT DATA

Project period	01.01.2008-31.03.2011 (Sweden) 01.01.2008-30.11.2010 (Finland)
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Other funding:

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ALS Scandinavia, Sweden	75 000 €
Kemira Oyj, Finland	0 €

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ABSTRACT

Soluble derivatives of cellulose are non-toxic and renewable polymers of increasing importance and use, e.g. in the pharmaceutical, material and consumer-oriented industries. The goal has been to on one hand to advance the basic knowledge on structure-property relationships from pulp production to cellulose derivative applications. On the other hand it is to improve existing products and to create new cellulose-based products for high-value applications. This has been in accordance with the goals specified by the European Forest-based Sector Technology Platform in the Strategic Research Agenda (RA1-3) and by the Swedish Forest Industry in the National Research Agenda (NS-6).

The approach has been to improve and/or to design new cellulose derivatives with well-defined properties suitable for pharmaceutical-, health care, food, building and other high-value applications. This goal has been to achieve assessment of important manufacturing parameters, e.g. optimisation of cellulose reactivity and controlled synthesis. A systematic approach using commercial substances of relevance to the industrial partners and model substances (produced by small scale cellulose preparation and modification) has been employed. Steam explosion, biochemical pre-treatments and dissolution of cellulose in ionic liquids for increase of cellulose accessibility have been studied in order to optimise cellulose reactivity. The research has also been focused on alternative synthesis paths for manufacturing new cellulose derivatives. The aim has been to enable for cost-effective and environmental friendly processes.

Chemical structure elucidation has been a central role in the proposed project and structure analysis of cellulose derivatives consisted of three interplaying parts: application of enzymatic, chemical, and modern analytical techniques. Endoglucanases, a type of cellulose degrading enzymes, have been applied for sample pre-treatment (depolymerisation). The objective was to find new tools for studying the substituent distribution in cellulose derivatives. To obtain quantitative structure information, chemical derivatisation methods have been developed, both for the fragments obtained in the enzymatic depolymerisation, and for the undegraded cellulose derivatives. State-of-the-art chromatographic and spectroscopic techniques, e.g. mass spectrometry and nuclear magnetic resonance spectroscopy, have been studied to assess the complex chemical structure of cellulose derivatives.

The overall aim of the chemical characterisation research has been to explain correlations between the chemical structure and the technologically important physicochemical properties of cellulose derivatives. Improved analytical methods for product and production control were of high priority for producers and end-users of cellulose derivatives.

Optimisation of target products result in improved technological properties, e.g. the possibility to manufacture well-defined biomedical formulations and other high-value chemical products.

1.1 Introduction

1.1.1 Background

Soluble derivatives of cellulose are non-toxic and renewable polymers of increasing importance and use, e.g. in the pharmaceutical, material and consumer-oriented industries. The goal is on one hand to advance the basic knowledge on structure-property relationships from pulp production to cellulose derivative applications. On the other hand it is to improve existing products and to create new cellulose-based products for high-value applications

The aim of the WP1 was to develop improved modification procedures for cellulose fibre materials in order to make them readily applicable and accessible to chemical reactions for various products, including cellulose derivatives. The raw materials include wood-based fibres from various sources, e.g. softwood dissolving pulps and various kraft pulps. The modification methods are primarily based on the action of specific enzymes on structurally modified pulps and suitable solvents, i.e ionic liquids (ILs), possibly combined with mechanical or other processing of pulps. Cellulose derivatives with targeted properties can be developed and synthesized by graft copolymerization.

The chemical structure of cellulose derivatives is very complex. Molecular dispersity is found at several levels. One example of molecular dispersity is the molecular mass distribution (MMD). Beside the influence of the natural origin, the whole production chain, from processing of pulp to production of the final product, will also greatly affect the MMD. Another and until now less studied type of molecular dispersity is the substituent distribution heterogeneity, which can be described at different hierarchical levels: the regioselectivity within the glucosyl unit, the distribution of the substituents along the cellulose polymer, and between different polymer molecules. Therefore, for structure elucidation, analysis must be performed on intact cellulose derivatives as well as partially and/or completely depolymerised. To differentiate between the different levels of substituent distribution, fractionation with respect to chemical composition need to be performed with consecutive chemical analysis. A combination of different strategies, use of enzymes as selective tools, appropriate chemical sample preparation, and use of sophisticated analytical techniques must be employed for the complex structure analysis. On one hand better understanding is required of physicochemical properties (dissolution behaviour, temperature dependent gelation, and flocculation), which are commonly determined in industry.

Electrospinning is a method for producing nanofibrous nonwovens from a wide variety of polymers. The high surface-to-volume ratio of electrospun nonwovens, their similarity to native extracellular matrix and the unlimited possibilities of functionalization makes them useful in many biomedical applications, from wound care to pharmacology and tissue engineering. In WP6, the technological properties of the cellulose derivatives developed within the project were to be evaluated by means of electrospinning. One important challenge in electrospinning cellulose derivatives is finding optimal solvent systems, to give the polymer solutions adequate viscoelastic properties and allow for sufficient solvent evaporation. Many research groups have investigated inclusion of a carrier polymer in the spinning solution, however when it comes to electrospinning pure cellulose derivatives there is an obvious lack of knowledge and research. For application purposes nanofibers of cellulose derivatives in their pure form would be beneficial and will therefore be investigated in this project.

