Combining multiple NMR spectroscopic approaches in study of cellulosic materials

Tommi Virtanen

University of Helsinki
Faculty of Science
Department of Chemistry
Laboratory of Polymer Chemistry
P.O. Box 55 (A.I. Virtasen Aukio 1)
FI-00014 University of Helsinki, Finland

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Abstract

The aim of this thesis was to combine several advanced NMR spectroscopic techniques for studying the cellulose in materials still possessing, at least partly, the original complexity of wood fiber. The NMR spectroscopic approaches can be roughly divided into two classes: First, solid state NMR methods were used to characterize cellulose and its response to various treatments directly. Second approach involves utilization of molecular probes, namely solvent components and water, to provide information about interactions between solvent species and cellulose in pulp fibers, and about effect of certain reactivity enhancing pretreatments on cellulose accessibility and fiber wall structures up to micrometer size scale. When comparing the different approaches, it was observed that the often used quantity, cellulose crystallinity, turned out to be a rather poor indicator for changes taking place in complex cellulose systems, as its value remained mostly inert while the other techniques already revealed changes in specific surface area of the fibers, average pore size at a size scale of ten nanometers, and in accessibility of chemicals through the fiber wall and to the cellulose fibrils. This shows the importance of combination of different type of NMR experiments when studying materials with considerable complexity.
Acknowledgments

This thesis is based on the work that was carried out during the years 2004–2013 in the Laboratory of Polymer Chemistry, University of Helsinki. It deals with the application of various NMR methods in study of cellulosic materials.

My first thanks go to my supervisor prof. Sirkka Liisa Maunu for giving me the chance to work in a such free environment, and for her flexibility and seemingly everlasting patience with me. I know that occasionally I must have put it in quite a test. I am also grateful for the Head of the Laboratory prof. Heikki Tenhu for the possibility to work in his laboratory, and in showing considerable understanding for a person (i.e., Yours Truly) who has not been blessed with any practical laboratory skills what so ever. I would also like to express my gratitude to Dr. Sami Hietala, Dr. Vladimir Aseyev, and to my room mate Erno Karjalainen, who repeatedly acted as a SampleChange'o'Matic™. I’m also grateful for the help I got from our laboratory engineer Juha Solasaari, and Mr. Matti Keinänen and Dr. Sami Heikkinen from Organic Chemistry department for their help with our older NMR instrument. Without your contribution our 300 MHz NMR instrument would have been retired already years ago. Wealthy thanks go also for all my collaborators that helped me in finalizing this work. Jonina and Ossi are acknowledged for providing child care services. K. Viikate is acknowledged for providing music background.

And finally: Thanks to my family, that is Milja, Nuuti, Seine, and Mirkka, just for being around. In this part it is usually a tradition to tell how much you helped and encouraged me in my work. However, I believe that everyone who can recall the days with children under school age, and still have the bite marks of reality itching at their rear ends, understands if I skip the honey–dripping part. But know this: Even though my work would probably have been done in half of the time should you have not been here with me, it would have lacked all purpose.
List of Abbreviations

\[ D_a \]  
Apparent diffusion coefficient

CP  
Cross polarization

CR  
Cross relaxation

CS  
Chemical shift

CSA  
Chemical shift anisotropy

DP  
Degree of polymerization

FID  
Free induction decay

FSP  
Fiber saturation point

GS  
Goldman–Shen experiment

HR–MAS  
High resolution magic angle spinning

LFAD  
Lateral fibril aggregate dimension

LFD  
Lateral fibril dimension

MAS  
Magic angle spinning

MC  
Moisture content defined as mass of water divided by mass of dry matter

MCC  
Microcrystalline cellulose

NFW  
Nonfreezing water

NMMO  
N-methylmorpholine-N-oxide

NMR  
Nuclear magnetic resonance

PFG  
Pulsed field gradient

r.f.  
Radio frequency

SE  
Spin echo
<table>
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<tr>
<td>SSA</td>
<td>Specific surface area</td>
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<td>STE</td>
<td>A stimulated echo type diffusion experiment</td>
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List of Publications


Author’s contribution to the publications

Paper I The author wrote the manuscript, except the parts describing pulp cooking and bleaching and sugar analysis. The author was responsible for all the NMR work, and sample preparation related to that, and for analysis of the NMR data.

Paper II The author wrote the Introduction part of the manuscript, and parts related to the solid state NMR and thermogravimetric work. The author was responsible for the NMR work and data analysis, and prepared the final form of the manuscript from the results obtained from other experimental methods.

Paper III The author made the research plan, was responsible for the NMR work and analysis, and wrote the manuscript.

Paper IV The author made the research plan concerning the NMR work, was responsible for the NMR work and data analysis, and wrote the manuscript, excluding the part describing pulp pretreatments in Materials section, and the parts describing X-ray scattering in Experimental section and X-ray results.
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1 Introduction

Cellulose has been an important material for Finnish society and economics for decades through the paper industry. Currently, the foundations of traditional papermaking in Finland are becoming unstable, as production is been re-located for saving costs and electric media is becoming more important, displacing the printed media. At this stage, new ways to utilize cellulose are sought. Cellulose does indeed have lot to offer besides papermaking: It has some rather remarkable properties, that have already been exploited in various industrial fields, from food industry to pharmaceutics [1–5]. As the ways to utilize cellulose become more sophisticated, new demands for analysis methods used to characterize it are set: When a design of a material starts from molecular level it is also necessary to apply tools that are capable of providing information from the same size scale.

This thesis describes the application of nuclear magnetic resonance (NMR) spectroscopy in characterizing cellulosic materials. One of the most fascinating aspects on NMR spectroscopy is its versatility: Not only it can provide structural information needed by organic chemists carrying out synthetic work, but it can be used to create a picture of a material’s properties from nanometer scale assembly of molecules up to micrometer scale structures. The principal aim of this thesis is to bring together a wide selection of different NMR approaches, in order to sharpen the image from cellulosic materials.

The first two publications of this thesis are related to characterization of cellulose assemblies at nanometer size scale, i.e., crystallinity and grouping of cellulose fibrils, using solid state NMR spectroscopy. The two other publications deal with a problems associated with bringing cellulose into dissolved state. Cellulose will not dissolve into any conventional organic solvent, nor does it dissolve in water. Yet dissolution is often a step that is required before cellulose can be processed any further. The interactions between molecules of a known cellulose solvent and cellulose are probed with several NMR approaches. Wood pulp can be used as a source for cellulose when preparing solutions. In that case it is necessary to pretreat the pulp in order to make it more reactive towards the solvent chemicals. The last paper in this thesis describes the changes that take place in pulp fiber wall due to mechanical and enzymatic pretreatments. Again, several NMR approaches, including NMR diffusometry and solid state
NMR spectroscopy, are taken in order to bring information from various size scales of the fiber. The combination of NMR techniques offers a wide perspective in the events that take place at molecular level and at larger size scale in these rather complex systems.
2 Cellulose

2.1 Cellulose structure

Cellulose is a polysaccharide built up from celllobiose as a repeating unit (Figure 2.1.1), where glucose rings have been connected by \( \beta-1,4 \) glucosidic bonds between carbon C1 and C4 of adjacent glucose units. The degree of polymerization (DP) depends on origin of cellulose, but it can be higher than 10 000, given as number of glucose units [6]. Cellulose has four major crystalline forms (designated as I, II, III, and IV), but the cellulose produced by biosynthesis in higher plants has crystalline form I. It was shown by VanderHart and Atalla [7] that the crystalline form I is actually a composition of two modifications, designated as \( I_\alpha \) and \( I_\beta \). These have the same heavy atom configuration, but their hydrogen bonding patterns deviate from each other. The structure and the polymeric nature of cellulose has been known for decades [6,8,9], and the arrangement of cellulose chains in different crystalline forms and intra/intermolecular hydrogen bonding has been resolved by using solid state NMR spectroscopic methods and X-ray and neutron scattering [10–12]. Based on electron diffraction [13] it was shown that the two components of cellulose I differ in alignment of cellulose chains: while cellulose \( I_\alpha \) has a one-chain triclinic unit cell, the unit cell for cellulose \( I_\beta \) is a two-chain monoclinic. In both forms the chains are in parallel alignment, i.e., their reducing ends are pointing in same direction. The \( I_\beta \) form is thermodynamically more stable, leading to enrichment of this form when cellulose is exposed to high temperatures [14] as it happens in, for instance, during pulping process. On the other hand, the higher stability also induces a lower reactivity compared to cellulose \( I_\alpha \). The other crystalline forms are formed by chemical treatments, with a exception that cellulose II has been found forming natively in mutant bacterial strain [15,16].

Each anhydroglucose unit in cellulose contains three hydroxyl groups. This affects both chemical behavior of cellulose, and its physical properties. The hydroxyls contribute to the intramolecular hydrogen bonding, making cellulose a rigid polymer. Because of this, and because of \( \beta- \)glucosidic linkages between the glucose units that promote linear conformation, cellulose favors crystallization and formation of fibrillar structures [6]. The rigidity of a polymer chain also leads to a low entropy gain in a dis-
solution process, making it more difficult to be dissolved [17]. The longitudinal modulus for cellulose \( I_\beta \) crystal has found to be around 150 GPa, as determined by different experimental methods and by computational results [18–21]. Consequently, for cellulose crystal the axial specific Young’s modulus is comparable to that of Kevlar [22]. When also taking into account the availability, low cost, and renewability, it is no surprise that cellulose has been considered as a candidate for replacing conventionally used fibers in polymer composites [23].