1.1.2 Objectives

- To advance the basic knowledge on structure-property relationships from pulp production to cellulose derivative applications in order to enable for development of improved and new products for high-value applications.
- To transfer this knowledge to improve processes for production of industrially important cellulose derivatives.
- To use new controlled free radical polymerisation reactions on pulp celluloses to develop new cellulose derivatives.
- To develop a comprehensive methodology for chemical structure elucidation of cellulose derivatives enabling correlation of technological properties to chemical structure.
- To apply the improved and new cellulose derivatives into high-value biomedical products.

1.2 Results and discussion

WP 1 – Cellulose accessibility (VTT, KTH, Helsinki University)

Dissolving grade pulps were activated by various treatments, aiming at improving their solubility and thus applicability as starting material for cellulose derivatives. The activating treatments tested were steam explosion (SE) and enzymatic treatment with endoglucanases, and combinations of the two. Both commercial and experimental enzymes were studied. Steam explosion reduced pulp viscosity dramatically, but the following enzyme treatment did not affect it much further. The degradation by the enzymes was heterogeneous, leaving behind both intact high molecular cellulose and degraded material, whereas steam explosion led to low and monodisperse molar mass distributions. The effect of the enzymes on the solid fiber matrix was followed by the DNS method directly from the suspension. The combination of steam explosion followed by endoglucanase treatment led to high content of fiber-bound reducing sugars but low content of sugars in the filtrate. This is interpreted as high fiber activation with concomittant low yield loss due to dissolution. The selectivity was best with the pure experimental enzymes, but the tested commercial enzymes were potential as well.

Steam explosion and/or enzymatic treatments

Dissolving grade pulps from Domsjö were activated by steam explosion or endoglucanases alone or in combination. Both commercial and experimental enzymes were studied. The effects of the treatments were followed by intrinsic viscosity and molar mass measurements. Steam explosion reduced pulp viscosity dramatically, but the following enzyme treatment did not affect it much further. Without preceding steam explosion, the enzymes alone reduced viscosity, but to a lesser extent than steam explosion. Size exclusion chromatography showed that the degradation by the enzymes was heterogeneous, leaving behind both intact high molecular cellulose and degraded material, whereas steam explosion led to less broad molar mass distributions at the low molecular region (Figure 1).

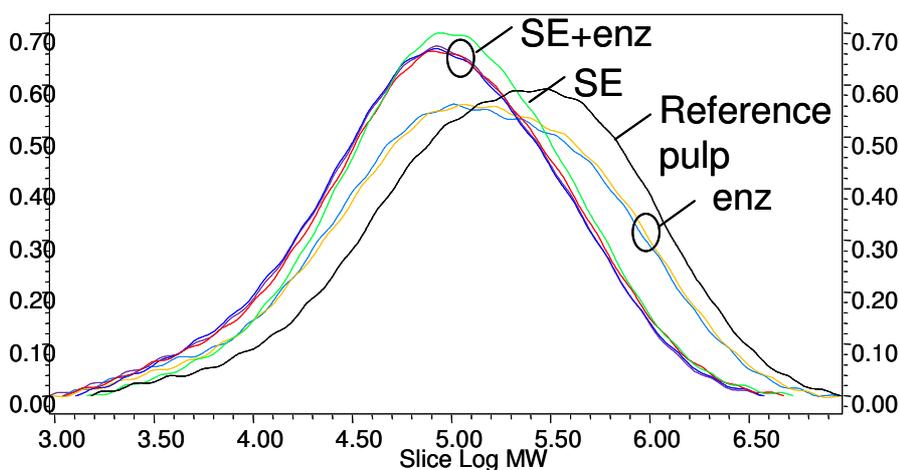


Figure 1. SEC curves of pulps treated with Steam explosion (SE), endoglucanases (enz) or both (SE+enz).

The DNS analysis of the filtrates indicated that SE pretreatment had a detrimental effect on the the following enzyme treatment. However, this negative effect was not detected when the analysis was performed directly from the sample slurry (Figure 2). In fact, this means that the SE lowered the yield loss in the form of dissolved sugars, still allowing the activating effect on the solid fiber matrix. As expected, the yield loss was found to be lowest with the pure experimental enzyme.

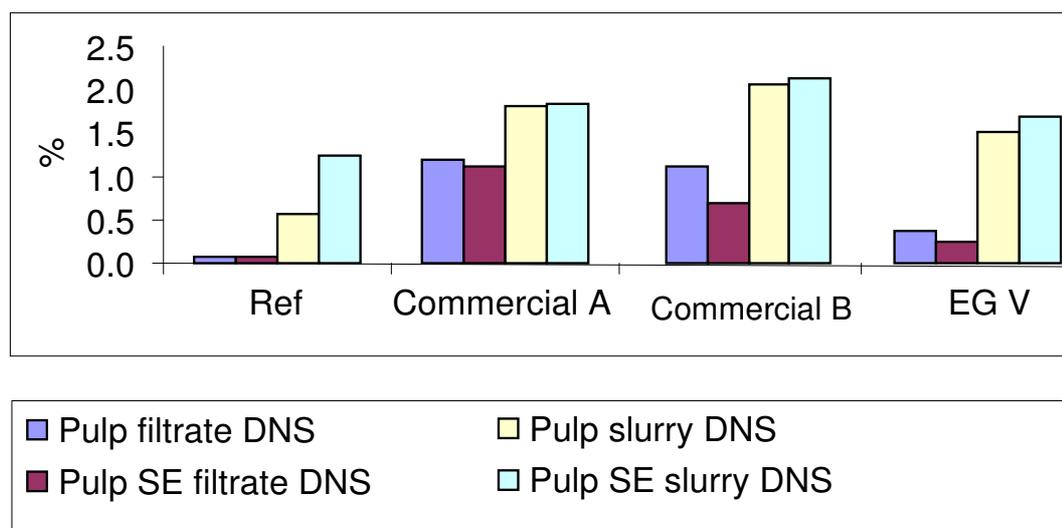


Figure 2. Effect of enzyme treatments on pulp with or without SE pretreatment, measured as formation of reducing end groups by the DNS method. The measurement was done either from the filtrate or from the whole pulp slurry.

Two series of pretreated Domsjö softwood bale pulp with viscosities of 460 and 560, totally 12 pulp samples, are got from VTT. Morphology and thermal properties are studied at University of Helsinki using ¹³C CP-MAS NMR, DSC and TGA. All samples are dissolved into NaOH/urea/water and DMAc/LiCl and the level of solubility is evaluated visually and using optical microscopy. Cellulose solutions are studied by light scattering methods and results compared to the viscosity and SEC results got from DOW and VTT. It is possible to conclude that steam explosion and endoglucanase activation affects fiber dissolution in different ways. Pretreatment procedures remarkably improve cellulose solubility. Steam explosion decreases molar masses more than enzyme treatment. Broad molar mass and hydrodynamic size distributions are obtained for all samples.

Cellulose dissolution in ionic liquids (KTH)

The utilization of cellulose or cellulosic materials has not been developed entirely because of its poor solubility in common organic solvents. Ionic liquids (ILs) are relatively new family of solvents for dissolution of cellulose. They are organic salts with low melting temperature, contain only cations and anions which make them suitable for the solubilization of cellulose. Moreover, ILs are non-volatile, non-toxic, non-flammable and thermally and chemically stable. Cellulose dissolved in ILs can be regenerated with anti-solvents as water, ethanol and acetone.

In this study, both the dissolution and regeneration of cellulose with and from ILs were investigated. Furthermore, the impact of treating cellulose with ILs was also evaluated by using different analytical techniques, such as size exclusion chromatography (SEC), thermal gravimetric analysis (TGA) and scanning electron microscopy (SEM).

WP 2 - Designed properties by graft copolymerization (Helsinki University)

In WP 2 new cellulose derivatives with targeted properties have been developed and synthesized by graft copolymerization. Starting material has been Domsjö softwood bale pulp (V450). Controlled/living radical polymerization (LRP) is used to copolymerize cellulose-graft-polyacrylamides and more precisely the mechanism for polymerization is proposed to be SET-LRP, single electron transfer – living radical polymerization.

Four cellulose-macroinitiators (V450-BiB) for LRP with different numbers of initiating sites along the cellulose backbone (i.e., with different degrees of substitution (DS)) were successfully synthesized by direct acylation of cellulose with 2-bromoisobutyryl bromide in homogeneous LiCl/DMAc solution. Cellulose-macroinitiator synthesis have been shown to be repeatable. These macroinitiators have been characterised by ATR-FTIR, ^1H NMR and ^{13}C NMR. Further macroinitiators have been characterised by elemental analyses (E.A.) at Technical University of Braunschweig. DSs have been calculated from Br content from E.A. and from ^1H NMR and also from ^{13}C NMR when possible. Dynamic light scattering (DLS) studies revealed that cellulose-macroinitiator molecules in DMSO exist primarily as individual chains with small amounts of intermolecular aggregates. Macroinitiators have also been sent to Lund University for enzymatic hydrolyses.

LRP of acrylamide (Aam) and N,N-dimethylacrylamide (DMAam) with the prepared cellulose-macroinitiators were carried out in DMSO solution with different reagent ratios yielding cellulose-*g*-PAam and cellulose-*g*-PDMAam with different substitution level and chain lengths of grafts.

Formation of cellulose-*g*-copolymers was confirmed by ATR-FTIR and ^1H and ^{13}C NMR, and the products were found to be water-soluble. The thermal properties of cellulose-*g*-copolymers were studied by DSC and TGA and solution properties by SEC, DLS and rheological measurements.

DLS study of aqueous solutions of cellulose-*g*-copolymers reveals that when DS is sufficiently high and side chains are long enough, interchain association is suppressed and intermolecular aggregation is diminished.

WP 3 Analytical techniques for solid-state and chemical characterisation of cellulose derivatives (KTH)

The chemical composition of the monomer residues in cellulose ethers greatly influences the technological properties. Consequently, there is a need for new versatile methods by which the chemical structure and composition can be analyzed. Workpackage 3 has focused on the

development of liquid chromatography (LC) coupled with UV and mass spectrometric (MS) detection for chemical analysis of cellulose ethers.

Commercial samples of carboxymethyl cellulose (CMC), methyl cellulose (MC) and hydroxypropylmethyl cellulose (HPMC) were subjected to total acid hydrolysis by trifluoroacetic acid (TFA). The monomers thus obtained were chemically labelled with a chromophoric group to allow for UV detection and quantification of the chemical composition. As no standard compounds are commercially available for quantification this challenge can be overcome by transforming the analyte constituents into compounds with similar (ideally equal) response factors in UV detection.

Some of the most common types of LC stationary phases, i.e. C₈, C₁₈, graphitized carbon and diol-based hydrophilic, were screened in order to find the optimum separation conditions. Figure 1 shows chromatograms of HPMC as measured by LC/MS (Fig. 1a) and LC/UV (Fig. 1b).