2.2 Supermolecular arrangements of cellulose

The intra– and intermolecular hydrogen bonding along the cellulose chains and the van der Waals interaction between chain layers allows the formation of long–range ordered crystalline structures. Even though the crystalline form \( I \) is the basis for almost all naturally produced cellulose, there exists a wide variation in the size, shape, and packing of the cellulose crystallites. The width of the fibrils varies from 3 to 4 nm found in wood cell walls, up to approximately 60 nm found in certain algae [23,24]. Typical feature for all cellulose sources is that the length of the fibrils is considerably higher than the lateral width. Experimental data regarding the supermolecular cellulose structures has been gathered by means of electron microscopy [25–27], atomic force microscopy [28,29], X–ray scattering [30], and solid state NMR spectroscopy [24,31]. Cellulose, as any polymer, is a semi–crystalline material. Besides the crystallites there exists domains with varying degrees of order, like the surfaces of the fibrils, and non–crystalline regions separating the crystals, often referred as discontinuity sections [23,32]. The naming convention for the cellulose supermolecular structures shows considerable variation: The threadlike entities formed from individual cellulose crystals and discontinuity sections are referred in texts as ‘fibrils’, ‘elementary fibrils’, or ‘microfibrils’, and the grouping of fibrils are referred as ‘macrofibrils’ or ‘fibril aggregates’. In this thesis the term fibril is used for cellulose crystallite with the discontinuity sections, and the fibrils that are grouped together are entitled as fibril aggregates.

The supermolecular assemblies of cellulose can be affected to some extent by chemical treatments. It is known that in pulping conditions the lateral dimension of fibril
aggregates increase, and drying of the pulp also leads to same effect [33]. Recently the importance of the supermolecular structure of cellulose crystallites has drawn attention because of its close relation to the reactivity of cellulose towards processing chemicals and cellulose solvents [6,34].

When the discontinuity sections in cellulose fibrils are cleaved by acid hydrolysis [35–37], thus leaving only the crystalline sections of cellulose, a product known as microcrystalline cellulose (MCC) is obtained [38]. MCC is commonly used in pharmaceutical industry as an excipient [39–42], and in food industries as a gelling agent, stabilizer, emulsifier, and thickener [1,43,44]. It has also found applications as a reinforcement in biocomposites [45–47] and as a starting material to produce nanocrystalline cellulose [48–50]. Besides the acid hydrolysis, MCC can be extracted by mechanical treatments [51] and biological treatments [52]. The choice of the processing method, together with the choice of starting material, determines the properties of the final MCC.

2.3 Cellulose from different sources

There exists also other potential sources for cellulose than wood. Cellulose has been extracted from jute [53], flax [54], from agricultural wastes like orange mesocarp [55], and from oil palm biomass residue [56]. Many cellulose containing materials that already are been utilized in some context, have a cellulose rich component that is rejected as an unsuitable for the primary use. This is also the case with rice husks and hemp stalks. Rice husk is a surface layer of a rice grain that protects it during its growth. It is indigestible for humans, and therefore it is removed in rice production. Rice husk has found use as an energy source in rice producing countries, but its combustion has certain environmental issues due to its pollutive nature [57]. Hemp is environmentally friendly to produce, as it does not have a high demand for pesticides, and it does not require any herbicides. As is the case with rice husk, hemp also has already found several applications [58–61], but there has not been any attempts for cellulose extraction and refining. The situation is similar with coniferous needles: They have been studied due to their potential as indicators for pollution [62–64], or because of their extractives. There has been only minor interest in cellulose found in them [65].

2.4 Wood pulp

In wood pulping the wood cell wall is broken with purpose to extract cellulose from fiber wall by dissolving lignin that holds the fibers together. In chemical pulping wood is first prepared into chips that are then cooked with pulping chemicals under high pressure. The choice of chemicals can be used to further classify chemical pulps:
The most common process uses sodium hydroxide and sodium sulfide, and is known as kraft process. Another common method is the sulfite pulping. The end product determines the choice of pulping method, as each of them have their benefits. From kraft pulping a mechanically strong pulp is obtained that can stand bleaching to very high brightness. The sulfite pulp can be bleached easily with hydrogen peroxide, and is used for chlorine–free products [66].

Dissolving grade pulp is a pulp with very low hemicellulose, lignin, and resin contents. It is typically used as a raw material for multiple cellulose products, like various derivatives and microcrystalline cellulose. Dissolving pulp is manufactured by acid sulfite and pre-hydrolysis kraft process, with acid sulfite process being the more common one [67].

Wood pulp in water swollen state is porous material, with pore sizes extending from sub-nanometer scale up to lengths in micrometer scale [68]. The pores are partially a by–product of the pulping process: The cavities that are formed when hemicelluloses and lignin are dissolved become filled with water [69]. The large variance in size makes the characterization of the pore structure challenging, and several approaches have been used to achieve this, including thermoporosimetry [70, 71], cryoporometry [72], and NMR diffusometry [73]. The pore structure of pulp is closely associated with the accessibility of processing chemicals into the interior of fiber wall, as it sets the limit for the size of the molecules that are able to enter [74].

2.5 Cellulose solvents

Many of the new applications of cellulose outside the traditional papermaking industry require cellulose to be dissolved first. Dissolving of cellulose, however, is notoriously difficult. Despite its high hydroxyl group content it is not water soluble, but it does not dissolve into any common organic solvent either. While it is possible to chemically modify cellulose to make it more water soluble, the direct dissolution would be also beneficial. The origin for difficulty in bringing cellulose into dissolved state is often attributed to its strong tendency to form inter– and intramolecular hydrogen bonds, but recently the amphiphilicity of the cellulose chain has been considered to be more important factor [17]. There exists several well known solvent systems for cellulose: Dimethylacetamide with LiCl (LiCl/DMAc), N-methylmorpholine N-oxide (NMMO) with a low water content, ionic liquids (IL), an aqueous alkali hydroxide solution with added ZnO, and a mixture of NaOH or LiOH with urea in aqueous solution [75–79]. Also certain ionic liquids with high hydrogen bond basicity and a moderate hydrogen bond acidity are excellent cellulose solvents [80, 81]. Of the mentioned solvent systems NMMO/water is used at industrial scale (Lyocell process). The water based solvent systems Na(Li)OH/urea are extremely interesting due to their non–
toxicity and low cost, but so far they have been only proved to work in laboratory scale. The above mentioned solvents differ notably in conditions they require to work: For instance, the aqueous LiOH/urea system requires a temperature below 0 \(^\circ\)C [82], while the NMMO monohydrate (13.3 % water by weight) has to be heated above the melting temperature 76–78 \(^\circ\)C [83]. The common nominator for all cellulose solvents mentioned here is that their operating mechanism at molecular level is mostly unknown, and because of this the interactions between cellulose and solvent species have been under investigation by many researchers [84–86].

### 2.6 Pulp pretreatments

Dissolution of wood pulp poses additional difficulties in addition to those already associated with cellulose. There exists remnant cell wall structures that inhibit the dissolution process, or slow it down to an extent where it is no longer feasible. Moreover, if the pulp has been dried, the water removal causes the pore structure to collapse, leading to an coalescence of cellulose fibrils and fibril aggregates [29,33]. These form tight structures that prevent the fiber to retain its original form when rewetted [28,34]. This process is known as hornification, and it decreases the reactivity of the cellulose fibrils towards processing chemicals.

To overcome the challenges in directly dissolving the wood pulp fibers several strategies have been developed to increase their reactivity towards solvents. There exists multiple different pretreatments that can be applied on wood pulp, like enzymatic hydrolysis, steam explosion, and different kinds of mechanical pretreatments. Steam explosion is used to break the interactions between cellulose fibrils, and it is known to decrease the degree of polymerization [87]. The effect of enzymes varies depending on the type of enzyme used. Endoglucanases mainly cleave the less–ordered regions of cellulose fibrils [88–90], leading to separation of fibrils, thus increasing their reactivity.
3 NMR spectroscopic methods

This chapter shortly introduces the basic concepts of NMR spectroscopy [91,92], and gives a short description of the NMR spectroscopic methods that were used in the work belonging to this thesis.

3.1 Nuclear magnetic resonance

Nuclei with a non-zero spin possess a magnetic moment. When such nuclei are placed in a magnetic field, the degeneracy in their nuclear spin energy states is broken. In the case of spin-\frac{1}{2} nuclei they acquire two energy eigenstates \( |\alpha\rangle \) and \( |\beta\rangle \) with distinct energies. These states correspond the situations where one component of a spin is aligned parallel or anti-parallel with the applied field, and the slight difference in the energy between \( |\alpha\rangle \) and \( |\beta\rangle \) is proportional to the magnetic flux density \( B_0 \) of the applied field. The proportionality varies between magnetically active nuclei, and is known as a gyromagnetic ratio (\( \gamma \)) of the nucleus. For proton the gyromagnetic ratio is approximately \( 2.675 \times 10^8 \text{s}^{-1}\text{T}^{-1} \). Basically, the magnitude of \( \gamma \) tells how magnetic the nucleus is: High absolute value of \( \gamma \) makes the nucleus easy to detect in NMR experiment. The majority of the nuclei are in a superposition states of the energy eigenstates, with population of \( |\alpha\rangle \) being slightly higher due to lower energy of \( |\alpha\rangle \).

The response of the spins to the applied magnetic field is precession around this field. Time evolution of these superposition states is the origin for spin precession, with precession rate equal to difference in energies of \( |\alpha\rangle \) and \( |\beta\rangle \):

\[
\omega_0 = -\gamma B_0.
\]  

(3.1.1)

\( \omega_0 \) is known as the Larmor frequency of the nucleus. Due to population difference the spin polarizations end up to have a slight orientation preference to the direction of applied field, which leads to macroscopic magnetization in the sample along the field. The population difference can be converted into a coherence in precession by applying a radio frequency (r.f.) radiation, with frequency set up to match the precession rate
of the spins. In modern NMR spectrometers the irradiation is performed by applying short r.f. pulse, which produces a small (compared to the external main magnetic field) transversal magnetic field. Due to the fact that this field is close to resonance with the Larmor frequency of the spins, it is capable to tilt the magnetization from the direction of the main field to the transversal plane (or to some other direction, depending on the experiment). The macroscopic magnetization, now precessing in the transversal plane, is detected in a NMR spectroscopic experiment by observing the current it induces to a detecting coil. The signal from the detecting coil is usually called a free induction decay (FID).