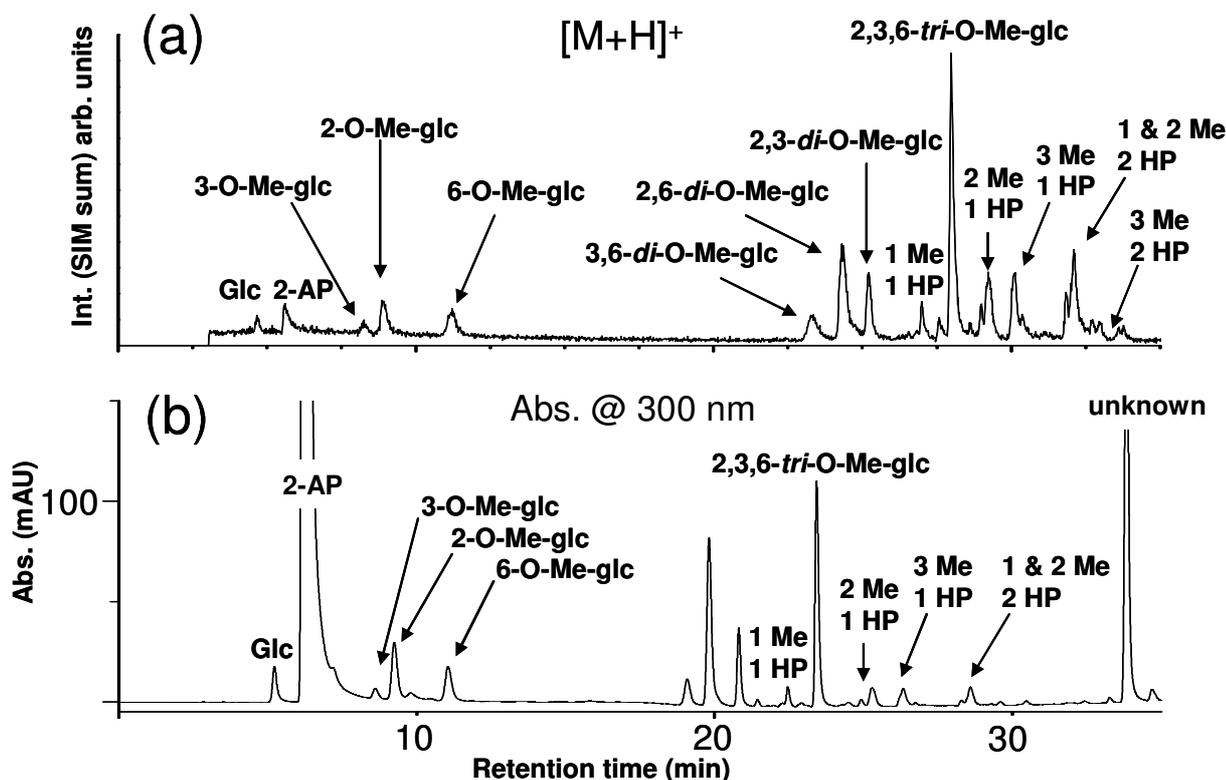


Figure 1. Separation of monomers obtained by TFA hydrolysis of HPMC. The monomers were subjected to reductive amination by 2-aminopyridine (a-AP). Separation was achieved on a Waters Symmetry C₈ column with 0.1 % TFA/methanol as mobile phase. Identification of the analyte constituents was performed by LC/MS/MS.

As can be seen in Figure 1 the different monomeric constituents of HPMC could be separated from one another and from the excess reagent (2-AP). Quantification of the chemical composition from the LC/UV chromatogram (Fig. 1b) corresponded well to the data supplied by the manufacturer. However, further investigation by independent methods will be performed in order to validate the method.

WP 4 Chemical structure analysis of cellulose derivatives (TU Braunschweig/Dow Wolff, Germany)

The detailed analysis of substituent's distributions of cellulose ethers is a puzzling task, dealing with very complex mixtures characterized by unknown dispersity in chemistry and size. In this work package all structural levels were addressed. On the level of the glucosyl monomers ("monomer analysis"), an existing method was optimized with respect to accuracy and time required and thus compatible with the requirements of routine analysis in an industrial lab.

On the next hierarchical level, the substituent distribution in the polymer chain ("oligomer analysis") the range of evaluation could be extended from DP2-5 to DP2-10 by introducing a label and LC coupling of ESI-MS. By this improvement, presently applied to methyl celluloses, significantly more data are obtained which is the basis for the development of advanced models. To allow reliable quantitative analysis of the more complex hydroxyalkylmethyl celluloses, fundamental studies with respect to quantitative mass spectrometry, including the study of various labeling procedures, have been performed and are now applied to these class of compounds.

All steps involved in sample preparation have been studied with respect to discrimination, e.g. randomness of partial hydrolysis, which is a prerequisite of quantitative evaluation.

With respect to the third level, the distribution over the bulk material, fractionation studies have been performed including various strategies like Soxhlet extraction, stepwise precipitation and chemical modification prior to separation.

ESI-MS has been applied in Braunschweig, while MALDI-MS was employed during a 4 month research stay of Kristin Voiges, PhD student in the project, at the partner institute at KTH Stockholm.

Further cooperation was performed with the partner group of Prof. Maunu at the University of Helsinki, Finland. Grafted cellulose derivatives were characterized with respect to the DS of macro-initiator and later with regard to the acrylate side chain distribution.

Further grafted PAam and PDMAam chains were isolated from cellulose backbone by hydrolyzing with HCl. Molecular weights and molecular weight distributions (PDI) of grafted PAam and PDMAam chains have been characterized by ¹H NMR and SEC. PDI of grafted PAam and PDMAam chains were found to be quite narrow from 1,06 to 1,46 determined by SEC. Samples of cellulose-*g*-PDMAam copolymers have been sent to Lund University for enzymatic hydrolyses and to Technical University of Braunschweig for acid hydrolyses and ESI-MS and MALDI-TOF-MS analyses.

WP5 "Enzymes as tools for chemical characterization of cellulose derivatives" (Lund University)

In this workpackage enzymatic tools for characterisation of cellulose derivatives were developed. For this we have applied enzymatic hydrolysis using several pure endoglucanases, and analysed the fragments with matrix-assisted time-of-flight mass spectrometry (MALDI-TOF-MS) (contribution to WP3). In addition, we have developed methods for the determination of rate and end-point degree of enzymatic hydrolysis of cellulose-derivatives.

We have previously shown that the degree of enzymatic hydrolysis of cellulose derivatives reflect the distribution pattern of substituents, which in turn affect the polymer properties; a heterogenous distribution pattern yield higher degree of hydrolysis and the stretches unsubstituted regions in heterogenous polymers is expected to show associative behavior.

To advance enzymes as general tools for characterisation of cellulose derivatives we have in this project analysed rate of enzymatic hydrolysis, in addition to degree of enzymatic hydrolysis and characterisation of hydrolysis products. Furthermore, in addition to the unspecific analysis of reducing ends during or after hydrolysis, we have advanced methods for determination of release of unsubstituted glucose. Here we developed, and evaluated, a new rapid and convenient glucose biosensor approach with carbohydrate HPLC (HPAEC, high performance anion-exchange chromatography). The used biosensor is based on the use of un-immobilised enzymes as recognition element; in our case enzymatic oxidation of glucose using glucose oxidase and electrochemical monitoring via an electrode. The following substrates were used for enzymatic hydrolysis: methyl-cellulose (MC), carboxy-methyl-cellulose (CMC), unsubstituted cellulose (Avicel) and, produced within the project, modified cellulose (macroinitator) and ready co-polymer (partner Helsinki university, WP1, WP2). Selective hydrolysis of three MC preparations with different degree of substitution (DS) using three pure endoglucanases (Cel5A, Cel7B, and Cel12A), and subsequent analysis using MALDI-TOF showed that two of the enzymes (Cel7B and Cel12A) could distinguish between the three preparations but Cel5A could not. The resolution of MALDI-TOF makes it possible to calculate the DS for each degree of polymerization (DP) of the enzymatically produced fragments. These results point in the direction that MC preparation 1 differs from the other two preparations, correlated with functional analysis.

We selected Cel5A and Cel7B, for further analysis of enzymatic hydrolysis using CMC (DS 0.7) as a substrate when comparing quantification of released glucose by the biosensor and HPLC (HPAEC). When using the single component endoglucanases we got some discrepancy of the results (approx. 25-30% higher values for HPLC). The correlation between biosensor and HPLC was significantly improved when adding β -glucosidase to the enzyme incubations, explained by the fact that β -glucosidase converts cellooligosaccharides to glucose, which is the analyte.

When performing enzymatic hydrolysis using a mixture of cellulases (endoglucanases, cellobiohydrolases) supplemented with β -glucosidase we got a very good agreement between biosensor and HPLC (approx. 1-2% higher values for biosensor), showing that the biosensor can be used as a fast alternative to HPLC when analysing rate and degree of hydrolysis in characterisation of cellulose derivatives.

The initial rate of CMC hydrolysis is 11-fold higher than for Avicel, but the final degree of hydrolysis 7-fold lower, or 8% glucose released. The profile indicates heterogenous distribution of the CMC substituents.

Derivatised cellulose (macroinitiator) from partner Helsinki University (synthesised in WP1, WP2) with DS 0.3 were enzymatically degraded to a degree of approx. 20%. As for CMC hydrolysis, the initial rate of the glucose release from the macroinitiator is higher than for Avicel, indicating that the substituents contribute to initial enzyme accessibility. The glucose release profile indicates heterogenous distribution of the macroinitiator substituents. Macroinitiators with DS 2 or higher were not enzymatically degraded. Also the ready made copolymer made from DS 0.27 macroinitiator were degraded by a mixture of cellulases to a limited extent (about 2% degradation), showing that the copolymer is not inert against enzyme degradation.

In conclusion, enzymes (cellulases) are valuable tools for characterisation of cellulose derivatives where the quantification of hydrolysis products are crucial. In addition to previous approaches with detailed characterisation of hydrolysis products and general reducing ends assays (for analysis of degree of hydrolysis), also quantification of glucose released by hydrolysis give additional information. Furthermore, glucose-specific enzyme-based biosensors are promising tools in this respect

WP 6 - Technological properties of improved and new cellulose derivatives (Swerea IVF)

The fiber spinning properties of the new cellulose derivatives (WP 2) were investigated by Swerea IVF by means of electrospinning method. Unfortunately fiber formation was not successful.

Nanoparticles were prepared successfully at Aalto University (Janne Raula) from water solutions of cellulose-*g*-PAam and cellulose-*g*-PDMAam copolymers by aerosol flow reactor method.

1.3 Conclusions

Steam explosion decreases cellulose molar mass. Endoglucanases after steam explosion had no further effect. Endoglucanases as such also decreased molar mass, but less than steam explosion. SEC measurements grouped the samples according to pretreatment based on the shape of the curve and molar mass as starting pulps and those pretreated by SE, SE+enzyme treatment and enzyme treatment only.