NMR spectroscopy takes advantage from the fact that total magnetic field that a nucleus experiences is a sum from the external field and all local magnetic fields. Therefore nuclei in different chemical environments have slightly different Larmor frequencies, and they come into resonance at a different frequency. This sensitivity of Larmor frequency to local fields, known as a chemical shift (CS), is one of the main tools in interpretation of NMR spectra.

3.2 Solid state NMR spectroscopy

From NMR spectroscopic point of view there are two issues that distinguish solid material from liquid: Close packing of the spin bearing particles, and lack of mobility. Most of the interactions that give the shape for NMR spectrum are anisotropic, i.e., their magnitude has an orientational dependence with respect to the applied magnetic field. In liquid state these anisotropic interactions are averaged out by rapid molecular tumbling, but in solids the lack of motion leaves the effects of anisotropies to be seen in the spectrum. The close packing of molecules causes the dipolar coupling, inversely proportional to cube of distance between the spins, to increase. Combined with the anisotropic nature of dipolar interaction this leads to severe line broadening. Similar problem arises with chemical shift. The lack of motion also leads to considerable increase in longitudinal relaxation times of nuclei, causing the repetition rate of the NMR experiment to become unfeasible. These problems were solved by introduction of magic angle spinning (MAS), high power decoupling, and cross polarization (CP) [93, 94]. MAS averages the anisotropic interactions that are proportional to second order Legendre polynomial,

$$P_2(\cos \theta) = \frac{1}{2} (3 \cos^2 \theta - 1)$$  \hspace{1cm} (3.2.1)

to their isotropic averages [95]. Such interactions are chemical shift anisotropy (CSA) and dipolar coupling. Magic angle spinning introduces a manifold of spinning side bands into solid state NMR spectrum, with separation from main signal, and from each other, given by spinning frequency. The number of side bands having intensity
Figure 3.2.1: A cross polarization pulse sequence.

The dependence of Hartmann-Hahn condition on spinning rate $\omega_r$ becomes more crucial when spinning rate increases, and in modern systems an amplitude ramp in cross polarization is commonly used [99]. When using cross polarization the relaxation behavior of spins $I$ sets the lower limit for the repetition rate of the experiment. Even though the proton longitudinal relaxation time in solids may also be quite long (in some rigid small molecular weight compounds the time constant for it may be as high as several hundred seconds), it is considerably shorter than that for carbon. For cellulose the proton longitudinal relaxation time constant is typically less than one second. The use of cross polarization also gives an additional bonus in sensitivity, as it provides a (theoretical) signal enhancement by a factor of $\gamma_I/\gamma_S$ [98]. In the case
Figure 3.2.2: A solid state $^{13}$C CP–MAS NMR spectrum from softwood pulp. The insert shows an expansion of the C4 signal, together with the peak fitting model that is used to calculate the lateral fibril and fibril aggregate dimensions.

of cross polarization from proton to carbon this factor is $\sim 4$. Cross polarization is based on dipolar coupling between the spins, therefore it is more efficient for those regions in the sample where molecular mobility is restricted.

NMR spectroscopy is inherently a quantitative technique: The obtained signal intensity is directly proportional to the number of spin bearing particles. There are, however, several experimental details that need to be taken into account even in liquid state NMR spectroscopy in order to obtain a quantitative spectrum, like the requirement for an adequate repetition delay between successive scans and adequate bandwidth of the excitation pulse [100]. In solid state all these aspects need to be considered as well, and additionally there are extra concerns [101]: In a cross polarization experiment the cross polarization dynamics may vary between different sites, and this leads to a non–quantitative result. It has been shown that the cellulose carbon sites have similar cross polarization dynamics, thus making the cellulose CP–MAS $^{13}$C spectrum quantitative [24,102]. This forms the basis of the coming analysis regarding the solid state $^{13}$C CP–MAS spectra of cellulose.
3.2.1 Interpretation of solid state NMR spectrum from cellulose

The typical solid state $^{13}$C CP–MAS NMR spectrum for a cellulose in wood pulp sample is shown in Figure 3.2.2. The most informative region of solid state NMR spectrum obtained from cellulose is the signal region from carbon site 4 (C4) at 80–92 ppm, which has a clear division into crystalline ($\sim 86–92$ ppm) and non–crystalline (80–86 ppm) components. The C4 region can be used to determine the crystallinity of cellulose ($C$) as the relative areas of the crystalline and non–crystalline signals [103–105],

$$C = \frac{A_{cr}}{A_{cr} + A_{noncr}} \times 100\%,$$

and to estimate the average lateral dimensions for cellulose fibrils (LFD) and fibril aggregates (LFAD) [102]. The calculation of LFD and LFAD is based on capability to distinguish between signals originating from non–equivalent surfaces of cellulose fibrils, namely solvent accessible and inaccessible surfaces (see Figure 3.2.2). By comparing the signal area that originates from solvent accessible surfaces to the whole signal area the number of cellulose chains at the aggregate surface can be counted, and knowing the dimension that each chain occupies in lateral direction, and assuming a square cross section for the fibrils and aggregates, the LFAD can be calculated. This is based on assumption that all non–crystalline signal is originated from cellulose fibril surfaces from a one–chain thick layer.

The information about LFAD can be combined with a fiber saturation point (FSP) measurement to yield an average pore size for the fiber. With the assumption of a square cross sectional cellulose fibril aggregate, the specific surface area (SSA) $\sigma$ can be expressed as [34]

$$\sigma = \frac{4}{LFAD \times \rho_{cel}},$$

where $\rho_{cel}$ is the density of cellulose fibril aggregate. The FSP may be determined by, e.g., solvent exclusion method [106], and it is given by

$$FSP = d\sigma \rho_{liquid},$$

where $d$ is the thickness of the liquid layer (uniformly covering the solid surfaces) and $\rho_{liquid}$ is the density of the pore–filling liquid. For water $\rho_{liquid}$ is taken to be 1000 kgm$^{-3}$. The average pore size $\langle a \rangle$ is then obtained as [107]

$$\langle a \rangle \equiv 2d = LFAD \times \frac{FSP}{2} \frac{\rho_{cel}}{\rho_{liquid}}.$$

There has been some questioning about the justification of the assumption that all non–crystalline cellulose would be located at fibril surfaces, neglecting the discontinuity sections in the fibril. If, however, the length of the discontinuity sections is
low enough compared to the length of crystalline regions, it could be argued that due to inherent insensitivity of the solid state NMR experiment the discontinuity sections would not then contribute to the observed solid state NMR spectrum, and the whole observed signal would indeed originate from the non-crystalline cellulose located at fibril surfaces.

3.3 HR–MAS spectroscopy

There exists a variety of materials, like polymer gels, that are not liquids but lack the dense packing and low molecular mobility typically associated with solid materials. In these materials the intermolecular spacing and molecular mobility is high enough to reduce the homonuclear coupling between protons, but still the inhomogeneous nature of the material prevents the acquisition of a high quality proton NMR spectrum. This is due to inhomogeneity of magnetic field susceptibility [108], which luckily can be averaged out by MAS. This technique is entitled commonly as high-resolution magic angle spinning (HR–MAS) spectroscopy. HR–MAS probeheads are equipped with a lock coil and gradient coils, giving thus an access to normal high resolution NMR experiments performed typically for liquid samples. HR–MAS is not capable for acquiring a proton spectrum from a rigid solid, and it can not be used for cross polarization experiments. It does, however, provide a clear proton spectrum from small molecular weigh species located in a heterogenous environment. As the proximity of, for instance, cavity walls affect the chemical shifts of a chosen probe molecule’s proton signals, this method allows detection of interactions between the probe and the bulk matrix.

Currently all major NMR spectrometer manufacturers have HR–MAS probeheads commercially available, and the popularity of the method has increased especially in biological and biomedical fields. HR–MAS spectroscopy has been used extensively in medicinal chemistry [109] to, for instance, identify metabolites in intact biopsy tumors [110]. Another field taking advantage of HR–MAS spectroscopy is polymer science, where swelling of a polymer by a suitable solvent can be used to increase the mobility of polymer segments enough to achieve a semi-solid material, which then under moderate spinning rates can provide high-resolution NMR spectra. Ng et al. [111] used HR–MAS NMR spectroscopy for demonstration of the cyclic polyamide receptor threading onto the highly flexible polyethylene glycol (PEG) polymer chain attached to a polystyrene bead. They were able to identify between threaded and non-threaded PEG based on their HR–MAS spectra, and further on 2D NOESY spectra showed cross-peaks between the aromatic protons of the rotaxane and the methylene protons of the PEG polymer chain. de Miquel et al. [112] showed in their study that changes in dynamics and supermolecular interactions in complexation of
3.3. HR–MAS spectroscopy

Figure 3.3.1: A HR–MAS $^1$H NMR spectrum of water swollen pulp sample. The region for sugar CH signals is shown. Excitation sculpting was used for water suppression. From bottom to top: Microcrystalline cellulose, hardwood kraft pulp, steam exploded and enzymatically hydrolyzed softwood pulp. Lower signal to noise ratio of MCC spectrum is due to lower amount of scans.