Endoglucanase treatment was more selective in terms of fiber activation versus carbohydrate dissolution, when performed after steam explosion compared to untreated pulp. The most selective enzyme tested was the experimental EGV. Minor difference was detected between the two commercial enzymes tested.

Varying reaction time and/or reagent ratios soluble cellulose-macroinitiators with different DS for copolymerization were obtained. New water-soluble cellulose-*g*-PAam and cellulose-*g*-PDMAam copolymers were prepared in homogeneous reaction medium using SET-LRP. PDI of grafted PAam and PDMAam chains were found to be quite narrow from 1,06 to 1,46 determined by SEC.

Improvement of quantifiability and accuracy of analyses in the field of substituent analysis of cellulose ethers by enhancing fundamental knowledge and implementing new approaches.

Enzymes (cellulases) have shown to be valuable tools for characterisation of cellulose derivatives. A new enzyme-based biosensor is a promising tool for quantification of glucose released by enzymatic hydrolysis.

In WP6 new understanding has been obtained of the electrospinning of pure carboxymethyl cellulose and the effect of added heat, salt and ethanol on fiber formation. Also, the importance of side chain length and solvent choice in the electrospinning of cellulose graft copolymers has been investigated and will be of importance in future utilization of these polymers in electrospinning.

1.4a Capabilities generated by the project

Several analytical methods were developed and tested for the evaluation of the effects of the pretreatments on pulp. This knowledge will be valuable in future research in this field. Also the activating pretreatments may be applicable for practical purposes in cellulose derivative industry

Comprehensive fundamental knowledge was gained during the project concerning all steps of analysis involved. This has not all been published yet but will be used as a valuable basis for further research in this field and hopefully be of long-term fruitfulness. New approaches have been tested, and partly been discarded and partly been selected for further promising studies.

Examples for this are Soxhlet extraction for fractionation, various labeling strategies including reductive amination, hydrazone formation, choice of tags and reducing agent, application of LC-coupling in the oligomer analysis, ultrasound treatment prior to partial degradation to oligomers to enhance accessibility and improve randomness, and others.

The industrial partner identifies this project as key during the development of cellulose ethers with tailor-made physico-chemical properties. The complex analytical approach is a fundamental part to understand structure-property relationships. A significant acceleration of new product development is expected due to the simplification of the analytical procedures.

Since the published research on electrospinning pure carboxymethyl cellulose (CMC) is very limited, although the polymer is widely used in many different areas of application, the knowledge generated within this project will be very useful in future work on producing nanofibrous nonwovens of CMC. Also, an increased understanding of the importance of side chain length and choice of solvent in the formation of electrospun nanofibers from cellulose copolymers has been obtained. Further research is needed to successfully produce nanofibrous nonwovens of cellulose derivatives, however such materials have great potential within many biomedical applications

1.4b Utilisation of results

The results will be further discussed with the industrial partners of the consortium in order to define the practical potential of the developed pretreatment techniques

The research work done under ReCell project has opened a way to tailor new cellulose derivatives by graft-copolymerization. In here novel living radical polymerization methods have been used to modify cellulose in homogeneous media in a way that product properties can be tailored and controlled.

A method to determine the substituent distribution of cellulose ethers was optimized and validated at TU Braunschweig and was implemented as a routine method at Dow Wolff.

Another method, concerning the determination of the substitution pattern of hydroxyalkyl celluloses on various structural levels is still under investigation and planned to implement at the partner company.

Electrospun nonwovens of cellulose derivatives have many possible application. Further research is needed to successfully produce such materials for industrial applications, however the obtained results and identified challenges in this project will be of importance in future electrospinning of cellulose derivatives.

1.5 Publications and communication

a) Scientific publications

1. Articles in international scientific journals with peer review

Enebro, J., Dane Momcilovic, Matti Siika-Aho, Sigbritt Karlsson, Liquid chromatography combined with mass spectrometry for the investigation of endoglucanase selectivity on carboxymethyl cellulose, *Carbohydrate Research*, 344, 2173-2181, 2009

Hiltunen M, Maunu SL (2010) Synthesis of Water Soluble Cellulose-g-Copolymers in Homogeneous Media Using Single-Electron Transfer Living Radical Polymerization, submitted to *Polym. Int.*

Hiltunen M, Riihelä S, Maunu SL, (2009) New Associative EHEC-g-PAam Copolymers: Their Syntheses, Characterisation and Rheological Behaviour, *J. Polym. Sci., Part B: Polym. Phys.* 47, 1869-1879.

Vollmer A, **Voiges K**, Bork C, Fiege K, Cuber K, **Mischnick P** (2009) Comprehensive analysis of the substitution pattern in dextran ethers with respect to the reaction conditions. *Anal Bioanal Chem.* 395: 1749-1768. DOI 10.1007/s00216-009-3013-4

Nagel MCV, Koschella A, Heinze T, **Voiges K**, **Mischnick P** (2010) Homogeneous methylation of wood pulp cellulose dissolved in LiOH/urea/H₂O. *Euro Polym J.* 46: 1726-1735.

Unterjeser I, **Mischnick P** (2011) Labeling of Oligosaccharides for Quantitative Mass Spectrometry. *Carbohydr Res.* 346: 68-75. DOI 10.1016/j.carres.2010.11.001

Voiges K, **Adden R**, **Rinken M**, **Mischnick P** (2011) Critical Re-Investigation of the Alditol Acetate Method for Analysis of Substituent Distribution in Methyl Cellulose. *Macromol Symp.* submitted 19.12.2010

Unterjeser I, **Cuers J**, **Voiges K**, **Mischnick P**, Quantitative Aspects in ESI-IT and MALDI-TOF Mass Spectrometry of Malto-Oligosaccharides. *Rap. Commun. Mass Spectrom.* (since Dec 2010 at Dow Wolff for approval, submission planned in January 2011)

2. Articles in international scientific compilation works and international scientific conference proceedings with peer review

Ek, M. and Karlsson, S., The 4th Workshop on Cellulose, Regenerated Cellulose and Cellulose Derivatives, Karlstad University, Karlstad, Sweden, proceedings

Hiltunen M, Maunu SL, (2009) Cellulose graft copolymer synthesized via controlled polymerization method in homogeneous media, The 4th Workshop on Cellulose, Regenerated Cellulose and Cellulose Derivatives, Karlstad University, Karlstad, Sweden, proceedings

Hiltunen M, Maunu SL, (2009) Cellulose graft copolymers through RAFT polymerisation method, 15th International Symposium on Wood, Fibre and Pulp Chemistry (ISWFPC), Oslo, Norway, proceedings

3. Articles in national scientific journals with peer review

4. Articles in national scientific compilation works and national scientific conference proceedings with peer review

5. Scientific monographs

Mischnick P, Momcilovic D (2010) Chemical Structure Analysis of Starch and Cellulose Derivatives
Adv Carbohydr Chem Biochem. 64: 117-210.

6. Other scientific publications, such as articles in scientific non-refereed journals and publications in university and institute series

Ek, M., and S. Karlsson, Oral presentation at “ The 1st EPNOE Conference on Polysaccharides as a Source of Advanced Materials”, September 21-24, 2009, Turku, Finland.

Ek, M., Cellulose accessibility and reactivity, lecture in the ReCell Summer School, August 2010, Bomlitz, Germany

D. Momcilovic, J. Enebro and S. Karlsson, Enzymatic hydrolysis and mass spectrometry of cellulose derivatives: Studies of enzyme selectivity and structure-property relationships, Oral presentation at “ The 1st EPNOE Conference on Polysaccharides as a Source of Advanced Materials”, September 21-24, 2009, Turku, Finland.

Enebro, J., The 4th International Symposium on Separation and Characterization of Natural and Synthetic Macromolecules – SCM 4, Amsterdam, Netherlands (Oral presentation), Jan. 28-30 2009

Karlsson, S., The 237th ACS National Meeting & Exposition (Oral presentation) March 22-26, 2009, Salt Lake City, USA

En ny metod för tillverkning av nanoporösa filmer från polysackarider. Master Thesis, KTH, 2010

Li, Dongfang, Cellulose solvents – ionic liquids. Master Thesis, KTH/Chalmers, 2010

Ek, M., 15th ISWFPC2009, Oslo, Norway, June 15-18 2009

Nordic Polymer Days 2009, Copenhagen, Denmark, May 25-27, 2009

The 4th Workshop on Cellulose, Karlstad, Sweden, November 17-18, 2009

Ståhlbrandt, H. 8th Carbohydrate bioengineering meeting, Ichia, Italy (Poster presentation)

Sirkka Liisa Maunu, Vladimir Aseyev, Helena Parviainen, Tarja Tamminen, Tiina Liitiä and Matti Siika-Aho; Dissolution behaviour of activated dissolving pulps evaluated microscopically and by light scattering methods, Pacifichem 2010, December 15-20, 2010, Honolulu, Hawaii; Invited oral presentation.

Vladimir Aseyev, Laura Heiskanen, Sirkka Liisa Maunu, Helena Parviainen, Satu Vuorela, Tarja Tamminen; Cellulose solutions from pretreated dissolving grade kraft pulps; 239th ACS meeting, March 21-25, 2010, San Francisco, CA, USA, oral presentation

Sirkka Liisa Maunu, lecture in the ReCell Summer School, August 2009, Stockholm-Helsinki

Sirkka Liisa Maunu, Laura Heiskanen, Miia Hiltunen, Pirita Uschanov, Tommi Virtanen; Modification of Dissolved Cellulose; European Polymer Congress, July 12-17, 2009, Graz, Austria, oral presentation

Miia Hiltunen, Janne Raula and Sirkka Liisa Maunu, Water-Soluble Cellulose-g-Copolymers Synthesized by ATRP in homogeneous medium, Nordic Polymer Days 2010, (NPD2010), Helsinki, Finland, May 24.-26., 2010, oral presentation

Miia Hiltunen and Sirkka Liisa Maunu, Graft copolymerization of acrylamide and N,N-dimethylacrylamide to cellulose in homogeneous media using atom transfer radical polymerization (ATRP), 239th ACS meeting, San Francisco, CA, USA, 21.-25.3.2010, oral presentation

Miia Hiltunen and Sirkka Liisa Maunu, Cellulose graft copolymers synthesized via raft polymerisation method in homogeneous media: EHEC-g-PAam copolymers, EPNOE 2009, Polysaccharides as a Source of Advanced Materials, Åbo, Finland, 21.-24.9.2009, poster

Miia Hiltunen and Sirkka Liisa Maunu, Cellulose graft copolymers synthesized via raft polymerisation method in homogeneous media, Nordic Polymer Days 2009 (NPD2009), Copenhagen, Denmark, 25.-27.5.2009, poster

Helena Parviainen, Vladimir Aseyev, Sirkka Liisa Maunu, Tarja Tamminen, Dissolution studies of pretreated dissolving pulps, Nordic Polymer Days 2010, (NPD2010), Helsinki, Finland, May 24.-26., 2010, oral presentation

Tommi Virtanen, Sirkka Liisa Maunu, Dissolution of cellulose into novel solvent systems, Pacifichem 2010, December 15-20, 2010, Honolulu, Hawaii; oral presentation.