Zn and Ru metalloporphyrins to beads functionalized with pyridyl ligands can be probed by HR–MAS techniques. In the work by Hofmann et al. [113] HR–MAS spectroscopy was used for studying solvent interactions in homo– and copolymer microgels of N–isopropylacrylamide and N,N–diethylacrylamide. Two different solvent species, bulk–like and restricted, were observed as separate signals in a proton HR–MAS experiment. Rather surprisingly though, there exists only few reports on application of HR–MAS spectroscopy in study of cellulosic materials [114, 115], even though for instance wood pulp in water swollen state would provide a rather interesting research field for this method. Figure 3.3.1 shows an example of a HR–MAS $^1$H NMR spectra obtained from microcrystalline cellulose, hardwood kraft pulp, and steam exploded and enzymatically hydrolysed pulp sample, all samples being wetted with D$_2$O. The observed signals may originate from chain ends, or from other regions with high mobility and loosened structure caused by swelling. In the case of enzymatically hydrolyzed pulp there are likely also contributions from cellulose oligomers, which is seen as increased fine structure of the spectrum compared to spectra obtained from MCC or non-treated wood pulp. In this thesis HR–MAS NMR spectroscopy is used to monitor the interactions between solvent species and cellulose during dissolution process. Figure 3.3.2 shows the HR–MAS spectrum from pulp–solvent mixture acquired with 3 kHz MAS, and as an insert a spectrum from the same sample without MAS. The static spectrum is more or less useless, while in the one acquired with MAS the NMMO and the residual HOD signals are clearly visible.
3.4 Quantitation of the water content in wood pulp

The proton NMR signal decay has a different time scale for liquid and solid materials due to rapid transversal relaxation in solids. The benefit of this is that in a NMR experiment the protons in solid state are easily distinguished from their liquid state companions. For instance, in a signal from a sample containing wood pulp and water, the time scale for signal decay from liquid water is in order of milliseconds, while the signal from cellulose protons decays to zero in a less than 100 µs. This offers a way to quantify the amounts of liquid and solid components in the sample. The spectrometer is set up to detect the first millisecond of a proton FID, and suitable functions describing the solid state and liquid state signal decays are fitted into the experimental data points [116]. The signal is extrapolated to time zero, taken as the middle of excitation pulse, and relative amounts of solid and liquid state material in sample can then be calculated from the initial intensities using a suitable conversion factor that takes into account possible different spin densities. For wood pulp the relative spin density (RSD) is 0.56 [116]. In the case of water swollen wood pulp sample the moisture content MC is therefore obtained as

\[
\frac{m_{\text{water}}}{m_{\text{pulp}}} = \frac{S_{\text{liquid}}^0}{S_{\text{solid}}^0} \times RSD,
\]

(3.4.1)
3.5 Cross relaxation in cellulose–water system

The longitudinal magnetization is capable to transfer between different phases in carbohydrate–water system. This process is known as cross relaxation (CR), and it poses a problem when analyzing NMR diffusometric data, or data from longitudinal relaxation experiments [120]. On the other hand, the cross relaxation rate is dependent on proximity of the interacting phases, and thus it can be used to provide at least qualitative information about how closely associated the interacting phases are [121]. The magnetization exchange processes between the solid and liquid phases can be
described using a two–site model, with rate constants $k_{\text{sol}}$ and $k_{\text{liq}}$ for magnetization exchange between the phases, and constants $R_{\text{sol}}$ and $R_{\text{liq}}$ for longitudinal relaxation processes of the phases. Rate constants for cross–relaxation can be obtained by performing a Goldman–Shen (GS) experiment that creates a controlled disturbance on both liquid and solid proton pools \[122\]. The pulse sequence for GS experiment is shown in Figure 3.5.1. The first $\frac{\pi}{2}$–pulse creates a transversal magnetization, from which the contribution from the solid state protons is let to dephase during the delay $\tau_1$. The second $\frac{\pi}{2}$–pulse restores the magnetization to the z–axis. During the $\tau_2$ delay the cross relaxation process and longitudinal relaxation take place, affecting the intensity obtained after the final reading pulse. In GS experiment both delays are varied in a systematic way to create dataset like shown in Figure 3.5.2. The magnetization that is observed in GS experiment can be shown to be given by equation \[117,123\]

$$M_{\text{liq}}(\tau_1, \tau_2) = M_{\text{liq}}^{eq} \left\{ 1 + c^+(\tau_1) e^{-R^+ \tau_2} + c^- (\tau_1) e^{-R^- \tau_2} \right\}, \quad (3.5.1)$$

where $R^\pm$ are terms formed from the rate constants of the two–site model

$$R^\pm = \frac{1}{2} \left\{ k_{\text{liq}} + k_{\text{sol}} + R_{\text{liq}} + R_{\text{sol}} \pm \sqrt{(k_{\text{liq}} - k_{\text{sol}} + R_{\text{liq}} - R_{\text{sol}})^2 + 4k_{\text{liq}}k_{\text{sol}}} \right\}, \quad (3.5.2)$$

and the $c^\pm$ are also functions of $\tau_1$ delay:

$$c^\pm(\tau_1) = \pm \left\{ \frac{M_{\text{liq}}(\tau_1) - M_{\text{liq}}^{eq}}{M_{\text{liq}}^{eq}} \times \frac{k_{\text{liq}} + R_{\text{liq}} - R^\mp}{R^+ - R^-} + \frac{k_{\text{liq}}}{R^+ - R^-} \right\}. \quad (3.5.3)$$

The $M_{\text{liq}}(\tau_1)$, i.e., the magnetization of the liquid phase after delay $\tau_1$ in Eq. 3.5.3 can be estimated from a single pulse experiment used for water content determination by using Equation 3.4.3. The rate constants for cross relaxation are then obtained by performing a global fit of Eq. 3.5.1 into experimental data (Figure 3.5.2).

### 3.6 NMR diffusometry

Self–diffusion of a molecule is caused by random thermal motion of molecules. For a spherical particle the self–diffusion coefficient can be described by a Stokes–Einstein
3.6. NMR diffusometry

Figure 3.5.2: An example of the analysis of the data from Goldman–Shen experiment for quantifying the cross relaxation rate constants. Dots correspond experimental data, curves are result from the global fit of Equation 3.5.1. Each curve represents data obtained with a different $\tau_1$ value, with $\tau_1$ increasing from top to bottom.

![Graph showing analysis of data from Goldman–Shen experiment](image)

The equation for the diffusion coefficient, $D$, is given by

$$D = \frac{kT}{6\pi \eta r_s},$$

(3.6.1)

where $k$ is the Boltzmann constant, $T$ temperature, $\eta$ viscosity, and $r_s$ the hydrodynamic radius of the molecule. NMR spectroscopic method for detecting diffusion is based on spatial labeling of the molecules using magnetic field gradients [124,125]. A short gradient pulse creates a phase encoding on the spins, which after a user–defined diffusion time is decoded by a second gradient pulse with an opposite sign. If the molecules remain static during the diffusion time, the decoding of the phase is complete, and an echo signal $E$ with no intensity reduction is obtained. If the molecules have moved from their original position during the diffusion time, the phase decoding is effective only partially, and there will be an attenuation in the observed echo signal. For a given gradient strength and duration the amount of attenuation depends on length of the experimentally set diffusion time and on the diffusion coefficient of the particle. In practice the echo signal is measured as a function of gradient pulse strength, and obtained data is fitted with a Stejskal–Tanner equation [124]

$$E(k) = e^{-kD},$$

(3.6.2)

with

$$k = (\gamma g \delta)^2 \left( \Delta - \frac{\delta}{3} \right).$$

(3.6.3)

In Equation 3.6.3 $g$ is the gradient strength, $\delta$ is duration of the gradient pulse, and $\Delta$ is the diffusion time. Pulse programs for diffusion measurements can be divided into
two families: Spin echo (SE) type, or stimulated echo (STE) type, for which a pulse sequence is shown in Figure 3.6.1. The difference between these two types is the type of relaxation mechanism that sets limit for the length of diffusion time in pulse sequence. In SE experiments the limit is set by transversal relaxation, while in STE this comes from longitudinal relaxation. The STE type experiments are more favorable in the case of inhomogeneous systems, like when monitoring molecular motion in confined space.

When the mobility of the molecules is limited, e.g., by a confinement inside some porous structure, the diffusion coefficient obtained with NMR method becomes a function of diffusion time [126]. This is because molecules are being reflected from pore walls back to the original volume where they were located during the first gradient pulse. The intensity reduction caused by gradients is thus less efficient, and results to a lower value for diffusion coefficient compared to that of free diffusion ($D_0$). The diffusion coefficient for this seemingly slower diffusion is called apparent diffusion coefficient ($D_a$). As the diffusion time is increased a larger portion of the molecules becomes affected by the pore walls around them. Since the observed diffusion coefficient is an average over the whole population, it continues decreasing until it reaches a level-off value $D_\infty$. The decay towards the leveling-off value can be described as [127]

$$D_a(\Delta) = D_\infty + \frac{D_0 - D_\infty}{D_0} \frac{\langle a^2 \rangle}{2d\Delta},$$  \hspace{1cm} (3.6.4)

where $\langle a^2 \rangle$ is the average squared pore diameter, $d$ is the dimensionality of the system, and $\Delta$ is the diffusion time. For a completely isolated pores $D_\infty$ is zero, while for a connected pore network its value depends on the tortuosity of the network. Tortuosity describes how much the path of a molecule moving from one point to another deviates from a straight line, i.e., how twisted the pore network is. The tortuosity $\alpha$ is defined to be [128,129]

$$\frac{1}{\alpha} = \frac{D_\infty}{D_0},$$  \hspace{1cm} (3.6.5)

and it can be obtained from diffusion data analysis, assuming that the confinement
inside the pores does not affect the true diffusion rate of confined water, thus changing
the value of $D_0$. The temperature dependence of $D_0$ has to be taken into account when
carrying out diffusion experiments at varying temperatures. In the work belonging
to this thesis $D_0$ at temperatures below 0 °C was calculated using data given by Price
et al. [130] The determination of $\langle a^2 \rangle$ and $D_\infty$ is carried out by measuring the $D_a$ as
a function of diffusion time, and performing a linear fit to the data after expressing
$D_a$ as a function of $\frac{1}{\Delta}$.