Vladimir Aseyev, Laura Heiskanen, Sirkka Liisa Maunu, Helena Parviainen, Satu Vuorela, Tarja Tamminen, Cellulose solutions from pretreated dissolving grade kraft pulps. 239th ACS meeting, March 21-25, 2010, San Francisco, oral presentation

Tarja Tamminen, Matti Siika-aho, Tiina Liitiä, Roland Adden, Helena Parviainen, Sirkka-Liisa Maunu, Enzymatic activation of softwood dissolving pulps, 14th International Biotechnology Symposium and Exhibition, 14-18 September 2010, Rimini, Italy, poster

Tarja Tamminen, lecture in the ReCell Summer School, August 2009, Stockholm-Helsinki

a) Other dissemination

The results have been presented to the industrial partners in steering groups meetings about twice a year.

Voiges K, Mischnick P (2009) Standard Operation Procedure (SOP) - Monomer Analysis of Methyl Cellulose (MC) by Gas Liquid Chromatography (GLC) – (for Dow Wolff Cellulosics)

Materials (Lectures etc) disseminated at two International Summer Schools:
24.-26.08.2009 Stockholm- Helsinki, Sweden/Finland: "Cellulose based polymers – Characterization and application"
30.08.-01.09.2010 Villa Wolff, Bomlitz, Germany: "Cellulose – Combined Contradictions"

Poster:

EPNOE, Turku, Finland, September 2009:

Unterieser, I, Müller R, Mischnick P(2009) Fraction of methyl celluloses according to chemical structure

Voiges K, Vollmer A, Mischnick P (2009) Critical investigation of the analysis of the substituent distribution in cellulose ethers by the alditol acetate method

Tamminen T, Adden R et al.: Poster at the 14th International Biotechnology Symposium and Exhibition, 14-18 September 2010, Rimini, Italy on "Enzymatic activation of softwood dissolving pulps",

Tamminen T, Adden R et al.: Presentation at ACS meeting, March 2010 on "Evaluation of pretreated dissolving pulp solutions",

Zellcheming, Wiesbaden, June 2010:

Voiges K, Adden R, Rincken M, Mischnick P (2010) Successive Labeling in Polysaccharide Derivative Analysis

Unterieser I, Voiges K, Adden R, Rincken M, Mischnick P (2010) Labeling Studies for the Quantitative Analysis of Cellulose Ethers by Mass Spectrometric Methods

Kapanen, E., Electrospinning of carboxymethylcellulose, (2010) Master Thesis, Chalmers University of Technology, Gothenburg, Sweden

1.6 National and international cooperation

The following project meetings were conducted:

2008

Kick-off meeting, KTH, Stockholm, Sweden, May 29, 2008

Steering Group and project presentations, Technical University of Braunschweig, Germany, Dec. 5, 2008

Phone conference on August 18, September, October 16

2009

- Steering Group and project presentations, VTT, Finland, June 2, 2009
- Steering Group and project presentations, KTH, Sweden, November 30, 2009
- Steering Group phone conference, March 19, 2009
- Several bilateral phone meetings/conferences

2010

Phone conferences: Jan and March 9th 2010

Steering Group meeting August 31, 2010 (in connection with the 2nd Summer School)

Guest professorship of P. Mischnick, TU Braunschweig, at KTH, Fiber and Polymer Technology, group of Prof. Sigbritt Karlsson (50%, 2008-2012)

Research stay of PhD student Kristin Voiges from TU Braunschweig, at KTH Stockholm, Fiber and Polymer Technology from Aug-Nov 2011; MALDI-MS, Ultrasound treatment, SEC

Cooperation with University of Helsinki, Prof. Sirkka-Liisa Maunu: Analysis of macroinitiators of prepared from cellulose and of the grafted polymers produced from these macroinitiators by ATRP with acrylamide

An in depth cooperation between Dow Wolff Cellulosics (DWC) and the TU Braunschweig took place, regular project meetings were scheduled. Implementation of a standard operation procedure developed in the project at DWC

Extensive networking between Dow Wolff and the University Helsinki and VTT has taken place leading to the Poster and the Presentation on the topic "Enzymatic activation of pulp".

Organization of two Summer Schools about Cellulose: structure, modification, properties, application, analysis.

1st: 24.-26. August 2009, Stockholm/Sweden – Helsinki/Finland (ferry), 48 participants, 35 PhD students. The first ReCell Summer School with the title "Cellulose Based Polymers - Characterization and Applications" was held during August 24-26 in Stockholm, Sweden. The Summer School was organized by: Momcilovic D, Karlsson S, Mischnick P, KTH Stockholm. A total of 35 students from Sweden, Germany, Finland and Austria participated. The lecturers were recruited both internally (ReCell researchers) and externally and were from both academy and industry. Two full days of lectures and group exercises were rounded of with a written home assignment, which was taken by the majority of the students. The course evaluation was very positive (also among the lecturers).

2nd: 30. August . 01. September 2010, at Dow Wolff Cellulosics, Bomlitz, Germany (organized by Adden R, DWC, and Mischnick P, TU BS)
additional funding by Deutsche Forschungsgemeinschaft, DFG, 71 participants, 44 PhD students