### 3.6.1 Impact of cross relaxation on diffusion experiments

Cross relaxation between diffusing species and the matrix that forms the pore walls
causes the spatial labeling created by PFG:s to become lost into the matrix, thus
affecting the value of apparent diffusion coefficient. The time scale for cross relaxation
is similar to that of diffusion time: As the diffusion time increases, so does the impact
of CR, and the $D_a$ becomes more affected. Unfortunately, CR induces a very similar
dependence to $D_a(\Delta)$ as does restricted diffusion. If CR is not taken into account
when analyzing diffusion data, this may lead to overestimation of the dependence
of the diffusion coefficient on diffusion time, and thus lead to erroneous conclusions
about the nature of the confinement of the diffusing species [120,123].

The effect of cross relaxation can be accounted in diffusion data analysis by in-
cluding a correction term into Stejskall–Tanner equation (Equation 3.6.2) using the
parameters obtained from the GS experiment [117,123]:

$$E(k) = \exp(-kD) f_{CR}(k, D, \tau, A', k_{liq}k_{sol}),$$

(3.6.6)

where

$$f_{CR}(k, D, \tau, A', k_{liq}k_{sol}) = \exp(A\tau/2) \times \frac{\cosh(B\tau/2) - \frac{A + A'}{2} \sinh(B\tau/2)}{\cosh(B_0\tau/2) - \frac{A + A'}{2} \sinh(B_0\tau/2)},$$

(3.6.7)

and

$$A = kD / (\Delta - \delta/3)$$
$$A' = k_{liq} + R_{liq} - (k_{sol} + R_{sol})$$
$$B = \sqrt{(A + A')^2 + 4k_{liq}k_{sol}}$$
$$B_0 = \sqrt{(A')^2 + 4k_{liq}k_{sol}}.$$  

### 3.6.2 Anisotropy of diffusion

In NMR experiment the diffusion is measured in the direction of the applied magnetic
field gradient. Fibers, however, are scattered into every possible direction, with the
direction of the fiber length axis being the preferred direction for diffusion. Because
of this, the echo becomes an integral over all possible fiber orientations:

\[
E(k) = \int_{0}^{\pi/2} d\theta P(\theta) \exp(-kD\theta),
\]

(3.6.9)

where \( \theta \) is the angle between the fiber director and magnetic field gradient, \( D_\theta \) is the diffusion coefficient corresponding to this orientation, and \( P(\theta) \) is the normalized probability distribution for the orientation \( \theta \). In the case of three dimensional powder \( P(\theta) \) equals \( \sin(\theta) \). Distribution of orientations can be transformed into distribution of diffusion coefficients [131], leading to following expression for the echo:

\[
E(k) = \int_{0}^{\infty} dD \int_{0}^{\pi/2} d\theta P(\theta) \xi(D,\theta) \exp(-kD)f_{CR}.
\]

(3.6.10)

In Equation 3.6.10 the term \( f_{CR} \) is the correction term for cross relaxation introduced in Equation 3.6.6, and

\[
\xi(D,\theta) = \frac{1}{D\lambda\sqrt{2\pi}} \exp \left[ -\frac{1}{2} \left( \frac{\ln(D/D_\theta)}{\lambda} - \lambda \right)^2 \right]
\]

(3.6.11)

is a function that is used to approximate Dirac’s delta function in numerical integrations by setting the width parameter \( \lambda \) small enough. The main purpose for using the Equation 3.6.10 in this thesis for diffusion data analysis is the numerical stability it provides, which is a prerequisite for automation of the data processing. It would be a tempting idea to try to characterize the possible changes in anisotropy in the fiber caused by pretreatments. However, the integration carried out for obtaining \( E(k) \) is an inverse Laplace transform by nature, and it is a well known fact these transforms are not unique, i.e., the same experimental data can be reproduced with several distributions [131]. Because of this, the attempt of quantifying the anisotropy based on distributions originating from the calculation of \( E(k) \) might yield unreliable results.
4 Experimental

This section gives a brief overview of the experimental work that was carried out for this thesis. The experimental approaches can be roughly divided into two classes: The first one deals with characterization of cellulose itself, i.e., its response to various physico-chemical processes seen as changes in crystallinity, or in changes of supermolecular assemblies of cellulose fibrils (papers I and II). The NMR spectroscopic method for observing these was solid state $^{13}$C CP–MAS spectroscopy. The second approach taken was to monitor the structure of pulp fibers indirectly via molecular probes. This was done by observing the NMR signals from the solvent species in the study of interactions during the dissolution process (paper III), and by monitoring the water located inside the fiber wall (paper IV).

4.1 Cellulose fibril aggregation in varying kraft pulping conditions

It is well known that changes in kraft pulp properties can be affected by varying the cooking conditions [132–134]. It has been also shown that during the cook changes in cellulose superstructure take place: There is an increase in both crystallinity and in LFAD [25, 28, 135, 136]. However, the connection between observed macroscopic properties and molecular level cellulose structures has not been fully understood. In order to clarify this, physical properties that are relevant for papermaking and changes that take place at supermolecular arrangements of cellulose fibrils in varying kraft pulping conditions were studied using solid state NMR spectroscopy (paper I). Four high alkalinity (HiA) cooks were performed with varying impregnation and cooking conditions, one of which having added polysulfide (PS), which is an additive used in kraft cooks to improve the yield. Besides these, a low sulfidity cook was performed to provide a pulp with a long cooking time, and a cook with standard kraft cooking conditions, with and without PS, to serve as a reference. The strength properties of the pulps were then analysed and compared to their LFADs, which were calculated by the method developed by Larsson et al. [102].
4.2 Microcrystalline cellulose from unconventional cellulose sources

The purpose of paper II was to study the physico-chemical properties of microcrystalline cellulose manufactured from various unconventional cellulose sources, i.e., from spruce needles (SN), pine needles (PN), hemp stalks (HS), and rice husks (RH). The MCC from purified starting materials was prepared by acid hydrolysis to a level-off degree of polymerization [137, 138]. Starting materials and the MCC produced from them were characterized using multiple different methods, including electron microscopy, X-ray scattering, and solid state $^{13}$C CP–MAS NMR spectroscopy. The role of solid state NMR experiments was to provide information about crystallinity and the relative amounts of crystalline forms [139] present in the MCC prepared from the starting materials. The crystallinity of MCC is known to affect, e.g., the drug releasing rate from MCC formulations [140].

4.3 Cellulose–solvent interactions

The dissolution process of softwood pulp into NMMO/D$_2$O solvent system was studied in paper III. The water in the solvent system was replaced by D$_2$O to reduce the signal from residual water in proton detected experiments. Wood pulp sample with enhanced reactivity, achieved by steam explosion and enzymatic hydrolysis, was mixed with a low amount of the solvent so that no complete dissolution would take place. Purpose of this was to mimic the initial stage of dissolution when the swelling of the fiber takes place, and to obtain samples that could be studied with both solid state NMR spectroscopic methods and HR–MAS experiments. Samples from this mixture were then kept at elevated temperature with varying time periods, and after designated heat treatment times (15 minutes, 30 minutes, and 90 minutes) they were cooled down to room temperature to stop (or at least considerably slow down) the reactions between solvent and cellulose. The aim of this was to create snap-shots of the solvent–pulp system at different dissolution stages, from which the progress in dissolution could be analysed. The time for the pulp–solvent mixture at the elevated temperature will be referred as the solvent exposure time hereafter. Besides of monitoring the changes at cellulose matrix, the solid state NMR methods were used to observe the signals from NMMO, and HR–MAS $^1$H experiments were used to observe those solvent species that were not in solid state. Cellulose–solvent interactions have been earlier studied by using liquid state NMR experiments in a system where cellulose has been completely dissolved [85, 141], but these approaches suffer from the fact that most of the solvent signal comes from bulk solvent molecules that are not interacting with cellulose. With HR–MAS the concentration of the solvent can be kept low enough
to ensure that most of the signal is gathered from molecular species that are in close contact with cellulose matrix.

4.4 Effect of pretreatments on pulp fiber wall

The response of the wood pulp fiber wall to mechanical treatment and enzymatic hydrolysis was studied in paper IV. A softwood dissolving grade pulp was exposed to mechanical shredding by a Baker Perkins mixer, with varying shredding time, and to subsequent enzymatic hydrolysis with a commercial endoglucanase. The effects of the treatments to fiber wall were then analysed by using solid state NMR spectroscopy and X-ray scattering, providing information about cellulose crystallinity and lateral fibril dimensions, and by studying the behavior of water confined inside the fiber wall using NMR diffusometry, cross relaxation measurements, and quantitation of the amount of nonfreezing water (NFW).

One difficulty in NMR diffusometry experiments in this case arises from the presence of bulk water, which is not interacting with cell wall. This is the source for the most intense signal when fibers are in water swollen state, i.e., their moisture content is above the fiber saturation point, and it obscures the signal from the water that is within the porous network. A solution to this was to carry out the diffusion experiments in a temperature below the freezing point of bulk water [119]. When a liquid is confined inside porous media, its freezing temperature is shifted down due to osmotic and capillary effects [142]. This ensures that the detected signal comes from water that is confined inside the fiber wall, thus having a depressed freezing temperature. The drawback of this approach is the formation of ice, which inevitably affects the pores. The alternative approach involving drying the fiber to a certain moisture content could, however, also lead to shrinkage of the porous network, and it was therefore rejected.
5 Results and discussion

5.1 Observation of cellulose using solid state NMR methods

Solid state $^{13}$C CP–MAS NMR experiments were used in all papers I–IV contributing to this thesis to provide direct information about cellulose structure at molecular and supermolecular levels. Crystallinity of cellulose is the easiest quantity to obtain from solid state NMR spectrum, but by a deeper analysis also the dimensions of the fibrils and fibril aggregates can be estimated. The fibril aggregate size can be further used to calculate the specific surface area of the fiber in the water swollen state, and by including data from fiber saturation point analysis also the average pore size of the fiber can be calculated.

5.1.1 Cellulose fibril aggregation in varying kraft pulping conditions

When comparing the LFAD:s obtained from different cooks, it was observed that varying the temperature during impregnation or cooking did not affect the LFAD within experimental error. Overall, the high alkalinity cooks had LFAD about 2 nm larger than the reference cook. The presence of polysulfide, however, changed this pattern, as then there were no significant difference between reference PS and HiA–PS. This was attributed to be most likely due to a protective nature of PS towards hemicelluloses during the cooking process, thus inhibiting the aggregate growth.

When it comes to correlation between aggregate sizes and physical properties, no clear evidence of such was observed (Table 5.1). The Figure 5.1.1 shows tear index as a function of LFAD. It would first appear that the pulps having high tear index also are having a large aggregate size. However, by closer inspection it can be seen that even though the pulps PS and HiA90i180c have a considerable difference in their tear index, their average LFAD can not be distinguished within experimental error. This suggests that the grouping seen in the Figure 5.1.1 is not caused by the aggregate size.

One specific issue regarding the data analysis carried out here needs to be pointed
Table 5.1: Strength properties for the pulp series at tensile index 70 Nm/g.

<table>
<thead>
<tr>
<th></th>
<th>Beating (revs.)</th>
<th>WRV (g/g)</th>
<th>Density (kg/m³)</th>
<th>Tear index (mNm²/g)</th>
<th>Zero span, wet (Nm/g)</th>
<th>Scott Bond (J/m²)</th>
<th>LFAD (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref</td>
<td>1103</td>
<td>1.94</td>
<td>742</td>
<td>13.8</td>
<td>134</td>
<td>449</td>
<td>18.2(0.3)</td>
</tr>
<tr>
<td>HiA130i180c</td>
<td>1787</td>
<td>1.83</td>
<td>700</td>
<td>16.1</td>
<td>141</td>
<td>296</td>
<td>20.4(0.2)</td>
</tr>
<tr>
<td>HiA90i180c</td>
<td>1746</td>
<td>1.74</td>
<td>711</td>
<td>15.6</td>
<td>134</td>
<td>311</td>
<td>20.2(0.5)</td>
</tr>
<tr>
<td>HiA90i160c</td>
<td>1737</td>
<td>1.82</td>
<td>738</td>
<td>15.9</td>
<td>136</td>
<td>459</td>
<td>20.9(0.5)</td>
</tr>
<tr>
<td>PS</td>
<td>778</td>
<td>1.88</td>
<td>743</td>
<td>14.0</td>
<td>128</td>
<td>437</td>
<td>19.5(0.3)</td>
</tr>
<tr>
<td>PS–HiA</td>
<td>801</td>
<td>1.84</td>
<td>714</td>
<td>14.1</td>
<td>133</td>
<td>327</td>
<td>18.7(0.4)</td>
</tr>
</tbody>
</table>

*i: Impregnation temperature, c: Cooking temperature

Figure 5.1.1: Tear index of the pulp samples at tensile 70 Nm/g as a function of lateral fibril aggregate dimension. The error bars correspond to one standard error.
5.1. Observation of cellulose using solid state NMR methods

Table 5.2: Cellulose crystallinities (C) for MCC from different sources, and for wood pulp after solvent exposure, and for wood pulp after reactivity enhancements by mechanical and enzymatic pretreatments.

<table>
<thead>
<tr>
<th>MCC source</th>
<th>(Paper II)</th>
<th>Solvent exposure</th>
<th>C (%)</th>
<th>Pretreatments</th>
<th>C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine needles</td>
<td>60</td>
<td>REF–SE(^a)</td>
<td>58</td>
<td>REF</td>
<td>61</td>
</tr>
<tr>
<td>Rice husks</td>
<td>45</td>
<td>15 min(^b)</td>
<td>57</td>
<td>M30(^c)</td>
<td>62</td>
</tr>
<tr>
<td>Hemp stalks</td>
<td>62</td>
<td>30 min</td>
<td>57</td>
<td>M60</td>
<td>60</td>
</tr>
<tr>
<td>Cotton based</td>
<td>65</td>
<td>90 min</td>
<td>42</td>
<td>M150</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M300</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M300ELD(^d)</td>
<td>63</td>
</tr>
</tbody>
</table>

\(^{a}\)Steam exploded and enzymatically hydrolyzed starting pulp.  
\(^{b}\)Solvent exposure time.  
\(^{c}\)M\(_x\): Mechanical treatment for \(x\) minutes  
\(^{d}\)M300 followed by low dosage enzymatic treatment

out. In the fitting procedure for calculating LFAD only Gaussian shaped lines was used in the cellulose crystalline region. This has no physical justification, as it is a known fact that line shapes for signals originating from highly crystalline domains in solid state NMR are Lorentzian type. In the case of cellulose crystalline C4 signal this has also been proved theoretically [143]. The reason for choosing the Gaussian line shape was the stability it provided in the fitting process. When three Lorentzian functions were used to model the cellulose \(I_\alpha\), \(I_\beta\), and \(I_\alpha + I_\beta\) signals in C4 region, the outcome of the fitting procedure became highly dependent on the starting values of the fit. The use of Gaussian functions seemed to offer a remedy for this problem. A physically more acceptable solution to this, used in papers \(\text{III}\) and \(\text{IV}\), would have been an exclusion of the one function describing the \(I_\alpha\) signal. This had similar effect in stabilizing the fitting procedure by making it less sensitive to starting values.

5.1.2 Cellulose crystallinities

Crystallinity (or crystallinity index) of cellulose is an often used indicator for changes taking place in cellulosic materials during physicochemical processing [105, 144, 145]. In this thesis crystallinity of cellulose was used as one of the characterization methods of MCC manufactured from different cellulose sources (paper \(\text{II}\)), and in study of the solvent exposure effects (paper \(\text{III}\)), and the effects of mechanical and enzymatic pretreatments (paper \(\text{IV}\)) on pulp fibers. The results are gathered in Table 5.2.

The solid state \(^{13}\text{C}\) CP–MAS spectra for MCC produced from pine needles (PN), hemp stalks (HS), and rice husks (RH) is shown in Figure 5.1.2. The characterizations carried out showed that acceptable yield (calculated relative to pristine material) was achieved with hemp stalks and rice husks as sources. The yield is, however, only one of the factors that affect the usability of the product. The crystallinity for RH–MCC was found to be considerably lower as compared to MCC from other sources, and the
Figure 5.1.2: Solid state NMR $^{13}$C CP–MAS spectra of MCC from unconventional sources. From bottom to top: Hemp stalks, pine needles, rice husks.

Figure 5.1.3: The effect of solvent exposure on steam exploded and enzymatically hydrolyzed wood pulp as seen from solid state NMR $^{13}$C CP–MAS spectra. The signals originating from NMMO are at 60–69 ppm. From bottom to top: Reference pulp with no solvent, mixed with NMMO/D$_2$O and heat treated for 15 min, 30 min, 90 min.
5.1. Observation of cellulose using solid state NMR methods

Figure 5.1.4: Solid state $^{13}$C CP–MAS NMR spectra for mechanically and enzymatically treated pulps. From bottom to top: Reference, M30, M300, M300ELD. The naming convention is that given in Table 5.2.

Figure 5.1.4 shows the effect of solvent exposure on solid state $^{13}$C CP–MAS NMR spectra of the pulp samples (paper III). It can be seen that the signals from the non-crystalline C4 become broadened already after the shortest solvent exposure time. There is also a change in the fine structure of the C1 signal, seen as an increase of the low-field component at 106 ppm. Both of these observations can be traced back to changes taking place at the interface of cellulose fibrils [86,146]. The crystallinity of cellulose was observed to remain intact for the first 30 minutes in the reaction conditions that were used. After 90 minutes there was a clear decrease in crystallinity, from 57 % to 42 %. However, as the prolonged solvent exposure also caused the appearance of C4 signal to deviate notably from its typical outlook, with signs of regeneration into cellulose II, the crystallinity value obtained after 90 minutes solvent exposure should be considered as tentative.

The solid state $^{13}$C CP–MAS NMR spectra of pulps with mechanical and enzymatic pretreatments are shown in Figure 5.1.4 (paper IV). A visual inspection reveals almost no differences between them. This similarity continues in their crystallinity

ash content was observed to be slightly higher for RH–MCC than it was for HS–MCC. On the other hand, the degree of polymerization for HS–MCC was found to be only about one half of that of RH–MCC:s. The main crystalline form for all MCC:s was observed to be $I_\beta$, but for RH–MCC the signal from $I_\alpha$ was not detectable at all, indicating that most of the crystallites were of type $I_\beta$. This may have impact on RH–MCC:s reactivity, as the $I_\beta$ is thermodynamically more stable than the $I_\alpha$ form.

The solid state $^{13}$C CP–MAS NMR spectra of pulps with mechanical and enzymatic pretreatments are shown in Figure 5.1.4 (paper IV). A visual inspection reveals almost no differences between them. This similarity continues in their crystallinity
Table 5.3: Effect of solvent exposure on specific surface area of steam exploded and enzymatically hydrolyzed (SE) wood pulp, and effect of pretreatments on average pore size at nanometer size scale \((a)_{nm}\). Sample naming corresponds to that in Table 5.2.

<table>
<thead>
<tr>
<th>Solvent exposure time</th>
<th>SSA ((m^2 g^{-1}))</th>
<th>Pretreatment</th>
<th>((a)_{nm} (nm))</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF–SE 98 ± 7</td>
<td>REF 7.6 ± 0.4</td>
<td>M30 14.8 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>15 min 89 ± 6</td>
<td>M300 15.0 ± 0.6</td>
<td>M300ELD 15.8 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>30 min 133 ± 7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and fibril sizes: It was observed there were no changes in crystallinity caused by any of the pretreatments, which was also verified by wide angle X-ray scattering. An agreement between WAXS and solid state NMR was also obtained in the case of lateral fibril dimensions: There were no changes in LFD caused by the pretreatments.

5.1.3 Specific surface areas and average pore sizes

The analysis method for calculating LFAD that was used in paper I can be extended to yield also specific surface areas, and with additional knowledge of fiber saturation point, average pore sizes for pulp fibers in water swollen state [34,107]. This approach was taken to characterize the SSA of pulp fibers after different solvent exposure times (paper III), and to quantify the effect of mechanical and enzymatic treatments on pulp fiber wall porosity at nanometer size scale (paper IV). The results are given in Table 5.3.

Even though the cellulose signals for pulps with 15 and 30 minutes solvent exposure appear by eye inspection rather similar (Figure 5.1.3 on page 30), a more detailed analysis revealed that there was a notable increase in SSA after 30 minutes solvent exposure compared to that of reference sample and to sample with shorter solvent exposure. After 90 minutes exposure the appearance of the C4 signal was altered to such extent that application of the fitting model was no longer possible.

The average pore sizes between the pretreated pulps at the approximately ten nanometer size scale were not affected within experimental error by duration of the shredding process, nor by the subsequent enzymatic hydrolysis (paper IV). There was, however, a clear difference in average pore size between the REF sample and the pretreated samples. This was accounted to be due to fact that the starting pulp was prepared from dried sheets, and drying is known to lead to (partially irreversible) coalescence of cellulose fibrils, thus decreasing the pore sizes.

An interesting issue on the results so far is that according to crystallinity results (Table 5.2), it can be seen that in the case of solvent exposure there was no change in the crystallinity of cellulose for the first 30 minutes. Yet, there was a notable change in SSA already after 30 minutes solvent exposure. Additionally, there was no difference to be seen in crystallinities between REF and pretreated pulps, a result that was also confirmed by wide angle X–ray scattering results. However, the average
5.2. Utilization of molecular probes

Pore size had almost doubled after the shortest mechanical treatment. This suggests that determination of the crystallinity of cellulose in materials having considerable complexity, like in wood pulp fibers, offers only a rather limited view to the material’s structure. In the case of MCC the crystallinity may still be a reasonable quantity to be characterized, since during the preparation of MCC a large part of the original complexity is lost. It is to be noted, however, that also in the case of MCC there are some peculiarities when crystallinities are correlated to other physical properties. For instance, the dissolution rate of a drug molecule from MCC tablet has shown to be dependent on the crystallinity of MCC, but this dependence was not a simple linear correlation [140].

5.2 Utilization of molecular probes

From NMR spectroscopists point of view the results from papers I and II were, if not unsatisfactory, at least somewhat incomplete. The strength properties of pulp fibers emerge from a complex interplay of features like molecular mass of both cellulose and hemicellulose, oxidative damage of the polysaccharides, cell wall dimensions, and fibrillar angle. Thus the expectations of being able to correlate the solid state NMR spectroscopic results directly to the macroscopic pulp properties, that was attempted in paper I, were perhaps unrealistic in the first place. In the case of paper II the solid state NMR analysis that was carried out seemed to offer a rather narrow view to the properties of the studied materials. Even though the crystallinity and relative amount of crystalline forms do play an important role in reactivity of the cellulose, there still might be some other important structural aspects as well that could be observed by extending the scale of NMR spectroscopic methods from the solid state NMR. This led to search for additional NMR approaches that could be used in combination with the solid state NMR spectroscopy for creating a more complete view of pulp fiber in later studies. As wood pulp is typically rich in water content, a natural choice seemed to be to use water molecules as probes for pulp structure. On the other hand, when exposing pulp to solvent systems, the solvent molecules could be used to serve as probes for accessibility and to monitor how the interactions between cellulose and the solvent evolve as the dissolution proceeds. These approaches, along with solid state NMR experiments, were taken in papers III and IV where cellulose–solvent interactions and effect of pretreatments on pulp fiber wall were studied.

5.2.1 The progress of dissolution as seen from NMMO solid state spectra

The 15 % (by weight of D₂O) NMMO/D₂O solvent system is known to be a good solvent for cellulose [83,147]. The low solvent concentration that was used in preparing
pulp–solvent mixture ensured, however, that the samples did not dissolve completely during the heat treatment. It was assumed instead that due to the heat treatment the NMMO molecules would be located in several different phases with different freedom in mobility: Some of them would become more closely associated with cellulose, thus having a restricted mobility. This would make them easier to observe by cross polarization experiment, which, due to nature of the cross polarization, favors the rigid regions in the sample. In the solid state $^{13}$C CP–MAS NMR spectra of solvent–pulp mixtures (Figure 5.1.3 on page 30) it is actually seen that there is a clear increase in the intensity of NMMO signals at region $\sim 60–69$ ppm relative to cellulose signals after 30 minutes solvent exposure. This was accounted to be due to increase in amount of those NMMO molecules that were closely associated to cellulose fibrils.

The NMMO $^{13}$C signals in NMMO–pulp mixture are in same spectral region with those originating from cellulose C6. NMMO contains one nitrogen atom, and thus offers the option to observe $^{15}$N, which has no interference from other molecular species. The low sensitivity, combined with low natural abundance of $^{15}$N makes the detection more challenging compared to $^{13}$C, but using cross polarization this can still be achieved with reasonable signal to noise ratio in acceptable acquisition time. Figure 5.2.1 shows the $^{15}$N spectra from the pure NMMO/D$_2$O solvent, and from pulp–solvent mixtures with different solvent exposure times. In the heat treated samples two signals, with relative intensity changing with the heat treatment time, were observed. This was attributed to possible existence of two interaction sites between cellulose anhydroglucose unit and NMMO, with preference of these sites changing in the course of the dissolution process.
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Figure 5.2.2: $^1$H HR–MAS spectra of heat treated pulp samples mixed with NMMO/D$_2$O. Left: Steam exploded and enzymatically hydrolyzed pulp. Right: Reference pulp with no pretreatments. The spectra are from different heat treatment times, from top to bottom: 90 min, 30 min, 15 min.

5.2.2 Changes in accessibility

Solvent species within fiber wall pores, or in cell lumen are not visible for cross polarization based solid state experiments due to motional averaging of the dipolar coupling. Instead, they can be detected by HR–MAS spectroscopy, in which the applied magic angle spinning averages out the inhomogeneities in magnetic susceptibility that would otherwise make the spectra unreadable. The change in accessibility of solvents through the fiber wall, caused by steam explosion/enzymatic hydrolysis, and also the effect of the heat treatment to this accessibility could be observed from the HR–MAS spectra in the signals from both HOD and NMMO (paper III). In the case of NMMO the chemical shift of the signals was affected by the length of the heat treatment: Prolonging the heat treatment time caused the signals to move to the lower field (Figure 5.2.2). For comparison, a reference pulp that had not been pretreated was also studied by exposing it to the same solvent with same heat treatment times. In this case the first observation about chemical shift change was after a 90 minutes heat treatment.

It has been shown that with HR–MAS spectroscopy it is possible to distinguish between water located at different environments [148, 149]. The shape of the HOD signal was observed to be time dependent, with features depending on length of the heat treatment as shown in Figure 5.2.3. It was suggested that the high field component of the signal was due to HOD not yet penetrated the fiber wall, while the low field component was already entered the pore network of the fiber. Provided that this interpretation is correct, the change in the HOD signal would then reflect the migration rate of HOD inside the pore network of the fiber. The shape of the HOD signal is known to be extremely sensitive to temperature gradients inside the sample that can appear at high spinning rates [108]. This was actually the first interpretation
also here for the time evolution of the line shape, but the fact that the behavior of the line shape appears to be markedly different for samples with different heat treatment times, and that a relative low spinning rate of 3.0 kHz (there is a misprint in paper III) was used in the experiments, the temperature gradient explanation does not seem to be plausible. Another common artefact observed with biological samples is the centrifugal packing of the sample material to the rotor walls, causing a water environment in the middle of the rotor that is not interacting with the sample [150]. This water is seen in a HR–MAS experiment as a sharp signal, and it was first identified as an extracellular water signal. In the case of wood pulp sample it can be, however, argued that such strong separation does not take place, as pulp is much more solid–like material compared to tissue samples where the water separation has been observed.

A different level of accessibility was probed with cross relaxation experiments that were carried out in paper IV for pulps with different pretreatments. It was observed that in enzymatically treated pulps the cross relaxation rate constants for magnetization transfer from water protons to cellulose was increased by over a factor of two compared to those pulps that were pretreated only mechanically (Figure 5.2.4). The endoglucanases are known to favor the less–ordered regions in cellulose [151,152], which could improve the fiber’s swelling capability. An increase in the inter–fibrillar distance due to pretreatments was indeed observed by SAXS, with the distance increase being most prominent in the case of enzymatically treated samples. The increase in cross relaxation rate could be therefore interpreted as an increase in water accessible regions caused by swelling of the fibrillar structures.
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Figure 5.2.4: Cross relaxation rate constants ($k_{\text{liq}}$) for pretreated pulps at different temperatures. For naming see Table 5.2.

Figure 5.2.5: Results from NMR diffusometry experiments carried out at several temperatures for studying the effect of pulp pretreatments on fiber wall pore structure. Left: Average pore size at micrometer scale. Right: Inverse of tortuosity parameter $\alpha$. Higher value indicates lower tortuosity.
5.2.3 Effect of pretreatments on pulp porosity

In the previous sections the molecular probe approach was based on interpretation of the NMR signals from probe molecules (with an exception of the cross relaxation between water and cellulose), and thus obtaining information about the interactions and changes in accessibilities of the probe molecules. In the work carried out in paper IV to characterize the pore network in pretreated pulps, the role of the probe molecules was slightly different: Now their motion inside the pore network was studied via NMR diffusometry, or the effect of the confinement to the freezing behavior of water was monitored by quantifying the amount of nonfreezing water. The quantities that are obtained from diffusion experiments characterizing the pore structure are the average micrometer scale pore size and the tortuosity of the porous network.

The effect of pretreatments on average pore size at nanometer size scale was already discussed in section 5.1.3. NMR diffusometry operates at a different size scale: The observable pore size is at the same order than the average root mean square displacement of the probe molecule at given diffusion time. With diffusion times accessible to the NMR instrument used in the present work this corresponds pores that have at least one dimension at a micrometer size scale. In pulp fiber there exists water that remains liquid at temperatures at least as low as 249 K, located at cellulose fibril surfaces and nanometer sized cavities along the fibrils [119,153,154]. These structures are connected to such extent that diffusion of water over a micrometer length scale can take place during a diffusion time of 0.1 s [119], and are referred here as micrometer size scale pores. The results of the diffusion experiments carried out at various temperatures below the melting temperature of the bulk water are shown in Figure 5.2.5. The purpose of performing the diffusion experiments at multiple temperatures was merely to obtain trends to evaluate, so that it would be more easy to identify possible errors in the data analysis. According to diffusion results the average pore size at micrometer size scale does not differ significantly between pretreated samples. The connectivity of the porous network as seen by the tortuosity (Figure 5.2.5) appears to be slightly more affected, although the uncertainty in calculated results increases as the bulk melting temperature is approached. When comparing the values obtained at 263 K it can be seen that the connectivity in the micrometer size scale pores is increased by the mechanical treatment, with prolonging of the treatment time leading to larger effect, and is further increased by subsequent enzymatic hydrolysis. It is to be noted though that the calculated value for $\frac{1}{\alpha}$ presents the true tortuosity of the fiber only at temperatures relative close to the bulk melting temperature, because the increased ice formation inside the porous network at lower temperatures most likely creates additive blockages. It should also be noted that when deriving the Equation 3.6.4 on page 20, that was used in this work for analyzing the dependence of apparent diffusion coefficient on diffusion time, Valiullin and Skirda [127] assumed a
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Figure 5.2.6: The amount of nonfreezing water in pretreated pulp samples.

The quantitation of the amount of nonfreezing water in pretreated pulp samples (Figure 5.2.6) showed that there was a large difference between the reference pulp and the pretreated pulp samples. This was most likely due to sample history, as the REF pulp was prepared from sheets, and thus it has been subjected to at least partial drying. The mechanical treatment caused the NFW content to increase by a factor of three, while prolonged mechanical treatment led to a slight decrease in NFW content. The mechanical treatment is known to induce closing of the macropores [155], i.e., pores at size roughly from 1 nm to 30 nm [72]. This could explain the decrease in NFW at prolonged mechanical treatment. The increase of NFW after enzymatic hydrolysis could be due to increased swelling ability of the cellulose fibril aggregates, caused by degradation of the non–crystalline regions, leading to an increase in water accessible surfaces. Keeping in mind the large increase in cross relaxation rate constant caused by enzymatic treatment, it would be expected that enzyme treatment would also cause a more notable increase in NFW. This was not, however, the case. It was therefore suggested that the enzymatic treatment induces a change in the pore geometry to such having a larger specific surface area, while the pore volume increases only slightly. It is also possible that in addition to increase of water accessible sites, the increased inter–fibrillar distance also causes an increase in pore size in the nanometer size scale, thus decreasing the NFW content. According to diffusometric results, however, the connectivity of the structures supporting diffusion over a micrometer length scales should increase with enzymatic treatment. As the network of nanometer sized pores

pore model where spherical pores were connected by cylindrical cavities. This model is not well suited for those structures where diffusion of water is monitored in this work, and therefore the values obtained here should be taken as approximates.
is assumed to take part to the diffusion of water, the formation of additional ice into this network would appear to contradict the diffusion results. It may be though, that the diffusion taking place at the liquid water layer on fibril surfaces is capable of compensating the ice formation.

5.2.4 Molecular probe approach with other solvent systems (unpublished)

The method of utilizing solvent components in study of solvent–cellulose interactions that was used in the case of NMMO/D$_2$O may be extended also to other solvent systems, providing that the solvent molecules contain some suitable NMR–active nucleus. Such is the case with aqueous solvent systems of NaOH/urea (NaU) and LiOH/urea (LiU), where both $^{23}$Na and $^7$Li are NMR active. They also happen to be quadrupolar nuclei, i.e., their spin is higher than $\frac{1}{2}$, but as they both have high natural abundance and relatively low quadrupolar moments, they are not experimentally as demanding as most of the quadrupolar nuclei are. This does not, however, imply that the spectra obtained from them would be easy to interpret. The solid state line shapes from quadrupolar nuclei are rather complicated, making it difficult to, for instance, determine the number of chemically inequivalent nuclear sites. In liquid state NMR the use of quadrupolar nuclei is hampered by their fast relaxation that causes broad featureless signals. The line broadening in solid state NMR signals for quadrupolar nuclei is, however, due to anisotropy of the quadrupolar interaction, and it contains information about the local symmetries and structure. Due to their strong interaction with local electric field gradients, the quadrupolar nuclei are extremely sensitive probes for structural differences, and the relatively recent experimental advances have made their study possible without any special hardware [156,157]. There already exists several reports on application of $^7$Li and $^{23}$Na NMR spectroscopy in study of cellulose–solvent interactions [141, 158, 159]. In the mentioned works, however, only liquid state NMR experiments were performed. From solid state NMR experiments more information (at least in principle) can be extracted: The solid state spectrum line shapes carry information about the quadrupolar coupling constant and about the biaxiality of the of the electric field gradient tensor. In favorable case both of these parameters can be used to provide information about local structures [156].

In connection to dissolution studies carried out in paper III a preliminary work was carried out to test whether $^7$Li and $^{23}$Na solid state NMR signals are able to provide information about the pulp dissolution process in NaU and LiU solvent systems. Two samples from the same steam exploded and enzymatically hydrolyzed wood pulp that was used with NMMO/D$_2$O solvent system were mixed with NaU and LiU solvents, with a pulp to solvent mass ratio being approximately 1:5. The solvents were prepared to be 4% LiOH/12% urea and 7% NaOH/12% urea, for which a rapid
5.2. Utilization of molecular probes

Figure 5.2.7: $^7$Li (on left) and $^{23}$Na (on right) spectra from samples of steam exploded and enzymatically hydrolyzed pulp mixed with aqueous LiOH/urea and NaOH/urea solvents. Bottom: Sample without cold treatment. Top: the cold treated sample. Chemical shift scale was externally referenced to solid LiCl and NaCl by setting their signals to 0 ppm. $^7$Li spectra were acquired with a single pulse excitation, in $^{23}$Na experiments Hahn–echo pulse sequence was used.

dissolution of cellulose is known to take place at -10 °C [82]. Both mixtures were divided into two samples, of which one was stored at +8 °C, and the other was kept at -18 °C for two hours. Solid state $^7$Li and $^{23}$Na NMR spectra were then measured for both sample pairs at ambient temperature. Figure 5.2.7 shows the resulting spectra, with clear differences between the cold treated and the sample kept at +8 °C. According to these spectra, both $^7$Li and $^{23}$Na signals are sensitive for the changes that take place during the dissolution process, but the interpretation of the results is most likely going to be difficult. It is hard to tell, for instance, if there are several different chemical sites contributing to the $^7$Li spectra, or is the shape of the signal just due to the spread in quadrupolar interaction and in chemical shift, caused by the molecular disorder in the sample [160]. Even if it were possible to extract the quadrupolar coupling parameters that depend on local structure and environment, and that give the distinctive shapes to the signals, correlating these parameters to actual structures is far from trivial task [161]. One approach could be an application of ab initio calculations, together with experimental studies, on some model systems with lower complexity than the pulp–LiU (or NaU) system. Additional difficulty rises from the observed line shapes' sensitivity to the sample history, which in repeated experiments was especially pronounced in the case of $^7$Li spectra. The experimental conditions were required to be replicated very carefully in order to reproduce similar results. However, both $^7$Li and $^{23}$Na NMR spectroscopy have been successfully used to characterize complicated disordered systems [162,163], which encourages to further develop the approach presented here.
6 Concluding remarks

When the work contributing to this thesis begun, the main course for the planned studies was set to be on characterization of the cellulose supermolecular structures by solid state NMR methods in complex systems. However, as the work proceeded it became clear that additional NMR approaches were required in order to fully describe the properties of the cellulosic materials. This thesis shows that the often used quantity, crystallinity of cellulose, is a rather bold tool for characterizing complex materials, like cellulose in pulp fiber wall, and changes that take place in it during various treatments. Changes in the cellulose supermolecular arrangements and in accessibility of chemicals into the fiber were observed even though the crystallinity of cellulose in the pulp samples was not affected. The application of NMR methods that indirectly probe the fiber wall structure were shown to be a valuable addition into the more traditional solid state NMR spectroscopic studies, although additional work is still required for quantitative understanding of the observations. Apparently the combination of different NMR approaches is of great importance when analyzing the events that take place when cellulosic materials like wood pulp are processed for applications, or when studying interactions between cellulose and other molecular species, like cellulose solvents. Considering the work to be carried out in the future, the complexity of the systems studied here sets quite high demands for creating a thorough quantitative understanding of, for example, observations in the case of signals from quadrupolar solvent species. But come to think of it, should it be too easy, it would be only of little interest.


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